THE RELATIONSHIP OF ENDOCRINE DISRUPTORS WITH PITUITARY, GONADAL, THYROID HORMONES AND SOME RECEPTORS IN NIGERIAN WOMEN WITH BREAST CANCER

BY

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ABSTRACT

Endocrine Disruptors (EDs) such as lead, cadmium, arsenic, polychlorinated biphenyls (PCBs) and bisphenol-A (BPA) are associated with increased risk of Breast Cancer (BCa). Human epithelial receptor, oestrogen, progesterone and their receptors, gonadotrophins and thyroid hormones are also implicated in the aetiology of BCa but remains controversial. Although, several studies have been conducted on BCa, the relationship of EDs with the hormones and receptors has not been fully explored. This study was designed to examine the relationship of these endocrine disruptors with pituitary, gonadal, thyroid hormones and selected receptors in Nigerian women with breast cancer.

One hundred and seventy non-pregnant women aged 48.3±1.3 years were purposively selected from the Surgical Oncology Clinic, University College Hospital, Ibadan. The study comprised of 85 Histologically-Confirmed Breast Cancer pre-therapy (HCBCa) which were sub-divided into premenopausal-HCBCa and postmenopausal-HCBCa. These were matched with 85 Apparently Healthy Women without BCa (AHWB) comprising of premenopausal-AHWB and postmenopausal-AHWB according to age and menstrual phase. Anthropometry and Blood Pressure (BP) were determined with standard methods. Blood was obtained from participants and centrifuged to obtain serum. Serum lead, cadmium and arsenic were determined using atomic absorption spectrophotometry. Bisphenol-A and PCBs were determined using HPLC and gas chromatography, respectively. Oestradiol, progesterone, Luteinizing Hormone (LH), Follicle Stimulating Hormone (FSH), Thyroid Stimulating Hormone (TSH), Free Thyroxine (FT$_4$) and Free Triiodothyronine (FT$_3$) levels were determined using ELISA. Expression of Oestrogen Receptor (ER), Progesterone Receptor (PR) and Human Epithelial Receptor 2 (HER2) were determined using immunohistochemistry. Data were analysed using descriptive statistics, Student's t-test and multiple regression at $\alpha_{0.05}$.

Waist Circumference (WC), Hip Circumference (HC), weight, height, Waist-Hip-Ratio (WHR), Waist-Height-Ratio (WHtR) were significantly higher in HCBCa compared with AHWB (89.9±1.1 vs 82.6±1.2 cm; 101.8±1.1 vs 98.5±1.0 cm; 69.2±1.4 vs 62.1±1.1 kg; 1.63±0.0 vs 1.58±0.0 m; 0.9±0.0 vs 0.8±0.0; 55.3±0.7 vs 52.4±0.7, respectively). The HCBCa had significantly higher levels of lead, cadmium, arsenic, PCBs and bisphenol-A compared with AHWB (5.5±0.2 vs 1.8±0.0 µg/dL; 0.04±0.0 vs 0.01±0.0 µg/dL; 0.30±0.0 vs 0.04±0.0 µg/dL; 0.8±0.5 vs 0.3±0.0 µg/dL; 0.8±0.7 vs 0.4±0.0 mg/dL, respectively). The FT$_4$ was significantly higher in HCBCa than AHWB (17.8±0.4 vs 14.7±0.3 pmol/L). There was no difference in LH, FSH and TSH in HCBCa compared with AHWB. Progesterone and

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oestradiol were higher in postmenopausal-HCBCa compared with postmenopausal-AHWB. (2.09±0.35 vs 1.04±0.10 nmol/L; 156.48±12.42 vs 90.42±3.59 pmol/L, respectively) Regression analysis showed that in HCBCa, oestradiol and WC jointly predicted lead and cadmium (β>0.348, β>5.830, respectively). Diastolic-BP predicted cadmium (β=0.299), WHR and HC predicted arsenic (β>2.732) while FT$_3$, WHtR and height positively predicted BPA (β>0.404). Contrarily, these relationships were absent in AHWB. Fifty-five tumour samples (69.6%) exhibited ER-, PR- and HER2- expressions (triple negative) while 2 (2.5%) demonstrated ER+, PR+ and HER2+ expressions. Fifty-two tumour samples (100%) in premenopausal-HCBCa exhibited ER-/PR- co-expressions while 46 (88.5%) exhibited HER2- expression. However, 18 (66.7%), 18 (66.7%) and 17 (63.0%) postmenopausal-HCBCa exhibited ER-, PR- and H HER2tumour expressions, respectively.

Endocrine disruptors could have contributed to adiposity and be associated with negative receptor expression, which may induce cell proliferation and probably cause derangement in thyroid metabolism.

Keywords: Adiposity, Women with breast cancer, Endocrine disruptors, Hormone receptors.

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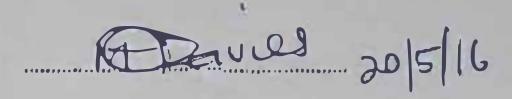
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CERTIFICATION

I certify that this work was carried out by Olulope Olufemi AJAYI in the Department of Chemical Pathology, Faculty of Basic Medical Sciences, College of medicine, University of Ibadan, Nigeria.

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DEDICATION

This work is dedicated firstly to the Almighty God, the fountain of knowledge and secondly to Rev Benjamin A. Orimolade (late), a worthy servant of God who lived a life of sacrifice to the glory of God and the betterment of humanity.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

As:	Arsenic
BCa:	Breast cancer
bcl-2:	B cell lymphoma/leukemia2
BM:	Basement membrane
BMI:	Body mass index
BPA:	Bisphenol-A
cAMP:	cyclic Adenosine Monophosphate
Cd:	Cadmium
CYP:	Cytochrome P450
DNA:	Deoxyribonuleic acid
E_1:	Oestrone
E_2:	Oestradiol
E_3:	Oestriol
ECM:	Extracellular matrix
EDs:	Endocrine disruptors
EIA:	Enzyme immune assay
ERα:	Oestrogen receptor-alpha
FSH:	Follicle stimulating hormone
FT_3:	Free triiodothyronine
FT_4:	Free thyroxine
GI:	Glycemic index
GL:	Glycemic load
GnRH:	Gonadotrophin releasing hormone
HC:	Hip circumference
hCG:	Human chorionic gonadotrophin
HER 2:	Human epithelial receptor 2

HRT: Hormone replacement therapy.

LH: Luteinizing Hormone

MMP-1: Matrix metalloproteinases-1

mRNA: Messenger ribonucleic acid

NADPH: Nicotine Adenine Dinucleotide Phosphate

OCs: Oral contraceptives

PA: Plasminogen-activator

Pb: Lead

PCBs: Polychlorinated biphenyls

PKA: Protein kinase A

PR: Progesterone receptor

PR-A: Progesterone receptor-α

PR-B: Progesterone receptor β

PRKO: Progesterone receptor knockout mice

SCFA: Short-chain fatty acids

SH: Sulfhydryl

SHBG: Sex hormone binding globulin

StAR : Steroidogenic Acute Regulatory Protein

TBG: Thyroxine-binding globulin

TDLU: Terminal ductal lobular unit

TIMP-1: Tissue inhibitor metalloproteinases-1

3β-HSD: 3, beta-hydroxysteroid dehydrogenase

TRH: Thyrotropin releasing hormone

TSH: Thyroid stimulating hormone

UCH: University College Hospital

UI: University of Ibadan

U S: United States of America

VEGF: Vascular endothelial growth factor

WC: Waist circumference

WHO: World Health Organization

WHR: Waist Hip Ratio

WHtR: Waist Height Ratio

CHAPTER ONE

1.0 INTRODUCTION

Breast cancer is one of the most common types of cancer that affects millions of women around the world with a noticeable fatality rate (Msolly et al., 2011). In Nigeria, the incidence which has been reported to be on the increase has been attributed to changes in demography, socio-economic status and epidemiological risk factors (Adebamowo and Ajayi, 2000; Privalsky, 2002). Several hormone-related factors, such as age at menarche, parity and age at menopause, are associated with breast cancer (Helzlsoucr et al., 1994). Moreover, high levels of endogenous sex hormones, especially oestrogens, are believed to increase breast cancer risk (Ho et al., 2009).

17β–oestradiol (E_2) is the most potent natural oestrogen and it is secreted by the granulosa and theca cells of the ovaries (Rotstein, 2011). This is under the control of the pituitary hormones, Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH). FSH stimulates the growth and recruitment of immature ovarian follicles in the ovary (Zhou et al., 2013). This is in addition to the fact that it regulates aromatase activity, whereas LH is responsible for the actual production of androgens in the ovarian theca cells, thus providing the substrate for aromatization to oestrogens in the granulosa cells (Powell et al., 2003; Rotstein, 2011). The principal function of the oestrogens is to cause cellular proliferation and growth of the tissues of the sex organs. This includes the development of the stromal tissues of the breast. It is thought that in promoting the growth of breast's end buds, oestrogens may contribute to an increase in cells that become prone to cancerous growth later in life (Russo and Russo, 1998; Brisken, 2008). Reports from animal studies and cultured human breast cells also suggest the induction of mammary tumours by oestrogens (Drabsch et al., 2007). Moreover, FSH has been linked with breast cancer cell proliferation and an increased risk of breast cancer development in females who have undergone infertility treatments (Zreik et al., 2010).

Progesterone, a sex hormone is primarily produced by the granulosa-lutein cells of the *corpus luteum* during the luteal phase of the menstrual cycle as well as the syncytiotrophoblast of the placenta during pregnancy. It enhances breast's lobular-alveolar development in preparation for milk secretion and facilitates implantation and maintenance of early pregnancy (Al-Asmakh, 2007). Although, the role of progesterone in breast cancer is controversial, it is suggested that its

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activity of opposing oestrogenic stimulation of the breast, decreases breast cancer risk (Ho et al., 2009). Conversely, it is thought that the risk of breast cancer is increased because breast mitotic rates are highest in the luteal phase (with high progesterone levels) of the menstrual cycle (Ho et al., 2009; Wang et al., 2009).

Thyroid hormones are the only iodine-containing substances of physiologic significance in vertebrates (Bello and Bakari, 2012). Thyrotropin Releasing Hormone (TRH) acts on the pituitary thyrotropes to stimulate both the synthesis and release of Thyroid Stimulating Hormone (TSH). Thyroid stimulating hormone controls the size and number of thyroid follicular cells. It stimulates the thyroid gland to produce thyroxine (T_4). Thyroxine, a prohormone, is converted to triiodothyronine (T_3), the active form of thyroid hormone in the peripheral tissues by 5'-deiodination (Krassas et al., 2010; Bello and Bakari, 2012). It is postulated that the thyroid gland interacts with the breast tissues, based on the common property of the mammary and thyroid epithelial cells to concentrate iodine by a membrane active transport mechanism. Additionally, TSH receptors in fatty tissues which are abundant in mammary gland have been reported to be a possible reason for this interaction (Turken et al., 2003; Ali et al., 2011). Thus, thyroid hormones appear to stimulate breast's lobular development, contributing to the differentiation of normal breast tissue (Lai et al., 2002; Neville et al., 2002). However, the relationship between breast cancer and thyroid hormone is controversial (Saraiva et al., 2005; Ali et al., 2011).

Hormone receptors are ligand-activated proteins that regulate transcription of selected genes. Oestrogen Receptor (ER) and Progesterone Receptor (PR) play important roles in the growth and differentiation of breast cancers making them important prognostic markers (Patel et al., 2013; Ramsey et al., 2015). The biologic, prognostic and predictive importance of assessment of ER expression in breast cancer is well established. However, the assessment of PR appears controversial in some regions of the world (Hefti et al., 2013; Qiao et al., 2013). Most evidence regarding the prognostic role of PR is based upon the assumption that its expression indicates a functioning ER pathway (Ravdin et al., 1992). Results from observational studies showed that loss of PR expression was associated with worse prognosis among ER+ breast cancer (Dunnwald et al., 2007; Prat et al., 2013). These results suggests that evaluation of PR status in ER+ breast cancer might be helpful in identifying those most likely to benefit from hormonal therapy (Hefti et al., 2013). Moreover, patients with ER- and/ or PR- breast cancer have been reported to have

higher mortality compared with those with ER+ and/ or PR+ (Anderson et al., 2001). Human Epithelial Receptor 2 (HER 2) also known as ErbB2-neu, located on chromosome 17q21 is also considered to be closely associated with the occurrence and development of breast cancer (Gown, 2008). HER 2 is inactive under normal physiological conditions but upon activation, it may enhance tumour invasion and metastasis (Guo and Bai, 2008). Hence, HER 2 status is important in the treatment of patients, particularly those with metastatic tumours who respond to Herceptin (Olayioye, 2001; Khokher et al., 2013). The knowledge of the different expression patterns of ER, PR and HER 2 is essential in planning the management of the disease (Low et al., 1992; Sacks and Baum, 1993).

Recent findings have suggested the contribution of environmental factors to the high incidence of breast cancer (Ragab et al., 2014). The current industrial revolution has brought about an increased use of various metals and compounds in industry, agriculture and medicine (Antila et al., 1996; Caserta et al., 2008). This is coupled to the wide spread environmental pollution which has been reported in Nigeria (Anetor et al., 2005). Environmental pollution has led to an increased exposure not only to occupationally exposed workers but also to consumers of the various products and the general public at large (Adachi and Tainosho, 2004). Some of these metals and compounds have been reported to adversely affect the endocrine signaling system and are referred to as endocrine disruptors (EDs).

Endocrine disruptors (EDs) may mimic, block or modulate the synthesis, release, transport, metabolism and binding or elimination of natural hormones. Even though EDs may be present in the environment at only very low levels, they may still cause harmful effects, especially when several different compounds act on one target (IPCS, 2002). EDs are widespread in food chains and in the environment. They include arsenic (As), cadmium (Cd), lead (Pb), bisphenol-A (BPA) and polychlorinated biphenyls (PCBs). Once in the environment, they are almost impossible to eliminate, because they do not decompose. These EDs are absorbed into the human system through different routes. Arsenic and cadmium compounds as well as PCBs are lipophilic, hence, they readily penetrate cell membranes (Carpenter et al., 2005). On the other hand, cadmium can bind to protein to form a complex, cadmium-metallothionein which is actively taken into the cell by endocytosis (Antila et al., 1996). Lead may be absorbed by passive diffusion while BPA is

absorbed upon ingestion of BPA contaminated food and water (Karnakar and Jayaraman, 1988; Kang *et al.*, 2006).

Arsenic is a metalloid that is ubiquitous in the environment. Human exposure includes ingestion of contaminated food and water, inhalation of contaminated air and by dermal contact. Arsenic compounds are lipid soluble and within 24 hours of absorption are distributed throughout the body where they can bind to sulfhydryl (-SH) groups on proteins. Arsenic may also replace phosphorus in bone tissue and be stored for years (Bartolome *et al.*, 1999). Methylation efficiency in humans appears to decrease at high arsenic doses and studies show that aging is associated with a diminishing capacity to methylate inorganic arsenic, resulting in increased retention of arsenic in soft tissues (Tseng *et al.*, 2005) including breast tissues. Interaction of arsenic compounds with thyroid hormone has been reported. Arsenite (AsO_3^{3-}) was reported to inhibit in vitro binding of triiodothyronine to its nuclear receptor at relatively high (millimolar) concentrations (Takagi *et al.*, 1990). Chronic exposure to arsenic compounds has been associated with several types of cancer (Frumklin *et al.*, 2001). Arsenite blocks the binding of E_2 to ER-alpha (ERα), acts as a ligand for ER thus, activating it in the absence of the hormone, suggesting that the metal interacts with the hormone binding domain of the ER. It increases cell growth and mimicked the effects of E_2, decreases the amount of ERα and increases the expression of the progesterone receptor (Stoica *et al.*, 2000a). However, there is paucity of information on arsenic in breast cancer patients in Nigeria.

Cadmium ranks close to lead as a metal of current toxicological concern (ATSDR, 2005). It occurs in nature in association with zinc and lead. Extraction and processing of these metals often lead to environmental contamination with Cd (Klaassen, 1996). Although, smoking is a well established source of cadmium exposure, the major route of cadmium exposure is ingestion of shellfish and certain food, particularly root vegetables, potatoes and grains (rice and wheat) grown on cadmium-rich soils. (McLaughlin *et al.*, 1997). Cadmium is a known cumulative toxicant with a biological half-life of more than 10 years in humans. Cadmium accumulation occurs in the adipose tissue, liver and kidneys (Sivrikoya *et al.*, 2013). Only a small fraction of inhaled or ingested Cd is excreted, resulting in increased body burden over time (Fujishiro *et al.*, 2012; Tekin *et al.*, 2012). Women tend to have higher Cd levels than men presumably because of lower iron stores, which increase Cd absorption (Olsson *et al.*, 2002; Reeves and Chaney, 2008)

Thus, comparable environmental exposures to Cd may disproportionately affect women compared to men (Reeves and Chaney, 2008). Chronic low Cd exposure will eventually result in accumulation to toxic levels (Sivrikaya *et al.*, 2013).

The ability of cadmium to induce cell proliferation, differentiation, apoptosis and signal transduction by enhancement of protein phosphorylation, activation of transcription and translation factors suggests its ability to induce breast cancer (Siewt *et al.*, 2010). Moreover, cadmium has the potential to disrupt endocrine function by behaving like sex hormones (Stoica *et al.*, 2000b). At low concentrations, the metal mimics the effects of oestradiol and binds with high affinity to the hormone-binding domain of ERα. This binding involves several amino acids, suggesting that cadmium activates the receptor through the formation of a complex with specific residues in the hormone-binding domain (Johnson *et al.*, 2003; Stoica *et al.*, 2000b). Circulating concentrations of pituitary hormones such as LH, FSH and TSH were altered in female rats exposed to Cd and Pb (Martin and Stoica, 2002).

Lead has been reported as a metal that can be found in drinking water, which is of great public health concern (ATSDR, 2005). Lead contamination in the environment, resulting in toxicity in several body organs and systems has been documented (Rothenberg *et al.*, 1994). This is in spite of the fact that Pb in gasoline, food cans and in paints was banned in the United States between 1980 and 1990. Recent reports showed that enamel paints with very high levels of Pb were sold freely in Nigeria (Clark *et al.*, 2007; Kessler, 2014). Lead adversely affects steroidogenesis by substituting for zinc in the DNA binding zinc (Zn^{2+})-linger motif of steroidogenic enzymes. These enzymes are Steroidogenic Acute Regulatory Protein (StAR), Cytochrome P450 side chain cleavage enzyme (CYP450cc) and 3 beta hydroxysteroid dehydrogenase (3β HSD). This results in decrease in the expression of the enzymes. (Huang *et al.*, 2002; Lutzen *et al.*, 2004). The reported mechanisms of Pb carcinogenesis are: direct DNA damage as a result of oxidative stress, inhibition of DNA synthesis and repair, and clastogenicity (Martin *et al.*, 2003; Anctor *et al.*, 2005; Ragab *et al.*, 2014). The results of epidemiologic studies investigating the association of Pb exposure with cancer are inconsistent and vary according to the type of cancers reported (Steenland *et al.*, 1992; Wong and Harris, 2000).

Bisphenol-A (BPA) also known as (BPA, 2, 2-bis (4-hydroxyphenyl) propane is a component of a variety of commonly used household items. It is used primarily in the manufacture of

polycarbonate plastic, epoxy resins and as a non-polymer additive to other plastics (Peretz et al., 2014). There is a wide spread and well documented human exposure to BPA. This is due to its extensive use in the manufacture of consumer goods and products, including polycarbonate food containers and utensils, dental sealants, protective coatings, some flame retardants, and water supply pipes (Calafat et al., 2005; Kang et al., 2006; Brody et al., 2007). Studies on the safety of BPA are inconsistent (Ochlmann et al., 2009). It is thought that BPA binds to the oestrogen receptors with almost the same strength as the oestradiol thereby eliciting oestrogenic effects (Welshons et al., 2006; Stahlhut et al., 2009). Moreover, BPA may also elicit a rapid response by binding non-classical membrane oestrogen receptors (ncmERs) (Alonso-Magdalena et al., 2005) or oestrogen-related receptors (ERRs) (Ben-Jonathan et al., 2009). BPA exposure increases adipose mass in rats by activating a key adipogenic regulator Peroxisome Proliferator Activated Receptor gamma (PPARγ), thus contributing to adiposity, a known breast cancer risk factor (Somm et al., 2009; van Kruijsdijk et al., 2009; Kwintkiewicz et al., 2010). There is currently paucity of information on the association of environmental exposure to BPA and the risk of breast cancer in Nigerian women.

Polychlorinated biphenyls (PCBs) are members of a chemical family that were widely used in the past in industry as lubricants, coatings and insulation materials for dielectric equipment like transformers and capacitors (Iyengar, 2005; Gray et al. 2009). The release of PCBs to the environment has been reported to be through poorly maintained hazardous waste dumps and city landfills, illegal or improper dumping of hydraulic fluids/coolants, leaks from electrical transformers and other equipment, burning of medical, industrial or city waste from older consumer goods like televisions (Gray et al., 2009). Human exposure to PCBs is through inhalation of contaminated air (outdoor or indoor), ingestion of contaminated food or non-food items, and dermal contact of contaminated surfaces. The primary route of exposure to PCBs is through consumption of contaminated lipid-enriched foods (e.g. fish and cooking oils) as PCBs can accumulate in these and other foodstuffs (Van-Emon et al., 2013). Studies on the association of PCBs and breast cancer aetiology are currently inconsistent. Some data indicate that high levels of PCBs are associated with oestrogen-negative tumours, which are more aggressive and have a faster rate of progression (Muscat et al., 2003 Kerdivel et al., 2013). One of the mechanisms involved include the induction of cytochrome P450 1A1 (CYP1A1) gene. An increased risk for breast cancer was reported for women with the highest blood levels of PCBs

who also possessed CYP1A1 variant (Muscat *et al.*, 2003). Conversely, other studies did not find association between PCBs and breast cancer aetiology (Sapozhnikova *et al.*, 2004; Martinez *et al.*, 2010).

There is a growing interest in understanding whether exposure to toxic metals and chemicals contribute to the increasing number of breast cancer cases worldwide. Unfortunately, relatively few studies have investigated the impact of these environmental chemicals on general human health and even fewer have addressed the roles endocrine disruptors may play in the initiation, promotion and progression of breast cancer (Martin *et al.*, 2003; Parkin and Fernandez, 2006). This present study was designed to identify the possible relationships of endocrine disruptors with pituitary, gonadal, thyroid hormones and selected receptors (ER, PR, HER 2) in Nigerian women with breast cancer.

1.1 Research Questions

1. Do participants with breast cancer have altered levels of reproductive and thyroid hormones?
2. What is the pattern of expression of ER, PR and HER2 in women with breast cancer?
3. Do participants with breast cancer have increased levels of EDs?
4. Are the alterations in hormone levels due to increased levels of EDs?
5. Are there relationships among the EDs, hormones and the receptors (ER, PR and HER2)?

1.2 Rationale for the Study

The incidence of breast cancer in Nigeria has been reported. 13.5 per 100,000 in the 1980s, 33.3 per 100,000 in 1992, 116 per 100,000 in 2001 (Adebamowo *et al.*, 2003). In spite of the numerous theories that have been proposed, the exact aetiology of breast cancer has not been clearly defined (Ijaduola and Smith, 1998; Adebamowo and Ajayi, 2000; Omar *et al.*, 2003).

Triple negative breast cancer is aggressive. It has been reported to be peculiar to African-americans and suggested to be common in African young women (Huo *et al.*, 2009) Studies aimed at determining the pathogenesis of the molecular subtypes that disproportionately affect young women of African ancestry are currently sparse.

High levels of endogenous sex hormones particularly oestrogens are thought to increase the risk of breast cancer (Ho et al., 2009). However, the role of progesterone and gonadotropins in the aetiology of breast cancer is controversial.

Thyroid hormones appear to stimulate breast's lobular development, thereby contributing to normal breast tissue differentiation (Neville et al., 2002). The role of thyroid hormones in the aetiology of breast cancer has not been systematically studied in indigenous women of Sub-saharan Africa.

Environmental exposure to EDs has been implicated in the aetiology of breast cancer (Ragab et al., 2014). However, there is currently paucity of information on the serum concentration of these EDs; lead, cadmium, arsenic, bisphenol-A, polychlorinated biphenyls.

There are reports of interactions of the EDs with the hormone signalling pathways (Caserta et al., 2008). There is paucity of information on the interactions of EDs with sex and thyroid hormones in Nigerian women with breast cancer.

1.3 Aim

This present study was designed to identify the relationships of endocrine disruptors with pituitary, gonadal, thyroid hormones and selected receptors (ER, PR, HER 2) in Nigerian women with breast cancer.

1.4 Objectives

a. To determine the contribution of reproductive and thyroid hormones to the pathogenesis of breast cancer.

b. To identify the pattern of expression of hormone receptors in Nigerian women with breast cancer.

c. To understand the role of endocrine disruptors in breast carcinogenesis.

d. To find possible relationships among endocrine disruptors, hormones and some receptors in Nigerian women with breast cancer.

1.5 Research Hypothesis

Exposure to Pb, Cd, As, BPA and PCBs which may result in altered serum levels of reproductive, thyroid hormones and expression of ER, PR and HER 2 could be associated with breast cancer development.

1.6 The Significance of the Study

Examining the roles of lead, cadmium, arsenic, bisphenol-A, polychlorinated biphenyls, reproductive and thyroid hormones in participants with breast cancer might help in the prevention, early diagnosis and treatment of breast cancer.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Epidemiology of Cancer

Cancer has been reported as the most dreaded non-communicable disease in developing countries, where it is invariably fatal. This is due to lack of adequate preventive and curative services. This is unlike the developed countries that have policies, strategies and programmes for cancer prevention and management (WHO, 2002; Thun, 2010; Nnodu, 2010; Kolawole, 2011). Although, the incidence of cancer is rising globally, the developing countries account for about 52% of this increase and about 70% of cancer deaths (Parkin, 2003; Kolawole, 2011) while possessing only 5% of global funds for cancer control and very few human and material resources (Jones, 1999). Cancer is the second most common cause of death constituting about 12% of all deaths after cardiovascular disease. Globally, cancer kills more people than tuberculosis, Human immune virus/Acquired Immune Deficiency Syndrome (HIV/AIDS) and malaria combined (WHO 2006a, 2006b). In 2007, there were 11 million cancer cases, 7 million cancer deaths and 25 million people living with cancer globally. This is projected to increase to 27 million cases, 17 million deaths and 75 million people living with cancer in 2050 (WHO, 2002; WHO, 2005). Africa carries an increasing cancer burden, 75% of the 650,000 annual cases present late and are at younger ages and about 510,000 deaths occur (Ngoma, 2006). In Nigeria, there are about 100,000 new cancer cases annually (Durosinmi, 2008). The incidence of cancer in Nigerian men and women by 2020 will be 90.7/100,000 and 100.9/100,000 respectively and the deaths rates will be 72.7/100,000 and 76.0/100,000, respectively (WHO, 2008). Cancer accounts for 4.4% of all deaths and is likely to increase to 6.8% in 2030. Out of 89,000 cancer deaths in 2005, 54000 were individuals younger than 70 years (WHO, 2008). Cancers will yet pose significant challenge to Nigeria and other developing countries which currently have insufficient cancer control programs directed at reducing cancer incidence and mortality and to improve quality of life (Kolawole, 2011). The aetiology of many cancers are still unknown, however there are risk factors which are either modifiable or non-modifiable.

2.2 Incidence, Morbidity and Mortality of Breast Cancer

Breast cancer is caused by the development of malignant cells in the breast and has been reported as a major health burden worldwide (Wang et al., 2009). It is the most common type of cancer among women in both high-resource and low-resource settings. It is responsible for over one million of the estimated ten million neoplasms diagnosed worldwide each year in both genders (Ferlay et al., 2001). It is also the primary cause of cancer death among women globally and was responsible for about 375,000 deaths in the year 2000 (Ferlay et al., 2001). As a consequence of changing exposures to reproductive and nutrition related determinants over time, women are at increasingly high risk of breast cancer, with incidence rates increasing in most countries and regions of the world in the past few decades (Bray et al., 2004). The most rapid rises in incidence rate are seen in developing countries, where breast cancer risk has historically been low relative to industrialized countries (Adebamowo et al., 2003). In Nigeria, the incidence of breast cancer has been reported to be on the increase (Adebamowo and Ajayi, 2000). It increased from 13.8-15.3 per 100,000 in the 1980s to 33.6 per 100,000 in 1992 and 116 per 100,000 in 2001 (Adebamowo et al., 2003). The increasing trends of breast cancer in the developing countries are often considered the result of the 'westernization' of lifestyles such as; delay in childbearing, dietary habits and exposure to exogenous oestrogen, towards a distribution closer in profile to that of women in industrialised countries (Barton et al., 1999; Bray et al., 2004).

An increase in the occurrence of breast cancer in premenopausal women in recent times has been reported (Abdulkareem, 2009). A report from the Niger Delta region of Nigeria showed that 65% of breast cancer cases occurred at 50 years and below; 50% occurred between ages 30 and 45 years (Sule, 2011). This was similar to other local reports in which premenopausal women accounted for between 57% and 67% of breast cancer cases (Adesunkanmi, 2006; Okobia, 2006; Kene, 2010) while postmenopausal women accounted for 20% of cases in certain studies (Oluwatosin and Oladepo, 2006). These reports illustrate the prominence of premenopausal breast cancer in Nigeria (Okonofua et al., 1990; Sule, 2011). Local investigators have attributed the higher incidence of premenopausal breast cancer to population demographics (Adebamowo and Ajayi, 2000). This was put in perspective with a life expectancy at birth in Nigeria of 51.56 years in the year 2000 and 46.94 years in the year 2009 (CIA, 2009). Higher life expectancy at 75 and 82 years was reported for United States and Britain respectively (CIA, 2009). Thus,

11

women in this country (Nigeria) may not live long enough for postmenopausal breast cancer. Emerging reports holds that the incidence of premenopausal breast cancer is higher in African Americans than in their Caucasian counterpart in spite of a life expectancy at 74 and 80 years respectively (Pinheiro, 2005). The reason for this is not yet clear.

Approximately 10-15% of patients with breast cancer has the aggressive type (tumour with triple negative hormone receptors i.e. ER-, PR- and HER2-) and develops distant metastasis within three years after the initial detection of the primary tumour (Gakwaya et al., 2008). This appears to be peculiar to Blacks (Thompson, 2006). This also results in unpredictable disease in which some patients present with relatively early stage disease and die of wide spread metastasis within six months and one year, while others present with advanced disease and yet survive longer (Gakwaya et al., 2008). The predominant feature of late presentation of breast cancer had been reported over three decades in Nigeria (Lawani et al., 1973; Khwaja et al., 1980; Chicdozie, 1985; Ihekwaba, 1992; Adebamowo and Adekunle, 1999; Okobia et al., 2006). This has been observed to be coupled with attendant poor outcome (Abdulkareem, 2009). Lack of established national screening program for breast cancer has been reported as one of the reasons adduced to late presentation with advanced breast cancer. Other factors are low social economic level (poverty), fear of mastectomy and ignorance (Ajekigbe, 1991; Elumelu et al., 2011). Poverty coupled with ignorance does not only impede access to health care system, but is associated with other co-factors that can relatively affect outcomes such as co-morbidity and lack of breast health awareness (Oluwole et al., 2003).

Metastasis is the leading cause of mortality in patient diagnosed with breast cancer (Schoppmann et al., 2002). Most breast cancer deaths are due to advanced cancer diagnosed when metastases have already disseminated to lymph nodes or distant organs (Autier et al., 2009). Despite many advances in diagnosis and screening, the disease is frequently discovered after it has spread to regional lymph node or even after dissemination of distant metastasis (Maki and Grossman, 2000). It has been reported that about 20 to 30% of patients with breast cancer will experience relapse with distant metastatic disease (Popoola et al., 2012).

In Nigeria, the most common histological type of breast cancer is invasive ductal carcinoma. This accounts for about 73-80% of cases (Sule, 2011). It was observed that the peak age of breast

women in this country (Nigeria) may not live long enough for postmenopausal breast cancer. Emerging reports holds that the incidence of premenopausal breast cancer is higher in African-Americans than in their Caucasian counterpart in spite of a life expectancy at 74 and 80 years respectively (Pinheiro, 2005). The reason for this is not yet clear.

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cancer in Nigerian women is about a decade earlier than Caucasian women (Okobia et al., 2006). The survival rate of breast cancer in Nigeria is low (about 10%) when compared with survival rate of 50% in East Africa. Moreover, a survival rate of 85% was reported for Americans (Olopade, 2004; Adetifa and Ojikutu, 2009).

2.3 Risk Factors of Breast Cancer

The two primary risk factors of breast cancer are increasing maternal age and female gender. Other risk factors are; longer reproductive span, exposure to exogenous hormones, socio-economic status, obesity, abnormal genes (BRCA 1, BRCA2 genes); less than 10% of all breast cancers can be attributed to genetic factors (Brisken., 2008). Other risk factors include; Obesity, lower levels of physical activity, diet, smoking, alcohol, previous breast lesion with atypical changes. Unoccupational exposure to endocrine disruptors (lead, cadmium, arsenic, bisphenol-A, polychlorinated biphenyls) in the aetiology of breast cancer has attracted little attention in the developing world like Nigeria.

2.3.1 Longer Reproductive Span: Early age at menarche, nulliparity, late age at first birth, late age at any birth, low parity, and late menopause-relate to the hormonal (largely oestrogen) milieu to which the breast is exposed from menarche to the cessation of ovulation at menopause (Pike et al., 1983; Bray et al., 2004).

2.3.2 Exposure to Exogenous Hormones: Exposure to exogenous hormones including oral contraceptives and hormone replacement therapy could result in an increase in the risk of breast cancer (CGHFBC, 1996; Beral, 2003). The risk conferred by oral contraceptive use could persist for up to 10 years after cessation. There is much evidence that the rate of exposure to endogenous and exogenous oestrogen is on the increase. This is consistent with upward trends in incidence of breast cancer (Bray et al., 2004).

2.3.3 Socio-Economic Status: The association between socio-economic status and risk of breast cancer has been suggested. Certain studies observed that women in higher socio-economic groupings were at higher risk. Conversely, women with low socio-economic status were reportedly at higher risk of breast cancer, owing to ignorance which made them report late in the hospital (Heck and Pamuk, 1997; Adams et al., 2004).

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2.4 Normal Mammary Gland Development

The development of mammary gland occurs throughout the female life time (Ronnov-Jessen *et al.*, 1996; Russo and Russo 1998). At puberty, the female mammary gland responds to the production of the ovarian steroid hormone, oestrogen, which makes the breast epithelium branch into numerous ducts with terminal end buds or alveoli, collectively referred to as the terminal ductal lobular unit (TDLU). In humans, the TDLU is composed of clusters of 6 to 11 ductules per lobule referred to as lobule type 1 (or Lob 1) (Russo and Russo, 1998). Lob 1 progresses to lobule type 2 (Lob 2) in the post pubertal virgin gland, with only modest alveolar proliferation producing a higher number of ductular structures per lobule during the menstrual cycle. Once pregnancy occurs, Lobs 1 and 2 are stimulated by the elevated levels of oestrogen and progesterone, thus resulting in Lob 3. Lob 3 is formed by epithelial expansion of existing puberial alveoli to 80 small lobules per alveoli. These changes prime the mammary gland for milk secretion from the alveoli, now called secretory lobules type 4 (Lob 4). After parturition, the lactating mammary gland becomes insensitive to oestrogen-dependent regulation of growth, during the post-weaning involution phase, responsiveness to oestrogen is restored. Finally, with the cessation of lactation, the alveoli collapse and the mammary gland regresses apoptotically to its resting, pre-pregnancy state, reverting to Lob 3 and Lob 2, retaining a more extensive framework of branching than Lob 1. Thus, the adult female mammary gland experiences recurrent cycles of regulated growth, differentiation and apoptosis, while oestrogen and progesterone play important roles in this process (Ronnov-Jessen *et al.*, 1996; Russo and Russo, 1998).

2.4.1 The Composition of the Mammary Gland

The mammary epithelium is a bilayered structure consisting of an inner continuous layer of luminal epithelial cells and an outer layer of myoepithelial cells. The epithelial bilayer is polarized; the apical layer (luminal epithelial cells) faces the lumen of the ducts and the alveoli, and the basal layer (myoepithelial cells) is in close contact with a laminin-rich basement membrane (BM). The epithelium is embedded in the mammary stroma, which makes up more than 80% of the breast volume (Ronnov-Jessen *et al.*, 1996). The breast stroma includes fat tissue, interstitial/interlobular dense connective tissue, intra lobular loose connective tissue and blood vessels. The stromal cells are also surrounded by extracellular matrix (ECM) that is

sometimes referred to as stromal ECM. Extracellular matrix refers to the insoluble proteinaceous components that exist in the mammary tissue. In the normal female breast, approximately 20% of the luminal epithelial cells are in direct contact with the basement membrane (BM), the remaining cells are adjacent to the myoepithelial cells (Gusterson *et al.*, 1982; Petersen & van Deurs, 1988). The precise relationship between the luminal epithelial cells, myoepithelial cells and the origin of these cells is largely unknown, making this an important problem for developmental biology of the mammary gland. It was recently reported that a portion of luminal epithelial cells, cultivated in culture to maintain correct functional characteristics gave rise to myoepithelial cells in an appropriate medium, but myoepithelial cells do not produce luminal epithelial cells (Pechoux *et al.*, 1999). This observation suggests a linear relationship between these two epithelial cell types and may be important to tumour biology because most breast cancers are luminal rather than myoepithelial in origin (Wellings *et al.*, 1975; Rudland, 1993). Myoepithelial cells have been hypothesized to play a 'tumour suppressive' role by maintaining the differentiated state of luminal epithelial cells (Bani *et al.*, 1994; Liu *et al.*, 1996). Moreover, it is believed that luminal epithelial cell transformation may prevent conversion to myoepithelial cells. This may explain why in premalignant lesions, there are fewer myoepithelial cells. In invasive breast cancer, myoepithelial cells are either missing or less differentiated (Gusterson *et al.*, 1982; Guelstein *et al.*, 1988; Rudland *et al.*, 1995). In more than 90% of cases, tumour cells are restricted to a luminal-like phenotype (Altmannsberger *et al.* 1986; Nagle *et al.*, 1986; Dairkee *et al.*, 1988; Guelstein *et al.*, 1988; Bocker *et al.*, 1992), and only a small proportion of these cells are in contact with myoepithelial cells (Gusterson *et al.*, 1982; Petersen & van Deurs, 1988). Although, breast cancer cells originate mainly in the epithelium, evidence suggests that the stroma is an active participant in cancer progression (and possibly even induction) and constitutes the majority of the tumour mass (Dvorak, 1986; Thomasset *et al.*, 1998). The tumour stroma contains changes in the cellular composition and in the amounts of certain protein constituents, often referred to as reactive stroma or desmoplasia when compared with normal mammary gland stroma. For example, the most prominent cellular change in tumour stroma is the appearance of myofibroblasts which are found in close proximity to tumour cell nests (Ronnov-Jessen *et al.*, 1996). Myofibroblasts produce proteases such as urokinase plasminogen activator and stromelysin-3 which degrade ECM and also contribute to tumour cell invasion (Wolf *et al.*, 1993; Unden *et al*, 1996).

15

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2.4.2 Mammary Gland Morphology and Breast Cancer Origin

The development of breast cancer is characterized by the acquisition or loss of discrete cellular functions. This results in altered tissue organization which has long been recognized by pathologists and used to classify breast tumours as specific morphological types (Beckmann et al., 1997). Observations have been made that specific morphological types of breast cancer are associated with specific breast structures or developmental stages of the mammary gland (Russo and Russo, 1998). For example, the common breast malignancy, ductal carcinoma which is thought to originate within the fairly undifferentiated epithelial cells of the terminal ductal lobular unit (TDLU), corresponds to Lob 1. Similarly, lobular carcinomas in situ are found in Lob 2, benign breast lesions originate in Lob 3, and lactating adenomas arise in Lob 4. It was however concluded from these observations that less functionally differentiated breast cells (Lob 1) are more susceptible to giving rise to the most undifferentiated and aggressive neoplasms (Russo and Russo, 1998). Thus, the developmental stage of the breast appears to affect neoplastic transformation. Supporting this hypothesis are studies demonstrating the higher risk of malignancy in nulliparous and late parous women (Lambe et al., 1996). In spite of this evidence, it is yet to be understood how the morphological and developmental stages of the mammary gland are associated with breast cancer. It is generally accepted that the development of invasive breast cancer occurs through the multistep transformation of epithelial cells via steps of hyperplasia, premalignant change, in situ carcinoma, and invasive carcinoma (Wellings et al., 1975; Gould, 1993; Beckmann et al., 1997). However, there is no evidence that each step is a necessary precursor of the next stage. This is because it has been difficult to develop model systems with cells representing various types of breast lesions from benign tumours to invasive carcinoma. Markers of malignant cells have been partially defined, however, the characteristics of the precursor cells are less well known, making identification difficult. Evidence does suggest, however, that certain regions of the mammary gland may be predisposed to tumour formation (Deng et al., 1996). Studies have indicated that whole regions of the breast may originate from the same cells, i.e. that they are clonal. If these cells are 'primed' for tumour formation by harbouring genetic mutations, one might expect to find normal-appearing cells with genetic abnormalities in the region surrounding tumours (Tsai et al., 1996). It is now known that morphologically normal breast epithelia could contain many genetic mutations which may give rise to cancer (Deng et al. 1996).

2.5 Anthropometric Measurements, Adiposity and Breast Cancer Risk

The World Health Organization (WHO) defines obesity as an abnormal or excessive fat accumulation in the adipose tissue to the extent that health is impaired. The classification of obesity for epidemiological purposes defines overweight as Body Mass Index (BMI) greater than $25kg/m^2$ and obesity as BMI greater than $30 kg/m^2$ (Gill *et al.*, 2003).

Adipose tissue is principally deposited in two compartments; subcutaneously and centrally. It is thought that centrally deposited or visceral fat is more metabolically active than peripheral subcutaneous fat (Kershaw and Flier, 2004; Vohl *et al.*, 2004; Galic *et al.*, 2010). Visceral adipose tissue largely comprises of omental adipose tissue but also includes other intra-abdominal fat sources such as mesenteric fat. Visceral fat has been reported to be more strongly associated with an adverse metabolic risk profile even after accounting for the contribution of other standard anthropometric indices (Pot and Simmins, 1994; Despress and Lemieux, 2006; Snijder *et al.*, 2006; Charles-Davies *et al.*, 2012). These systemic effects exerted by visceral adiposity are putatively involved in cancer biology (van Kruijsdijk *et al.*, 2009; Amadou *et al.*, 2013) and are the focus of much research (Donohoe *et al.*, 2011).

Studies have shown that obesity is marked by alteration in the production of adipocytokines; leptin and adiponectin. Increased leptin levels and decreased adiponectin levels promote breast carcinogenesis (Tworoger *et al.*, 2007; Mantovani *et al.*, 2009). Leptin is strongly angiogenic and may increase tumour angiogenesis by directly acting on the endothelium or by increasing local vascular endothelial growth factor (VEGF) secretion (Hanahan and Weinberg, 2000; Rutkowski *et al.*, 2009). Studies in Ibadan, Nigeria showed elevated leptin levels in apparently healthy premenopausal women with metabolic syndrome compared with those without metabolic syndrome. Leptin levels were similar in both pre and postmenopausal women with metabolic syndrome (Fabian *et al.*, 2015). Our earlier study showed that elevated levels of leptin in individuals with metabolic syndrome might reflect adiposity and could be a compensatory mechanism for maintaining weight/fat loss and blood pressure (Fabian *et al.*, 2015)

Body mass index (BMI) has been reported as a measure of overall adiposity. Its commonly used cut off values to diagnose obesity has been reported to have a high specificity (Okorodudu *et al.*, 2010). High BMI has been associated with an increased incidence of many types of cancer

(Renehan et al., 2008). There are reports that overweight or obesity is associated with poorer prognosis in most studies that have examined body mass and breast cancer risk (Ryu et al., 2001; Berclaz et al., 2004; McTieman, 2005; Dignam et al., 2005; Whiteman et al., 2005; Kroenke et al., 2005; Loi et al., 2005). This is because, the obese state may be thought of as a pro-tumourigenic environment which can act to facilitate tumour development by promotion of the acquisition of some of the hallmark properties that characterize cancerous lesions (Hanahan and Weinberg, 2000; Mantovani, 2009).

Women with a BMI of ≥25 had about 58% increased risk of breast cancer in a reported study (Hirose et al., 2007). Other studies reported an increased BMI or body weight to be a significant risk factor for recurrent breast cancer, breast cancer survival, or both (Ryu et al., 2001; Berclaz et al., 2004; McTieman, 2005; Dignam et al., 2005; Whiteman et al., 2005; Kroenke et al., 2005; Loi et al., 2005). In postmenopausal women, epidemiologic evidence suggest a positive association between body mass, body weight and breast cancer (Key et al., 2001; Carpenter et al., 2003; Feigelson et al., 2004; Sweeney et al., 2004; Ursin et al., 1995; van den Brandt et al., 2000; Friedenreich, 2001; Lahmann et al., 2004).

Moreover, Height and BMI were reportedly associated with postmenopausal breast cancer in another study (Trentham-Dietz et al., 1997; Shu et al., 2001; Iwasaki et al., 2007). This effect was most pronounced in women with oestrogen receptor positive (ER+) tumours. Ogundiran et al. (2010) demonstrated that height was a significant risk factor for female breast cancer in both premenopausal and postmenopausal women. The underlying mechanism could be that childhood energy balance is associated with mammary gland mass and increased insulin-like growth factors (Adami et al., 1998; Lovegrove, 2002). Attained height is determined by genetic makeup and environmental factors, including energy intake during childhood and adolescence. In societies with an insufficient food supply, caloric intake plays a more important role in determining height than in societies with an abundant food supply. Thus, energy intake in earlier life may play an important role in breast carcinogenesis.

Waist Circumference has been reported to be an accurate predictor of visceral adiposity, either alone or in combination with BMI or waist to hip ratio (Zhu et al., 2004). Its accuracy compared to waist to hip ratio has also been reported (Donohoe et al., 2011). This is because it directly reflects total abdominal fat mass (Lemieux et al., 1996, Bose and Mascie-Taylor, 199s.

18

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Women with a BMI of ≥25 had about 58% increased risk of breast cancer in a reported study (Hirose et al., 2007). Other studies reported an increased BMI or body weight to be a significant risk factor for recurrent breast cancer, breast cancer survival, or both (Ryu et al., 2001; Berclaz et al., 2004; McTiernan, 2005; Dignam et al., 2005; Whiteman et al., 2005; Kroenke et al., 2005; Loi et al., 2005). In postmenopausal women, epidemiologic evidence suggest a positive association between body mass, body weight and breast cancer (Key et al., 2001; Carpenter et al., 2003; Feigelson et al., 2004; Sweeney et al., 2004; Ursin et al., 1995; van den Brandt et al., 2000; Friedenreich, 2001; Lahmann et al., 2004).

Moreover, Height and BMI were reportedly associated with postmenopausal breast cancer in another study (Trentham-Dietz et al., 1997; Shu et al., 2001; Iwasaki et al., 2007). This effect was most pronounced in women with oestrogen receptor positive (ER+) tumours. Ogundiran et al. (2010) demonstrated that height was a significant risk factor for female breast cancer in both premenopausal and postmenopausal women. The underlying mechanism could be that childhood energy balance is associated with mammary gland mass and increased insulin-like growth factors (Adami et al., 1998; Lovegrove, 2002). Attained height is determined by genetic makeup and environmental factors, including energy intake during childhood and adolescence. In societies with an insufficient food supply, caloric intake plays a more important role in determining height than in societies with an abundant food supply. Thus, energy intake in earlier life may play an important role in breast carcinogenesis.

Waist Circumference has been reported to be an accurate predictor of visceral adiposity, either alone or in combination with BMI or waist to hip ratio (Zhu et al., 2004). Its accuracy compared to waist to hip ratio has also been reported (Donohoe et al., 2011). This is because it directly reflects total abdominal fat mass (Lemieux et al., 1996; Bose and Mascie-Taylor, 1998;

Kopelman, 2000; Kashihara et al., 2009; Chakraborty and Bose, 2009). These measures of adiposity have been widely recommended for epidemiological surveys because of their independent association with major non-communicable metabolic diseases including breast cancer (Chakraborty and Bose, 2009).

Waist Height Ratio (WHtR) is an index of assessing central fat distribution. Several studies have demonstrated that waist to height ratio (WHtR) is a better predictor of metabolic risk in oriental people (Ho et al., 2003; Hsieh et al., 2003; Tseng, 2005). Although, the mechanisms that explain the health risk predicted by WHtR are not firmly established, it is often suggested that the risk is explained by its association with elevations in abdominal obesity (Ashwell et al., 1996). WHtR has an added advantage over isolated waist circumference measurement, because its adjustment for height allows establishment of a single, population-wide cut off point that remains applicable regardless of gender, age, and ethnicity (Ashwell and Hsieh, 2005).

Cancer mortality associated with obesity has been reported. A prospective study of 900,000 adults in the United States reported that obesity could account for 20% of all deaths from cancer in women. Women with a BMI greater than 40kg/m² had a death rate of about 62% in when compared with those with normal weight (Calle et al., 2003).

2.6 Steroid Hormones Biosynthesis

Cholesterol is the building block of steroid hormones. *De novo* synthesis of all steroid hormones starts with the conversion of cholesterol to pregnenolone by CYP11A, one of the cytochrome P450 enzymes (Miller, 1988; Parker and Schimmer, 1995). CYP11A is bound to the inner membrane of the mitochondrion and is found in all steroidogenic tissues (Miller, 1988; Reincke et al., 1998). Pregnenolone is converted to progesterone by 3β-hydroxysteroid dehydrogenase (3β-HSD), one of the several non-CYP450 enzymes that are involved in steroidogenesis which is found in both mitochondria and smooth endoplasmic reticulum. 3β-HSD is widely distributed in steroidogenic and non steroidogenic tissues and consists of two isoenzymes (types 1 and 2, 3 β-HSD), which are regulated in a tissue-specific manner (Leers-Sucheta et al., 1997; Mason et al., 1997; Gingras et al., 2001; Simard et al., 2005). The type 2 3β-HSD is predominantly expressed in steroidogenic tissues including the adrenal gland and ovary, whereas type 1 is found in

placenta and in non steroidogenic tissues such as liver, kidney and skin. Pregnenolone and progesterone form the precursors for all other steroid hormones.

2.6.1 Steroidogenesis in the Ovaries

The main role of the ovary is to produce eggs for fertilization and steroid hormones for sexual and reproductive function. The ovum inside the developing follicle is directly surrounded by layers of granulosa cells followed by thecal cells, which is where steroidogenesis predominantly takes place. The *theca interna* is highly vascularized and produces large amounts of progesterone and androgens, which act as precursor for oestrogen synthesis in the granulosa cells. Androstenedione and testosterone diffuse into the neighbouring poorly vascularized granulosa cells where they are converted to predominantly oestradiol via the concerted action of aromatase and 17β-HSD types 1 and 7, which favour the conversion of oestrone to oestradiol (Luu-The, 2001; Mindnich *et al.*, 2004). In the pre-ovulatory follicular stage during which the follicle matures, oestrogen synthesis increases gradually due to up regulation of aromatase by LH and FSH. During this critical phase, oestrogen appears to be responsible for the up regulation of LH receptors and the initiation of the positive feedback loop responsible for the LH and FSH surge which triggers ovulation (Greenwald and Roy, 1994). Interference with the synthesis of oestrogens during this critical window of time would prevent ovulation. After the LH surge, the follicle enters the luteal phase and becomes a corpus luteum which predominantly synthesizes progesterone. Decreased LH concentration and subsequently decreased aromatase expression result in declining oestrogen production (Fitzpatrick *et al.*, 1997), while a concurrent increase in CYP11A and 3β-HSD activity promotes the synthesis of progesterone which via its receptor initiates the process of follicle rupture.

2.7 Oestrogens

The oestrogens are a family of steroid hormones synthesized in a variety of tissues including ovaries, placenta and adrenal cortex (Tsang *et al.*, 1980; Rotestein, 2011). They are responsible for the development and maintenance of the female sex organs and secondary sexual characteristics. More than 97% of circulating oestradiol is bound to plasma proteins. It is bound specifically and with high affinity to sex hormone binding globulin (SHBG) and non-specifically to albumin. Only a tiny fraction circulates as free (unbound) hormone (Martin *et al.*, 19 1, Smteri

20

et al., 1982; Rotstein, 2011). Both the free and albumin-bound fractions of oestradiol are thought to be available, but measurement of this (protein-bound) fraction has not been shown to be clinically important. In conjunction with progesterone, oestrogens also participate in the regulation of the menstrual cycle, breast and uterine growth as well as the maintenance of pregnancy (Carl and Edward, 2001). Oestrogenic activity is effected via oestrogenic-receptor complexes which trigger the appropriate response at the nuclear level in the target sites. These include ovarian follicles, uterus, breast, vagina, urethra, hypothalamus, pituitary and to a lesser extent the liver and skin (Carl and Edward, 2001). The principal function of the oestrogens is to cause the cellular proliferation and growth of tissues of the sex organs and other tissues related to reproduction (Tsang *et al.*, 1980; Guyton and Hall, 2000). In the female, oestrogens cause (1) the development of the stromal tissues of the breast (2) growth of an extensive ductile system and (3) deposition of fat in the breast, subcutaneous tissues, the buttocks and thighs. More than 20 oestrogens have been identified, but only 17β –oestradiol (E_2), oestrone (E_1) and oestriol (E_3) are known to have clinical importance (Heldring *et al.*, 2007). The most potent natural oestrogen secreted by the ovaries is 17β –oestradiol (Rotstein, 2011). It is a C18 steroid hormone with a phenolic ring and a molecular weight of 272.4 kDa (Tsang *et al.*, 1980). In pregnancy, relatively more oestriol is produced and this comes from the placenta.

2.7.1 Metabolism of Oestrogens

Glandular synthesis of oestrogen occurs in the granulosa and theca cells of the ovaries, as well as the corpus luteum, while extraglandular synthesis is by aromatization of androgens in non gonadal sites. This is a complex process that involves three hydroxylation steps, each of which requires O_2 and NADPH (Mark and Paul, 2001). Oestradiol is formed if the substrate of this enzyme complex is testosterone, whereas, oestrone results from the aromatization of androstenedione. The conversion of androstenedione to oestrone is the major source of oestrogens in postmenopausal women from the aromatization in extragonadal tissues such as the liver, muscle and adipose tissues (Saten *et al.*, 1986; Rotstein, 2011). Increase activity of the enzyme aromatase may contribute to excess oestrogen that characterizes such diseases as breast cancer among other chronic diseases. Oestrogens are catabolized mainly by hydroxylation reactions (Mark and Paul, 2001) resulting in the formation of: 2-hydroxyestrone and 2-hydroxyestradiol, 4-hydroxyestrone and 4-hydroxyestradiol and 16α-hydroxyestradiol and 16α-

21

hydroxyestrone. 4-hydroxyestrone and 16α-hydroxyestradiol of these metabolites are known to be oestrogenic and are thought to be carcinogenic (Mark and Paul, 2001).

2.8 Progesterone

Progesterone is a 21 carbon steroid that is primarily produced by the granulosa-lutein cells of the corpus luteum during the luteal phase and also by the syncytiotrophoblast of the placenta during pregnancy (Al-Asmakh, 2007). It is transported in the blood by transcortin and albumin with approximately 2% present in the free, unbound state. The half life of progesterone is approximately 5 minutes in the blood and its principal degradation product, pregnanediol, is formed in the liver. The plasma progesterone concentration is usually below 5 nmol/L (1.5 ng/ml) during the follicular phase of the menstrual cycle. However, it rises to the peak value of 40-50nmol/L (12-16ng/mL) in the luteal phase (Laycock and Wise, 1996; Pfeifer and Strauss, 1996; Al-Asmakh, 2007). Progesterone is essential for the regulation of normal female reproductive functions. Its major physiological actions are: facilitation of implantation and maintenance of early pregnancy in the uterus, lobular-alveolar development in preparation for milk secretion in the breast, neurobehavioral expression associated with sexual responsiveness in the brain and prevention of bone loss (Clark and Sutherland, 1990; Graham and Clarke, 1997; Genazzani *et al.* 2000; Balasch, 2003).

2.8.1 The Function of Progesterone during the Menstrual Cycle

Progesterone is essential for the implantation and maintenance of early pregnancy. The follicular phase of the menstrual cycle is oestrogen dominated, while the luteal phase is progesterone dominated (Cameron *et al.*, 1996). The secretion of progesterone converts an oestrogen primed proliferative endometrium into a secretory one, which is receptive to the blastocyst. The granulosa cells in the follicle biosynthesize and secrete oestrogen before ovulation takes place. Upon follicle rupture and release of the ovum, these granulosa cells mature to form the corpus luteum, which is responsible for secretion of progesterone and oestrogen in the latter part of the cycle (Al-Asmakh, 2007). In humans, if fertilization does not occur within 1 to 2 days, the corpus luteum continues to enlarge for 10-12 days; this is followed by regression of the gland and concomitant cessation of oestrogen and progesterone release. The corpus luteum then continues to grow and function for the first 2 to 3 months of pregnancy if fertilization occurs.

After this time, it slowly regresses as the placenta assumes the role of hormonal biosynthesis for the maintenance of pregnancy (Graham and Clarke, 1997; Al-Asmakh, 2007).

2.8.2 The Effects of Progesterone on Ovulation and Luteinization

In primates, luteinization and follicular rupture occur 36–38 hours after the onset of mid-cycle gonadotropin surge. During this pre-ovulatory phase, granulosa cells undergo changes in response to the ovulatory stimulus that result in terminally differentiated luteal cells. These differentiating (luteinizing) granulosa cells secrete large amounts of progesterone (Suzuki et al., 1994). Acute administration of 3β-hydroxysteroid dehydrogenase (3β-HSD) inhibitors or progesterone receptor antagonists prevented ovulations in monkeys (Hibbert et al., 1996) and mice (Loutradis et al., 1991). Moreover, follicles from progesterone-depleted monkeys and progesterone receptor knockout mice (PRKO) do not luteinize (Lydon et al., 1995). The increase in progesterone levels and in progesterone receptor expression within 12 hours of the ovulatory stimulus in the macaque (monkey) follicle supports a critical early role for progesterone in ovulation and luteinization (Chaffin et al., 1999). The pre-ovulatory surge of gonadotropins activates a cascade of proteolytic enzymes resulting in the rupture of the follicular wall and the release of a fertilizable ovum during ovulation. Several lines of evidence support a role for progesterone in the induction of proteolytic activity in the pre-ovulatory follicle of primate and non-primate species. The levels of mRNAs for matrix metalloproteinases-1 (MMP-1) and tissue inhibitor matrix metalloproteinases-1 (TIMP-1) increased dramatically within 12 hours of gonadotropin stimulus and were up-regulated by progesterone (Chaffin and Stouffer, 1999). Moreover, inhibition of progesterone synthesis or blocking progesterone action with RU486 decreased MMP activity in the rat and ewe in a reported study (Curry and Osteen, 2003). A regulatory role for progesterone in the activation of other ovulation-associated proteases, such as plasminogen-activator (PA), has been suggested as well, because administration of a selective progesterone receptor antagonist, Org 31710 to gonadotropin-treated rats resulted in lower PA activity levels (Pall et al., 2000).

2.8.3 The Effects of Progesterone on Cellular Proliferation in the Uterus during the Menstrual Cycle

The changes in proliferative activities of the glandular epithelium and stromal elements of the human endometrium correlate with the circulating levels of oestrogens and progesterone. During oestrogen-dominated follicular phase, cellular proliferations occur in both epithelial and stromal cells. This is followed by a decline in proliferation in the first half of the secretory, progesterone-dominated phase of the cycle. In the late luteal phase, while proliferative activity remains low in the epithelium, a second peak of proliferation, consistent with decidual changes, is seen in the stromal elements (Al-Asmakh, 2007). Oestrogen stimulates epithelial cell proliferation, while progesterone opposes the mitotic effects of oestrogen and inhibits proliferation (Graham and Clarke, 1997; Conneely et al., 2002). In progesterone receptor knockout (PRKO) mice, ablation of both progesterone receptor-o (PR-A) and progesterone receptor-β (PR-B) isoforms resulted in a marked hyperplasia in the endometrial epithelium due to unopposed proliferative oestrogen action (Lydon et al., 1995). However, in a PR-A knockout mice (PRAKO), in which the expression of the PR-A isoform is selectively ablated, the PR-B isoform functions to mediate rather than inhibit cellular proliferations. This gain of PR-B-dependent proliferative activity upon removal of PR-A indicates that PR-A is necessary not only to oppose oestrogen-induced proliferations, but also required to inhibit proliferations induced by progesterone acting through the PR-B proteins (Conneely et al., 2002; Mulac-Jericevic and Conneely, 2004; Al-Asmakh, 2007).

2.8.4 Progesterone's Effects on Cellular Differentiation

As a result of the inhibitory effects of progesterone on cellular proliferation, progesterone induces secretory differentiation in the glandular epithelium and stromal fibroblast. The differentiating action of progesterone is terminal: if implantation does not occur, the tissue is shed and endometrial renewal from the basal portion of the endometrium takes place. Progesterone's effect on the stromal decidualization is described as the progesterone mediated differentiation of small stromal fibroblast into large epitheloid decidual cells. This process occurs around day 23 of the menstrual cycle and is accompanied in fertile cycles by the implantation event (Mulac-Jericevic and Conneely, 2004). The decidual reaction is inhibited in PRAKO mice, but not PRBKO mice, suggesting a critical significance of PR-A in this process (Conneely, et al.,

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2002; Mulac-Jericevic and Conneely, 2004; Brosens *et al.*, 2004). In humans, decidual transformation occurs in stromal cells surrounding the spiral arteries approximately 10 days after the postovulatory rise in ovarian progesterone level, indicating that the expression of the deciduas-specific gene is unlikely to be under the direct control of activated PR. Evidence has emerged to suggest that the initiation of decidual transformation requires elevated intracellular cAMP levels and sustained activation of protein kinase A (PKA) pathway (Gellersena and Brosens, 2003; Al-Asmakh, 2007).

2.8.5 The Roles of Progesterone on Menstruation and Regenerative Phase

Menstruation is defined as the shedding of the superficial layer of the endometrium due to withdrawal of progesterone following luteolysis (Cameron *et al.*, 1996). It is the result of enzymatic autodigestion and ischaemic necrosis. During the first part of the secretory phase, acid phosphatase and lytic enzymes are restricted to the lysosomes. Progesterone plays a role in stabilizing the lysosomal membranes. In the second part of the secretory phase, these lysosomal membranes are degraded resulting in the release of lytic enzymes into the cytoplasm and intracellular membrane. The lytic enzymes digest cell elements, including intracellular bridges and desmosomes (Bergeron, 2000). Matrix metalloproteinases have an important role causing degradation of many components of the uterine extracellular matrix, including proteoglycan, glycoproteins and basement membrane collagen (Curry and Osteen, 2003). There is substantial evidence that MMPs are produced in the endometrium and that expression of their mRNAs is closely correlated with the process of normal menstruation (Hampton and Salamonsen, 1994). Studies showed that production of endometrial MMPs is modulated by progesterone withdrawal in vitro and in vivo (Salamonsen *et al.*, 1997; Zhang and Salamonsen, 2002). Progesterone has a role in maintaining coagulation and as a result any fall in serum progesterone level will engender fibrinolysis and initiate menstrual bleeding (Bergeron, 2000). Vasoconstriction of the spiral arterioles also plays a role in the breakthrough of the menstrual bleeding (Al-Asmakh, 2007). Prostaglandin F 2- alpha (PGF2α) causes vasoconstriction. It is negatively controlled by progesterone and causes reduction in blood flow to the corpus luteum, thus, it may cause luteolysis by depriving the gland of nutrients and substrates needed for steroidogenesis (Bergeron, 2000; Niswender *et al.*, 2000). Finally, apoptosis takes place. Apoptosis is a phenomenon regulated by the gene bcl-2 (B cell lymphoma/leukemia-2) causing gland cell death

and shedding of the menstrual blood (Bergeron, 2000). The protooncogene bcl-2 functions to prolong the survival of healthy and pathological cells by blocking apoptosis. Several studies showed a decrease in the expression of bcl-2 during menstruation and following the withdrawal of progesterone (Dahmoun et al., 1999; Mertens et al., 2002). Angiogenesis (new blood vessel formation) is rare in adult tissue, however, the female reproductive tract is an exception, with blood vessel formation taking place during regeneration, development of spiral arterioles in the late secretory phase and at the time of implantation. Three peaks of regeneration have been indicated in endometrial tissue. Two peaks of endometrial regeneration under the control of oestrogen occur immediately postmenstrually and during the mid proliferative phase of the cycle. The third peak is progesterone related and occurs during the secretory phase of the cycle. This peak involves the growth of spiral arterioles. The persistence of stromal progesterone receptors provides evidence that progesterone influence the development of spiral arterioles (Critchley and Healy, 1998).

2.9 Hormone Receptors and Breast Cancer

Oestrogen receptor (ER) and progesterone receptor (PR) play important roles in the growth and differentiation of breast cancers making them important prognostic markers (Patel et al., 2013; Mohamed et al., 2015; Deepti et al., 2015). Two isoforms of ER are known to exist; oestrogen receptor alpha (ERα) and oestrogen receptor beta (ERβ) (Green et al., 1986, Greene et al., 1986, Kuiper et al., 1996). A strong expression of ERα is reportedly observed in tissues related to female reproduction; ovary, womb, mammary gland (Kerdivel et al., 2013). Recent studies have reported a mild expression of ERβ in the mammary gland (Dotzlaw et al., 1997, Saji et al.2000). There is paucity of information on its role in mammary gland (Cowley et al., 1997; Kuiper et al., 1997; Pace et al., 1997; Pettersson et al., 1997; Hansen and Bissell, 2000; Saji et al., 2000).

Oestrogen receptor, PR and HER 2 are determined by immunohistochemistry (Recavarnu et al., 2011; Qiao et al., 2013). The biologic, prognostic and predictive importance of assessment of ER expression in breast cancer is well established. However, the added value of PR assessment appears controversial in some climes (Olivotto et al., 2004; Colozza et al., 2005; Fuqua et al., 2005; Hefti et al., 2013; Qiao et al., 2013). In spite of this, the American Society of Clinical Oncology and the College of American Pathologists recommend testing for both ER and PR on

all newly diagnosed cases of invasive breast cancer (Hammond *et al.*, 2010). Since the 1970s, it has been hypothesized that PR expression will be associated with response to hormonal therapies in ER+ breast cancer, as it is thought that ER and PR co-expression demonstrates a functionally intact oestrogen response pathway (Horwitz *et al.*, 1978; Horwitz and McGuire, 1978; Horwitz and McGuire, 1975; Horwitz and McGuire, 1979). Analyses from observational studies showed that loss of PR expression was associated with worse overall prognosis among ER+ breast cancers (Bardou *et al.*, 2003; Grann *et al.*, 2005; Dunnwald *et al.*, 2007; Cancello *et al.*, 2013; Prat *et al.*, 2013). These results suggested that evaluation of PR status in ER+ breast cancer might be used to help guide clinical management, as high levels of PR expression may identify a subset of ER+ patients most likely to benefit from hormonal therapy (Davies *et al.*, 2011; Hefti *et al.*, 2013).

The biological and clinical significance of the ER-/PR+ breast cancer subtype has been reported to be controversial, with some reports claiming it represents a distinct, clinically useful biologic entity (Thor et al., 1998; Seawn and Shousha, 2002; Rakha *et al.*, 2007; Rhodes and Jasani, 2009; Suvarchala and Negesrwararao, 2011; Al-Khafaji *et al.*, 2014) while others are of the view that ER-/PR+ classification is primarily a technical artifact (De Maeyer *et al.*, 2008; Nadji *et al.*, 2005) and too rare to be of clinical use (Chariyalerstak *et al.*, 1996; Olivotto *et al.*, 2004). In large published series, the percentage of ER-/PR+ cases has been in the range of zero (Nadji *et al.*, 2005) to four percent (Bardou *et al.*, 2003; Colditz *et al.*, 2004). In the Early Breast Cancer Trialists' Collaborative Group (EBCTCG) meta-analysis, PR expression was not significantly predictive of tamoxifen treatment response in ER-negative breast cancer, although, there was a slight trend, which failed to reach statistical significance (Davies *et al.*, 2011). In the EBCTCG analysis, the investigators noted that as methods for assessment of hormone receptor status have improved. The proportion of cases reported as ER-/PR+ has decreased from approximately 4% in the early 1990s to only 1% in recent SEER (Surveillance, Epidemiology, and End Results) cancer registry data. This suggests that as methods of ER testing and interpretations have improved, the rates of false negative ER results have decreased (Davies *et al.*, 2011). However. There are reports that breast cancer patients with tumours that are ER+ and/or PR+ have lower risks of mortality after their diagnosis compared to women with ER- negative and/or PR-negative disease (Fisher *et al.*, 1988; Parl *et al.*, 1984; Crowe *et al.*, 1991; Aaltomaa *et al.*, 1991,

Lethaby et al., 1996; Anderson et al., 2001). Clinical trials have also shown that the survival advantage for women with hormone receptor-positive tumours is enhanced by treatment with adjuvant hormonal and/or chemotherapeutic regimens (Smith and Good, 2003; Goldhirsch, et al., 2003; Fisher et al., 2004).

Human epithelial receptor 2 (HER 2), a proto-oncogene also known as ErbB2-neu, located on chromosome 17q21 is also considered to be closely associated with occurrence and development of breast cancer (Gown, 2008). Under normal physiological conditions HER 2 is inactive; however, once activated it may enhance tumour invasion and metastases and increase the degree of malignancy (Revillion et al., 1998; Guo and Bai, 2008), which may explain HER 2 association with intermediate to high grade tumours and large tumour sizes (Makanjuola et al., 2014). Status of HER 2 is important when considering treatment choice especially for patients with metastatic tumours, who respond better to additional medication such as Herceptin (Coblcigh et al., 1999; Shak, 1999; Khokher et al., 2013).

Different expression patterns of ER, PR and HER2 have been identified, making the knowledge of the receptor content of breast carcinoma essential in planning the management of the disease (Low et al., 1992; Sacks and Baum, 1993). ER over-expression has been predominantly observed in lower grade, smaller size-tumours, more likely to be node negative, and shows better survival outcome than ER-negative cancers (Fisher et al., 1988; Low et al., 1992; Grann et al., 2005). PR over-expression is also associated with well differentiated tumours with good overall survival (Reiner et al., 1990). The over-expression of ER is reported to occur in approximately 70-80% of invasive breast carcinoma at the point of diagnosis (SjÄgren et al., 1998). Over-expression of HER2 is associated with higher grade (SjÄgren et al., 1998) and ER-negative tumours (Gago et al., 2006) which demonstrate poor overall survival (Yamauchi et al., 2001). The HER2 over-expression is reported to occur in 10-30% of invasive breast cancers (Ciocca et al., 2006). Another subtype usually identified in breast cancer classification is the triple-negative. Triple-negative breast cancers are tumours characterized by their lack of hormone receptors (ER and PR) and HER2. They are the most aggressive form and account for 10-17% of all breast cancers (Nwachukwu et al., 2009). This subtype is reportedly more prevalent in African-Americans than in their white counterparts (Carey et al., 2006; Bauer et al., 2007; Ihemelandu et al., 2007; Yong et al., 2007).
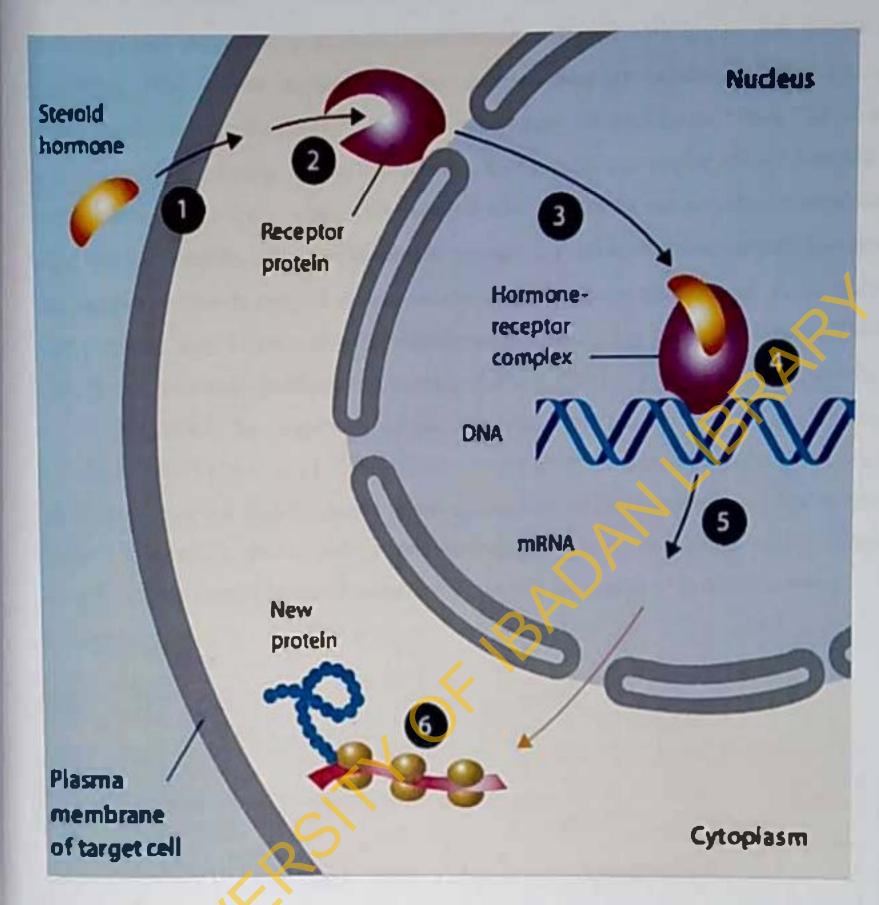
Figure 2.1: Mechanisms of Action of Sex Hormones (Bergman *et al.*, 2013).

2.10 Mechanisms of Action of Sex Hormones

Oestrogen and progesterone promote proliferation and differentiation in the normal breast epithelium. They function via binding to their corresponding intracellular receptors, ER and PR, which are members of the nuclear hormone receptor super-family (Evans, 1988). The process by which oestrogen and progesterone interact with their receptors is similar for all members of the nuclear hormone receptor family (White and Parker, 1998). In the absence of hormones, the receptors are inactive. When hormones pass through the cell membrane and bind the receptors, the inactive oligomeric complex dissociates and the receptors are transformed into an active state that regulates gene expression either directly as a transcription factor by binding DNA at a specific response-element (Beato and Sanchez-Pacheco, 1996; Glass et al., 1996; Horwitz et al., 1996), or indirectly by cooperative interactions with other transcription factors e.g. activator protein 1 (AP-1) (Gaub et al., 1990; Philips et al., 1993; Umayahara et al., 1994). As DNA-binding transcription factors, steroid hormone receptors do not function alone but interact with general transcription factors and receptor interacting proteins. In addition to this complexity, members of the nuclear hormone receptor super family are expressed in multiple forms.

2.11 Oestrogens and Breast Cancer

Oestrogens play a role in breast cancer. It is thought that in promoting the growth of breast's end buds, oestrogens may also contribute to an increase in cells that later in life become prone to cancerous growth (Russo and Russo, 1998). During the periods when the duct structures grow, especially during puberty, the breast is particularly vulnerable to cancer-causing influences (Russo and Russo, 1998). The cyclical secretion of oestrogen during a woman's life is now recognized as a key determinant of breast cancer risk. The more oestrogens reach the sensitive structures in the breast during her lifetime, the higher the overall risk. Thus, every year of delay in the onset of regular ovulations corresponds to 5% reduction in breast cancer risk. Conversely, every year of delay in menopause increases the risk by 3% (Travis and Key, 2003). On the other hand, pregnancies have a protective influence (Hinkula et al., 2001). Each child birth is thought to decrease the risk of breast cancer by 7% and this effect is even more pronounced before the age of 20 years (Travis and Key, 2003). The very high levels of oestrogen and other hormones that are secreted during pregnancy stimulate the full maturation of the duct system of the breast. It is thought that this leads to a reduction in the number of cells in the buds that are vulnerable to cancer-causing factors and thus to a decrease in cancer risk. Moreover, E_2 not only trigger cell proliferation/division but alter breast micro-environment. They change intercellular communication and have systemic effects with secondary consequences for breast tissue. All these changes are important for the formation of new milk ducts during normal breast development and may promote progression of breast cancer (Brisken, 2008).

2.12 Progesterone and Breast Cancer

Progesterone's role in breast cancer is controversial (Ho et al., 2009). It has been hypothesised that its activity of opposing oestrogenic stimulation of the breast decreases breast cancer risk (Kelsey, 1979; Foidart et al., 1998; Ho et al., 2009). On the other hand, some believe that the risk of breast cancer is increased because breast mitotic rates are highest in the luteal phase of the menstrual cycle (Harris et al., 1992; Foidart et al., 1998). In recent times, the findings of studies of serum progesterone levels in premenopausal women have been conflicting (Bernstein et al., 1990; Ho et al., 2009). A number of case-control studies have observed lower levels of serum progesterone in premenopausal cases (Key and Pike, 1988; Bernstein et al., 1990). Currently,

31

there is paucity of information on the serum progesterone levels in Nigerian women with breast cancer.

2.13 Follicle Stimulating Hormone (FSH)

Follicle stimulating hormone is a 35.5kD glycoprotein dimer. Its structure is similar to those of luteinizing hormone (LH), thyroid stimulating hormone (TSH) and human chorionic gonadotropin (hCG). The protein dimer contains 2 polypeptide units, labelled alpha and beta subunits. The alpha subunits of LH, FSH, TSH and hCG are identical and contain 92 amino acids. The beta subunits vary. Follicle stimulating hormone has a beta subunit of 111 amino acids (FSH β), which confers its specific biologic action and is responsible for interaction with the FSH receptor (Jiang et al., 2012). The sugar part of the hormone is composed of fucose, galactose, mannose, galactosamine, glucosamine, and sialic acid, the latter being critical for its biologic half-life. The half-life of FSH is 3-4 hours.

2.13.1 The Physiological Roles of FSH in Females

Follicle stimulating hormone stimulates the growth and recruitment of immature ovarian follicles in the ovary. FSH is the major survival factor that rescues the small antral follicles (2–5 mm in diameter for humans) from apoptosis. In the luteal-follicular phase transition period, the serum levels of progesterone and oestrogen (primarily oestradiol) decrease and no longer suppress the release of FSH, consequently FSH peaks at about day three (day one is the first day of menstrual flow). The cohort of small antral follicles is normally sufficient in number to produce enough Inhibin B to lower FSH serum levels. In addition, there is evidence that gonadotropin surge-attenuating factor produced by small follicles during the first half of the follicular phase also exerts a negative feedback on LH secretion amplitude, thus allowing a more favourable environment for follicle growth and preventing premature luteinization (Fowler et al., 2003). When the follicle matures and reaches 8–10 mm in diameter it starts to secrete significant amounts of oestradiol. Normally in humans, only one follicle becomes dominant and survives to grow to 18–30 mm in size and ovulate, the remaining follicles undergo atresia. The sharp increase in oestradiol production by the dominant follicle (possibly along with a decrease in gonadotropin surge-attenuating factor) cause a positive effect on the hypothalamus and pituitary gland,thus, rapid gonadotropin-releasing hormone (GnRH) pulses occur and an LH surge results.

32

The increase in serum oestradiol level causes a decrease in FSH production by inhibiting GnRH production in the hypothalamus (Dickerson *et al.*, 2008).

The decrease in serum FSH level causes the smaller follicles in the current cohort to undergo atresia as they lack sufficient sensitivity to FSH to survive. Occasionally two follicles reach the 10 mm stage at the same time by chance and as both are equally sensitive to FSH, both survive and grow in the low FSH environment and thus two ovulations can occur in one cycle possibly leading to non identical (dizygotic) twins. As a woman nears perimenopause, the number of small antral follicles recruited in each cycle diminishes and consequently insufficient Inhibin B is produced to fully lower FSH and the serum level of FSH begins to rise. Eventually, the FSH level becomes so high that down regulation of FSH receptors occurs and by menopause any remaining small secondary follicles no longer have FSH receptors (Radu *et al.*, 2010). FSH binding is thought to upregulate neo-vascularization via at least two mechanisms – one is the Vascular Endothelial Growth Factor (VEGF) pathway and the other VEGF independent - related to the development of umbilical vasculature when physiological. This presents possible use of FSH and FSH-receptor antagonists us an anti tumour angiogenesis therapy (Radu *et al.*, 2010).

2.14 Luteinizing Hormone (LH)

Luteinizing hormone, also known as lutropin or lutrophin is a hormone produced by gonadotroph cells in the anterior pituitary gland. In females, an acute rise of LH ("LH surge") triggers ovulation and development of the corpus luteum. (Louvet *et al.*, 1975). It acts synergistically with FSH in females. LH supports theca cells in the ovaries that provide androgens and hormonal precursors for E_2 production. At the time of menstruation, FSH initiates follicular growth, specifically affecting granulosa cells (Mahesh, 2011). With the rise in oestrogens, LH receptors are also expressed on the maturing follicle, which causes it to produce more E_2. Eventually, when the follicle is fully mature, a spike in 17-hydroxyprogesterone production by the follicle inhibits the production of oestrogen, leading to a decrease in oestrogen-mediated negative feedback of GnRH in the hypothalamus, which then stimulates the release of LH from the anterior pituitary (Carr, 1998). This increase in LH production only lasts for 24 to 48 hours. This "LH surge" triggers ovulation, thereby not only releasing the egg from the follicle, but also initiating the conversion of the residual follicle into a corpus luteum that, in turn, produces

progesterone to prepare the endometrium for a possible implantation (Yeh and Adashi, 1999). If pregnancy occurs, LH levels will decrease, and luteal function will instead be maintained by the action of hCG, a hormone very similar to LH but secreted from the new placenta). The release of LH from the pituitary gland, and is controlled by pulses of GnRH. When the levels are low, GnRH is released by the hypothalamus, stimulating the pituitary gland to release LH (Carr, 1998; Yeh and Adashi, 1999; Yen, 1999).

2.15 FSH, LH and Breast Cancer

FSH stimulates follicle growth and development in the ovaries (Zhou et al., 2013). FSH has been reported to be associated with certain cancers including prostate, endometrial and ovarian cancers (Ben-josef et al., 1999; Bax et al., 2000; Chen et al., 2009; Huhtaniemi, 2010). There are reports that FSH induces cancer cell proliferation, differentiation and metastasis by activating adenylyl cyclase, thereby resulting in increased cAMP levels (Tunizicker-Dunn and Maizels, 2006; Fan et al., 2007). High FSH levels have been associated with a significantly poor prognosis in patients with premenopausal breast cancer (Pujol et al., 2001). FSH has also been linked to breast cancer cell proliferation and an increased risk of breast cancer development in females who have undergone infertility treatments (Zreik et al., 2010). While the oestrogen signal pathway on tumourigenesis and tumour progression in breast cancer has been widely discussed, there is paucity of information on the FSH and LH pathway(s) in breast cancer. Moreover, the specific functions of FSH and LH have not been fully elucidated with regards to the progression of breast cancer (Zhou et al., 2013). There is paucity of information on the role of gonadotropin and breast cancer based on menopausal status.

2.16 Thyroid Physiology and Pathophysiology

Thyrotropin Releasing Hormone (TRH) acts on the pituitary thyrotropes to stimulate both the synthesis and release of TSH (Krassas et al., 2010). TSH in turn controls the thyroid gland and the synthesis and release of thyroid hormones. TSH also controls the size and number of thyroid follicular cells. Thyroid hormones are the only iodine-containing substances of physiologic significance in vertebrates (Bello and Bakari, 2012). Thyroid cells actively extract and concentrate iodide from plasma. A tightly controlled feedback system exists between the thyroid gland, the hypothalamus and pituitary gland (Bello and Bakari, 2012). These three glands

34

function closely thereby ensuring that thyroid hormone concentration in the blood are maintained within certain limits in the face of large changes in basal metabolic and physiological need for thyroid hormone (Surks et al., 2004). A rise in the serum thyroid hormone concentration elicits an inhibitory effect on the pituitary response to TRH (negative feedback). Thyroxine (T_4), a prohormone, is converted to triiodothyronine (T_3), the active form of thyroid hormone, in the peripheral tissues by 5'-deiodination. Normal thyroid gland produces all of the circulating T_4 and about 20% of the circulating T_3 (Surks et al., 2004). Most of the biologic activity of thyroid hormones is due to the cellular effects of T_3, which has a greater affinity for the thyroid hormone receptor and is approximately 4 to 10 times more potent than T_4 (Surks et al., 1973; Sawin et al., 1977). 80% of serum T_3 is derived from the de-iodination of T_4 in tissues such as the liver and kidney. Once T_4 and T_3 are released into the circulation, they are bound by Thyroxine Binding Globulin (TBG), transthyretin (thyroxine-binding pre-albumin), and albumin. Thyroxine Binding Globulin has the highest affinity for T_4 and T_3 and the lowest capacity, whereas albumin has the lowest affinity and the highest capacity. Only the free (unbound) fraction of T_4 and T_3 is able to bind to specific thyroid hormone receptors in peripheral tissues and possesses biologic activity. Normally, approximately 0.03% of T_4 and 0.5% of T_3 is free (Oppenheimer et al., 1972; TNACB, 1996). Changes in the binding capacity of thyroid hormone transport proteins may significantly affect the measurement of total thyroid hormone concentration and thereby complicate the diagnosis of hypothyroidism. The accurate diagnosis of thyroid disease is more difficult in patients with multiple abnormalities in thyroid hormone-binding proteins (Robbins, 1992).

Localized disease of the thyroid gland that results in decreased thyroid hormone production is the most common cause of hypothyroidism. Under normal circumstances, the thyroid releases 100 to 125 nmol of T_4 daily and only small amounts of T_3. Decreased production of T_4 causes an increase in the secretion of TSH by the pituitary gland. TSH stimulates hypertrophy and hyperplasia of the thyroid gland and thyroid T_4-5'-deiodinase activity. This in turn causes the thyroid to release more T_3. Deficiency of the hormone has a wide range of effects, because all metabolically active cells require thyroid hormone. The systemic effects are due to either derangements in metabolic processes or direct effects by myxedematous infiltration (that is, accumulation of glucosaminoglycans in the tissues).

2.16.1 The Metabolism of Thyroid Hormones

One of the earliest recognized physiologic actions of thyroid hormones was its effect on the basal metabolic rate (Dickennan and De Vries, 1997). In general, thyroid hormone deficiency results in a reduction in the metabolic rate. This is manifested as the intolerance to cold temperatures experienced by many hypothyroid patients. Thyroid hormone is also an important modulator of intermediary metabolism. Thyroid hormone replacement therapy may slow the progression of coronary artery disease, because of its beneficial effects on lipids (Sundaram *et al.*, 1997). Glucose homeostasis may be altered due to the slower rate of glucose absorption from the gastrointestinal tract. Insulin secretion in response to glucose load varies in hypothyroid individuals, but there is evidence of insulin resistance and reduced glucose utilization (Pedersen *et al.*, 1988; Fowler *et al.*, 1996). Hypothyroid patients generally exhibit decreased appetite (Bello and Bakari, 2012). Some studies have found an association between thyroid hormones and adiposity. Leptin regulates the hypothalamic-pituitary-thyroid axis by regulating TRH gene expression in the paraventricular nucleus in the hypothalamus thus, prompting TSH to stimulate leptin secretion (Feldt-Rasmussen, 2007; Menendez *et al.*, 2003; Oge *et al.*, 2005; Santini *et al.*, 2010; Mehran *et al.*, 2014).

2.16.2 Thyroid Hormones and the Reproductive System

A possible relationship between the thyroid hormones and ovarian function has been well documented in the literatures based on in vivo studies. The effects of hypothyroidism on fertility are mediated by a disruption of gonadotropin secretion and steroidogenesis. Serum levels of FSH and LH may be increased, normal, or decreased, and the preovulatory LH surge may be absent (Ottesen *et al.*, 1995). Delayed LH response to GnRH has been reported in some hypothyroid women (Valenti *et al.*, 1984; Marino *et al.*, 2006).

In females, hypothyroidism is associated with menstrual irregularities, i.e. changes in cycle length and amount of bleeding (Joshi *et al.*, 1993). The latter is probably due to oestrogen breakthrough bleeding secondary to anovulation (Krassas *et al.*, 1999). Defects in haemostasis factors (such as decreased levels of factors VII, VIII, IX, and XI) that occur in hypothyroidism may also contribute to polymenorrhea and menorrhagia (Ansell, 1996). Menstrual disturbances

specifically, amenorrhea, clinical metropathia haemorrhagica (haemorrhage during the menstrual cycle), and menorrhagia was reported in patients with primary myxedema (Krassas *et al.*, 2010).

Anovulation and infertility have also been reported in hypothyroid females (Stradtman, 1993). Hypothyroid women have decreased rates of metabolic clearance of oestrone and exhibit an increase in peripheral aromatization (Longscope *et al.*, 1990; Redmond, 2004). An increase in excretion of 2-oxygenated oestrogens has been reported in hypothyroid women (Gallergher *et al.*, 1966). Plasma binding activity of SHBG is decreased, which results in decreased plasma concentrations of both total testosterone and E_2 but their unbound fractions are increased. Alterations in steroid metabolism disappear when a euthyroid state is restored (Gordon and Southren, 1977).

2.16.3 Gonadotropins (LH, FSH) In Hyperthyroid Women

Gonadotropin dysfunction has been reported in women with hyperthyroidism. It was reported that the mean LH levels in both the follicular and luteal phases of the menstrual cycle are significantly higher in hyperthyroid women than in normal women (Akande and Hockaday, 1972). Similar results were obtained in women at the middle of the luteal phase of the menstrual cycle (Pontikides *et al.* 1990). Some authors found that LH secretion was increased, whereas, the pulsatile characteristics of LH and FSH secretion did not differ in patients when compared with controls in the early follicular phase of the menstrual cycle. However, LH peaks may be absent in patients with amenorrhea (Zähringer *et al.*, 2000). Serum LH levels decrease to normal after a few weeks of treatment with antithyroid drugs (ATD) (Akande, 1974). Baseline FSH levels may be increased, although, data on this are limited (Tanaka *et al.*, 1981; Pontikides *et al.*, 1990). However, some reports claim that FSH levels remain normal in thyrotoxic women (Distiller *et al.* 1975; Zähringer *et al.* 2000). The mechanism for the increase in serum LH and FSH in hyperthyroid women is unclear (Krassas *et al.*, 2010). It has been reported that hyperthyroxinemia resulted in an augmented gonadotropin response to GnRH (Tanaka *et al.*, 1981). Other studies, however, have been unable to confirm these findings (Distiller *et al.*, 1975).

The biochemical and hormonal abnormalities, nutritional disturbances and emotional upheavals that are commonly associated with hyperthyroidism may individually or in combination be the cause of the menstrual disturbances (Krassas, 2005). A study in India showed menstrual

37

irregularities in 65% of hyperthyroid women, compared with 17% among healthy controls (Joshi et al., 1993). These irregularities sometimes preceded the identification of thyroid dysfunction (Krassas et al., 2010). Similar results were observed in other studies (Krassas et al., 1994). Although, these findings indicate that menstrual disturbances are 2.5-fold more frequent in thyrotoxicosis than in the normal population (Krassas et al., 2010).

2.17 Thyroid Hormones and Breast Cancer

The growing and developing breasts require the coordinated action of several hormones such as oestrogen (E_2), progesterone, and thyroid hormones (Lai, 2002; Neville et al., 2002). While oestradiol has been reported to be a potent mitogen for normal mammary gland, thyroid hormones appear to stimulate lobular development, contributing to the differentiation of normal breast tissue (Neville et al., 2002). However, the relationship between breast cancer and thyroid hormone is controversial (Saraiva et al., 2005). Even though, many studies have shown that thyroid diseases are common in women with breast cancer, other reports have not confirmed this association (Gogas et al., 2001; Turken et al., 2003; Smyth et al., 1996; Smyth et al., 1998; Cengiz et al., 2004; Giustarini et al., 2006; Conde et al., 2006; Tosovic et al., 2010; Tosovic et al., 2012). Almost every form of thyroid disease including hyperthyroidism has been identified in association with breast cancer (Takatani et al., 1989; Goldman, 1990; Cengiz et al., 2004; Rasmusson et al., 1987; Lemaire and Baugnet-Mahieu, 1989; Takatani et al., 1989). For instance, it was speculated that subclinical hyperthyroidism in postmenopausal patients contributes to breast tumour growth (Saraiva et al., 2005). It has also been suggested that free triiodothyronine (FT_3) plays an important role in the physiology of fibrocystic breast disease (Martinez et al, 1995). There is currently paucity of information on the link of thyroid hormones with breast cancer in Nigeria. However, physiological concentrations of T_3, the more active form of thyroid hormone is reported to significantly enhance oestradiol growth stimulation of a number of human breast carcinoma cell lines (Shao et al., 1995). In T47D breast cancer cells, E_2 and T_3 similarly regulate cell cycle progression and proliferation raising the p53 level and causing hyperphosphorylation of pRb (Dinda et al., 2002). Moreover, it was demonstrated that in breast cancer cell lines, T_3 at supra-physiologic concentrations and in the absence of oestradiol mimics the effects of oestradiol, possibly through the ER (Nogueira and Brentani, 1996).

2.18 Contraceptives Use and Breast Cancer Risk

More than 100 million women worldwide use Oral Contraceptives (OCs), which are the most commonly used contraceptive method for US women (Bensyl et al., 2005). They are prescribed because they are reportedly safe, effective, well tolerated and convenient (Bensyl et al., 2005). The effectiveness of OCs and of the other combination hormonal contraceptives including the patch and ring is 99.7% if used exactly as directed and only slightly lower at 92% if the dose is occasionally taken late or not taken. The effectiveness of other contraceptives ranges from approximately 85% for barrier methods such as condom, sponge, and diaphragm, to upward of 99% for intrauterine devices, subdermal implants, progesterone injection and sterilization in both men and women (Trussell, 1998; Implanon, 2006). There have been suspicions for many years that the use of hormonal contraception is linked to an increased risk of breast cancer. These suspicions have been fuelled by the fact that widespread use of hormonal contraceptives, particularly OCs has paralleled an increased incidence of breast cancer in many countries. Increasing evidence that breast cancer is hormonally mediated has heightened concern about a possible link. Yet the numerous investigations of possible OC/breast cancer associations that have been carried out around the world have not provided conclusive answers (Trusell, 1998; Casey et al., 2008). In general, these studies have been characterized by weak, sometimes conflicting associations (PATH, 1997). It has been reported that 5 years of combined Hormone Replacement Therapy (HRT) of oestrogen and progesterone was associated with a 26% increased risk of invasive breast cancer in postmenopausal women (WGWHI, 2002). Moreover, the carcinogenic effect of oestrogen-progestagen contraceptives and replacement hormones has been reported (Cogliano et al., 2005). This has been confirmed and acknowledged by the World Health Organization (IARC, 2005). However, progestin-only pills were associated with a relative risk of breast cancer of 1.17 within 5 years of use, while the relative risk for use within 10 years is 0.99 (Casey et al., 2008).

Two major potential mechanisms have been postulated by which oestrogens (both endogenous and exogenous) increase the risk of breast cancer. The first mechanism is the stimulation of oestrogen receptor–mediated transcription that results in cell proliferation. The second mechanism is direct carcinogenesis via metabolic activation and direct binding of DNA. One

39

hypothesis is that these 2 mechanisms act in an additive or even synergistic fashion to induce carcinogenesis (Yager, 2000; Santen et al., 2004).

2.19 Induced Abortion (IA) and Breast Cancer

Childbearing has been consistently shown to reduce the risk of breast cancer in the long term (CGHFBC, 1996). Until recently, incomplete pregnancies were thought to have no effect, or perhaps slightly reduce the risk of breast cancer (Vessey et al., 1982). However, the outcome of a study involving literature review suggested that induced abortion might increase the risk of breast cancer (Remennick, 1990). This was further supported by similar findings (Brind et al., 1996). However, others who reviewed the evidence made by Remennick (1990) and Bind et al (1996) arrived at different conclusions (Michels and Willett, 1996; Wingo et al., 1997; Batholomew and Grimes, 1998). Induced abortion (IA) was reportedly significantly associated with an increased risk of breast cancer among Chinese females, and the risk of breast cancer increased as the number of IA increased (Huang et al., 2014). As of 2004, 41 studies had been published in the worldwide medical literature (including 16 American studies) reporting data on the risk of breast cancer among women with a history of induced abortion (AAPLOG, 2008). Twenty nine (70%) of these studies, reported increased risk. Thirteen of the 16 (81%) American studies reported increased risk, 8 (50%) with statistical significance (at least 95% probability that the result was not due to chance) irrespective of age at first full-term pregnancy. The relative risk increase of the 41 studies combined was 30% (AAPLOG, 2008). In the current American abortion experience, this would result in approximately 5,000 additional cases of breast cancer per year in the U.S. (There are about 190,000 new cases of breast cancer diagnosed in the US each year) (AAPLOG, 2008). Moreover, a 50% breast cancer risk increase by age 45 in United States' women who have had an induced abortion has been reported (Daling et al., 1994). However, a 12% lifetime chance of developing breast cancer was equally reported. Among women with a family history of breast cancer (mother, grandmother, sister or aunt), the increase in risk was 80% (Daling et al., 1994). Few studies have focussed on the association of induced abortion with breast cancer risk in indigenous African women.

2.19.1 The Proposed Mechanism of Induced Abortion in Breast Cancer

The hypothesized mechanism by which, induced abortion influence the development of breast cancer has been described (Russo et al., 2001). Prior to puberty, a woman's breast contains immature lobules, called type 1 lobules. After puberty, with increasing oestrogen levels, these lobules begin to increase in number and in maturity, and are called type 2 lobules. Pregnancy produces a huge increase in oestrogen levels (about 20 times non-pregnant levels). This causes an immense increase in the number of type 1 and 2 (relatively immature, in accelerated growth phase) lobules. More vulnerable lobules make more places where cancer can start. In the 3rd trimester and with lactation, the lobules complete their maturation into type 3 and 4 lobules, which have been reported to be more resistant to cancer influences/genetic mutations than are the less mature type 1 and type 2 lobules. The post abortive woman is left with a huge increase in the more vulnerable type 1 & 2 lobules. Thus, the process of lobular maturation in a full term pregnancy could account for "the protective effect" that is observed. Abortion abruptly interrupts this process before the 3rd trimester maturation of lobules happens by causing an immediate and marked drop in the oestrogen levels. This leaves the type 1 and 2 lobules, now greatly increased in number, in non-mature (only partially differentiated) growth phase. This could make them more susceptible to malignant change with exposure to carcinogens at a future time. This could be a major factor in the increased risk between induced abortion and subsequent breast cancer that many studies show (Russo et al., 2001; Beiler et al., 2003; Bull et al., 2012).

2.20 Diet and Breast Cancer Risk

2.20.1 Fibre-Based Diet

A plant based diet is naturally high in fibre. A diet rich in natural fiber obtained from fruits, vegetables, legumes (lentils, split peas, black beans, pinto beans etc.), and whole-grains may reduce cancer risk and/or reduce risk of cancer progression (Harris et al., 1993). Certain case-control studies have reported that the greater the fibre intake, the lower the incidence of breast cancer (Howe et al., 1990; Freudenheim et al., 1996; De Stefani et al., 1997; La Vecchia et al., 1997; Challier et al., 1998). A high fibre diet is also associated with less obesity (Stoll, 1996). However, data from prospective studies is mixed, reporting protective effects (Rohan et al., 1993; Mattisson et al., 2004) or no effect observed (Terry et al., 2002; Cho et al., 2003). Total

41

dietary fibre intake, particularly from cereals and fruit was found to reduce the risk of breast cancer in premenopausal but not postmenopausal women (Cade *et al.*, 2007). Moreover, a cohort study reported that high fibre intake was associated with a 42% lower risk of postmenopausal breast cancer (Mattisson *et al.*, 2004). Indigenous African women who ate beans and lentils at least twice a week had a 24% lower risk of developing breast cancer than women who ate them less than once a month (Adebamowo *et al.*, 2005).

Various mechanisms have been proposed for the protective effects of dietary fibre against cancer. These include: Increased faecal bulk and decreased intestinal transit time, which allow less opportunity for faecal mutagens to interact with the intestinal epithelium (Slavin, 2000), binding to bile acids, which are thought to promote cell proliferation (Slavin, 2003). Fermentation in the gut produces short-chain fatty acids (SCFA) which improves the gut environment and may provide immune protection beyond the gut (Slavin, 2000; Slavin, 2003). Additionally, whole grains are rich in antioxidants, including trace minerals and phenolic compounds, which have been linked to disease prevention (Slavin, 2003). Furthermore, there are reports that a high fibre diet works to reduce hormone levels that may be involved in the progression of breast cancer (Bagga *et al.*, 1995; Stoll, 1996; Slavin, 2000; Rock *et al.*, 2004; Wayne *et al.*, 2007). In a high-fibre, low-fat diet intervention study, fibre reduced serum E_2 concentration in women diagnosed with breast cancer, the majority of whom did not exhibit weight loss. Thus, increased fiber intake was independently related to the reduction in serum oestradiol concentration (Rock *et al.*, 2004). This decrease in oestrogen levels in the blood thereby may potentially reduce the risk of hormone-related cancers, such as breast cancer (Slavin, 2000; Rock *et al.*, 2004). Reduced levels of serum oestrone and oestradiol were observed in premenopausal women with a greater intake of dietary fibre (Bagga *et al.*, 1995). Similarly, a high intake of dietary fibre was significantly associated with low serum levels of oestradiol in postmenopausal breast cancer survivors (Wayne *et al.*, 2007). Dietary fibre intake increases the amount of oestrogen excreted in the stool (Goldin *et al.*, 1982).

.2.20.2 Fruits and Vegetables

Fruits and vegetables contain vitamins, minerals, fibre, and various cancer-fighting phytonutrients (i.e. carotenoids, lycopene, indoles, isoflavones, flavonols). Vibrant, intense colour is one indicator of phytonutrient content in fruits and vegetables. There is extensive and

42

consistent evidence that diets high in fruits and vegetables are associated with decreased risks of many cancers, and while results for breast cancer risk are not yet conclusive, they are promising (Riboli and Norat, 2003; Gaudet et al., 2004; Hirose et al., 2005; World Cancer Research Fund, 2007; de Lima et al., 2008). In a study of about 3000 postmenopausal women, a protective effect for vegetables was observed (Gaudet et al., 2004). Women who consumed 25 or more servings of vegetables weekly had a 37% lower risk of breast cancer compared with women who consumed fewer than 9 vegetable servings weekly. An epidemiological study reported a significant protective effect of vegetables against breast cancer when case-control and cohort studies were considered together (Riboli and Norat, 2003). A recent case-control study reported women who consumed more than 3.8 servings of fruits and vegetables daily had a lower risk of breast cancer when compared with women who consumed fewer than 2.3 daily servings (Shannon et al., 2005). Japanese women following a prudent dietary pattern (high in fruits and vegetables, low in fat) had a 27% decreased risk of breast cancer (Hirose et al., 2007). A Korean case-control study reported that a high intake of certain fruits and vegetables resulted in a significantly lower risk of breast cancer in premenopausal and postmenopausal women (Do et al., 2007). These observations indicate that regular consumption of fruits and vegetables could reduce the risk of breast cancer.

2.20.3 Refined Carbohydrates

When carbohydrates are refined, nearly all of the vitamins, minerals and fibres are removed leaving only calories. Certain products like white flour and sugars are refined and then enriched meaning that only certain nutrients removed in the refining process are added back into the product. In white flour, the kernel of the grain is processed to remove the germ portion. This removes about 33 nutrients. Enriching adds 4-6 nutrients back into the product. This creates the nutritive deficit. White flour is literally sugar in itself, and where it is mixed with fats in processed foods, the fats are commonly hydrogenated, increasing consumer's susceptibility to a number of disease processes (Sieri et al., 2007). A case-control study reported that carbohydrate intake significantly increased the risk of breast cancer; sucrose (table sugar, a refined carbohydrate) imparted the greatest risk (Romieu et al., 2004). This risk was lessened considerably with a higher fibre intake. Adding credence to the idea that blood sugar levels may affect disease progression. Women who consumed a high glycemic index (GI) and glycemic load

(GL) diet had a high risk of breast cancer. This effect was reportedly most pronounced in premenopausal women and those women of a healthy body weight (Sieri *et al.*, 2007). Similarly, GI and GL were both associated with an increased risk of breast cancer among postmenopausal overweight women; this effect was most pronounced for women with ER (negative) breast cancer (Lajous *et al.*, 2008). A meta-analysis showed that GI to modestly increased the risk of breast cancer (Barclay *et al.*, 2008).

2.20.4. Meat

Reports have associated the consumption of red meat with the risk of breast cancer (Zheng *et al.*, 1998; Taylor *et al.*, 2007). Meat consumption increased the risk of breast cancer risk by 56% for each additional 100 g (3.5 oz) daily of meat consumption in a French case-control study (Wakai *et al.*, 2005). Regular consumption of fatty red meat and pork fat greatly increased the risk of breast cancer in a Brazilian study (Di Pietro *et al.*, 2007). In a study of over 35,000 women, meat consumption significantly increased the risk of breast cancer in both premenopausal and postmenopausal women (Taylor *et al.*, 2007). Women who eat 1.75 ounces of processed meat daily, increased the risk of breast cancer by 64% in postmenopausal women compared to women who did not eat meat (de Lima *et al.*, 2008). Consumption of red and fried meat quadrupled the risk of breast cancer in a case-control study in Brazil (de Lima *et al.*, 2008). A large case-control study found that women who consumed meat for hamburger, bacon, and steak had a 54%, 64%, and 221% increased risk for breast cancer, respectively (Zheng *et al.*, 1998).

2.21 Alcohol Consumption and the Risk of Breast Cancer

Alcohol consumption has been considered a plausible risk factor of breast cancer (Qian *et al.*, 2014). Certain studies found a positive relationship between alcohol consumption and breast cancer (Nasca *et al.*, 1990; Bowlin *et al.*, 1997; Thun *et al.*, 1997; Bagnardi *et al.*, 2001; Hamajima *et al.*, 2002; Key *et al.*, 2006; Suzuki *et al.*, 2008) However, other studies did not (Kinney *et al.*, 2000; Zhang and Holman 2011; Llanos *et al.*, 2012; Chandran *et al.*, 2013). Studies of the relationship between alcohol consumption and breast cancer risk among African-Americans have found inconclusive results (Hiatt and Bawol 1984; Hiatt *et al.* 1988, Brinton *et al.*, 1997; Kinney *et al.*, 2000; Zhu *et al.*, 2003). The extent of alcohol drinking's effect on breast cancer risk may vary across races, possibly due to different drinking habits, metabolism and

genetic factors (Dumitrescu and Shields, 2005). In general, alcohol drinking is less common among African women than their counterparts in North America and Europe (Martinez *et al.*, 2011; Peer *et al.*, 2014). This could be due to racial differences in the distributions of genetic polymorphisms related to ethanol metabolism (McCarver *et al.*, 1998; Dumitrescu and Shields, 2005). As women in Africa are increasingly influenced by western cultures and begin to change their lifestyle and as the populations in African countries are becoming more affluent, more and more women may be exposed to alcohol (Martinez *et al.*, 2011; Francis *et al.*, 2014; Peer *et al.*, 2014). There is currently paucity of information on the association of alcohol consumption with the risk of breast cancer in indigenous sub-Saharan African women.

2.22 Endocrine Disruptors (EDs)

An endocrine disruptor is defined as an exogenous substance or mixture that alters the function(s) of the endocrine system and consequently causes adverse health effects in an intact organism or its progeny or (sub) populations (Sprangler, 1996; IPCS, 2002). There is increasing evidence that various chemicals introduced into the environment have the potential to adversely interfere with the endocrine system in humans and wildlife (IOMC, 2013). EDs are widespread in food chains and in the environment. Certain studies have found that potential EDs at very low levels in the environment may result in harmful effects especially when several different compounds act on one target. The homeostasis of sex steroids and the thyroid appears to be the main targets of endocrine disrupting substances (Cascita *et al.*, 2008).

Many EDs have been reported to act as agonists of oestrogen receptors (ER), e.g. bisphenol-A, or to antagonize androgen receptor (AR). Progesterone receptors are also a potential target for many chlorinated endocrine disruptor (Scippo *et al.*, 2004). Some of these endocrine disruptors could also inhibit hormone synthesis, transport or metabolism. Moreover, some could inhibit the conversion of androgens to oestrogens (Matsui *et al.*, 2005). Despite several studies done on endocrine disruptors, relatively few studies have addressed the roles of known carcinogens, such as metals in the initiation, promotion and progression of breast cancer (Adachi and Tainosho, 2004).

45

2.22.1 Metabolism of Toxic Metals

Humans have found an increasing number of uses for various metals in industry, agriculture, and medicine since the industrial revolution (Juracek and Ziegler, 2006). These activities have increased exposure not only to metal-related occupational workers, but also to consumers of the various products (Adachi and Tainosho, 2004). Metals like lead, cadmium and arsenic can be harmful pollutants when they enter the soil and water. Once in the environment, metals are almost impossible to eliminate because they do not decompose. Metals get into the body through air, food, water, or dermal exposure. They cross the plasma membrane to enter the cell in order to exert toxicity. Lipophilic metals like the arsenic and cadmium readily penetrate the plasma membrane (Lakowicz and Anderson, 1980). Cadmium can also bind to a protein, metallothioncin to form cadmium-metallothioncin, which allows cadmium to be actively taken into the cell by endocytosis (Antila et al. 1996). Other metals. like lead may be absorbed by passive diffusion (Karmakar and Jayaraman 1988).

These metals among other toxic metals have been reported as a major source of oxidative stress (Ragab et al., 2014). Oxidative stress describes the steady state level of oxidative damage in a cell, tissue, or organ caused by Reactive Oxygen Species (ROS). Oxidative stress occurs when the generation of ROS in a system exceeds that system's ability to neutralize and eliminate them. The imbalance can result from a disturbance in production or the distribution of antioxidants, as well as an overabundance of ROS from an environmental or behavioral stressor (Danilova 2006). Oxidative stress induces a cellular redox imbalance which has been found to be present in various cancer cells compared with normal cells; the redox imbalance thus may be related to oncogenic stimulation (Valko et al., 2007). The permanent modification of genetic material resulting from oxidative damage incidents represents the first step involved in mutagenesis and carcinogenesis. Elevated levels of oxidative DNA lesions have been noted in various tumours, strongly implicating such damage in the aetiology of cancer (Valko et al. 2007).

Moreover. these metals could also act through direct binding to DNA (De Bont and van Larebcke, 2004). They have been shown to directly modify and/or damage DNA by forming DNA adducts that induce chromosomal breaks (Chakrabarti et al., 2001). DNA damage can result in the arrest or induction of transcription, induction of signal transduction pathways, replication errors and genomic instability, all of which are associated with carcinogenesis (Valko

et al., 2006). DNA damage, mutations and altered gene expression are thus key players in the process of carcinogenesis (Hartwig et al., 2002; Valko et al., 2007).

2.22.2 Toxic Metals and Breast Cancer

Heavy metals are reported to play critical roles in cancer biology (Kirkwood, 2002; Ragab et al., 2014). A large number of epidemiological studies indicate a close association between heavy metals such as lead (Pb), arsenic (As), cadmium (Cd) and development of breast cancer (Ragab et al., 2014). There are reports that these metals are a major source of oxidative stress (Leonard et al. 2004; Wang et al., 2004; Hei and Filipic, 2004). Substantial data suggest that oxidative stress is involved in the development of breast cancer (Gammon et al., 2002; Wu, 2004; Rossner, 2006). Certain studies suggested that these toxic substances are agonists or antagonists for the oestrogen receptor in various in vitro systems. Although, usually with very low affinities relative to endogenous hormones such as 17β-oestradiol and oestrone (Stoica et al., 2000a; Johnson et al., 2003).

2.23 Cadmium (Cd)

Cadmium is a toxic, bio-accumulating, non-essential and highly persistent heavy metal with a variety of known adverse health effects (McElroy et al., 2006). It occurs naturally in the soil, rocks and water. It has been classified among the most important carcinogens (EPA, 1987; Garcia-Morales et al., 1994; Stoica et al. 2000b; Johnson et al., 2003). For non-occupationally exposed women who do not smoke, food is the largest source of Cd intake (Amzal et al. 2009). Particularly, root vegetables, potatoes, and grain, including rice and wheat, grown on Cd rich soils, and shellfish (Vahter et al., 1996; Mueller et al., 1996; McLaughlin et al., 1997; Olsson et al., 2005; Perez and Anderson, 2009; EFSA. 2009; Reuben, 2010). Inhalation of tobacco smoke is the predominant source of exposure (CDC, 2005) for smokers. The estimated daily intake of Cd in food in a non-hazardous environment for heavy metals is between 8 and 25 µg/day, whereas one pack of cigarettes is estimated to add 1 µg/day (Satarug et al., 2010). Only a small fraction of inhaled or ingested Cd is excreted, resulting in increased body burden over time (Klaassen, 1981; Fujishiro et al., 2012; Tekin et al., 2012). Women tend to have higher Cd levels than men presumably because of lower iron stores, which increase Cd absorption (Olsson et al.

2002; Reeves and Chaney, 2008). Thus, comparable environmental exposures to Cd may disproportionately affect women compared to men (Reeves and Chaney, 2008).

Cadmium-containing products are rarely recycled. Instead, they are frequently dumped together with household waste, thereby contaminating the environment, especially if the waste is incinerated. Cadmium is a known cumulative toxicant with a biological half-life of more than 10 years in humans. Thus, chronic low level exposure will eventually result in accumulation to toxic levels. Cadmium has the potential to disrupt endocrine function by behaving like sex hormones (Stoica et al., 2000b). At low concentrations the metal mimics the effects of oestradiol and binds with high affinity to the hormone-binding domain of ER-alpha. This binding involves several amino acids, suggesting that Cd activates the receptor through the formation of a complex with specific residues in the hormone-binding domain (Stoica et al., 2000b; Johnson et al., 2003). Cadmium affects cell proliferation, differentiation, apoptosis and signal transduction by enhancement of protein phosphorylation and activation of transcription and translation factors (Siewt et al., 2010). Early puberty has been associated with breast cancer (Colditz and Frazier, 1995; Hamilton and Mack, 2003; Johnson et al., 2003).

2.24 Lead (Pb)

The heavy metals of greatest concern for health with regard to drinking water exposure are Pb and arsenic (ATSDR, 2005). Lead in gasoline was removed during the early 1990s. Lead solder in food cans was banned in the 1980s and Pb in paint was severely restricted in 1978 in the U.S. Both the nervous and reproductive systems are susceptible targets for Pb toxicity. Results of epidemiologic studies investigating the association of Pb exposure with cancer are inconsistent and vary according to the type of cancers reported (Steenland et al., 1992; Wong and Harris, 2000). The ability of Pb to function as potent oestrogens suggests that it may be an important class of endocrine disruptors (Martin et al., 2003). There are reports from New Delhi, India and Cairo, Egypt that support an association between environmental exposure to Pb and the risk of breast cancer (Siddiqui et al., 2003; Ragab et al., 2014). There is currently a paucity of information on the role of Pb in breast cancer aetiology in Nigeria. It is however reasonable to examine the possible association between environmental exposure to Pb and risk of breast cancer, given the known impact of Pb on human health. The mechanisms of Pb carcinogenicity

involve direct DNA damage as a result of oxidative stress, clastogenicity, inhibition of DNA synthesis or repair (Martin et al., 2003; Ragab et al., 2014).

2.25 Arsenic (As)

The major source of human exposure to As is through food. Microorganisms convert As to dimethylarsenate, which can accumulate in fish, providing a source for human exposure (ATSDR 2005). Arsenic compounds are lipid soluble and within 24 hours of absorption distribute throughout the body where they can bind to sulfhydryl (SH) groups on proteins. Arsenic may also replace phosphorus in bone tissue and be stored for years (Bartolome et al., 1999). Methylation efficiency in humans appears to decrease at high As doses and studies show that aging is associated with a diminishing capacity to methylate inorganic As, resulting in its increased retention in soft tissues (Tseng et al., 2005). The oestrogenic-like activities of As have been studied in human ER-positive breast cancer cell line MCF-7 (Stoica et al., 2000a; MartÀnez-Campa et al., 2006).

Arsenite (AsO$_3^{3-}$) blocked the binding of oestradiol to ER-alpha, acted as a ligand for ER activating it in the absence of hormone, suggesting that the metal interacts with the hormone binding domain of the receptor. It increased cell growth and mimicked the effects of oestradiol, decreased the amount of ER-alpha and increased the expression of the progesterone receptor (Stoica et al., 2000a). Kaltreider et al. (1999) in a recent study examined the effect of single low-dose As, potentially directly relevant to human exposures, on binding of transcription factors in human MDA-MB-435 breast cancer and rat H4IIE hepatoma cells. These transcription factors were sensitive to the toxic metal at low doses. The specific effects were dependent on the transcription factor, time, dose, and cell line. This study showed that alteration in gene expression may play a role in long term effects of low dose environmental exposures, such as in metal induced carcinogenesis. Regulation and activation of transcription factors is an important part of mediating cellular response to target genes by metals. However, the pathways remain to be known.

2.26 Bisphenol-A (BPA)

Bisphenol-A is formed by the condensation of phenol with acetone. It has a low vapour pressure, high melting point and moderate solubility (Howard, 1989; Cousins et al., 2002; Shareef et al.,

49

2006). It is thus expected to have low volatility. Less than 1% of environmental BPA is thought to occur in the atmosphere, where it is believed to photooxidize and breakdown rapidly (Cousins et al., 2002; Howard, 1989). It is estimated that the largest environmental compartments of BPA are abiotic and are associated with water and suspended solids, soil, or sediments (Staples et al., 1998; Cousins et al., 2002; Environment Canada, 2008). Bisphenol-A has become ubiquitous in the environment within the past 80 years. This is because of its presence in a multitude of products including food and beverage packaging, flame retardants, adhesives, building materials, electronic components, and paper coatings (Staples et al., 1998; Flint et al., 2012). This has resulted in a widespread human exposure (Brody et al, 2007; Betancourt et al, 2012). As demand for these products has increased, so has BPA production. In 1964, 42 metric tons of BPA were produced in the United States (Dermer, 1977). As at 2003, global production of BPA was 3.2 million metric tons (Tsai, 2006), approximately one-third of which was manufactured in the United States (NIH, 2008). Global consumption of BPA in 2011 was predicted to exceed 5.5 million metric tons (Greiner et al., 2007). The oestrogenic effects of BPA were first reported in 1936 (Dodds and Lawson, 1936) but its use as a synthetic oestrogen was not pursued (Dodds et al., 1938). A study indicates that BPA may be as effective as oestradiol in triggering some receptor responses (Stahlhut et al., 2009) and it may act as an androgen receptor antagonist (Roy et al., 2004; Zoeller et al., 2005; Urbatzka et al., 2007). The safety of BPA is currently controversial. High levels of these endocrine disruptors have been suggested in the serum of breast cancer patients (Brisken, 2008; Calafat et al, 2013).
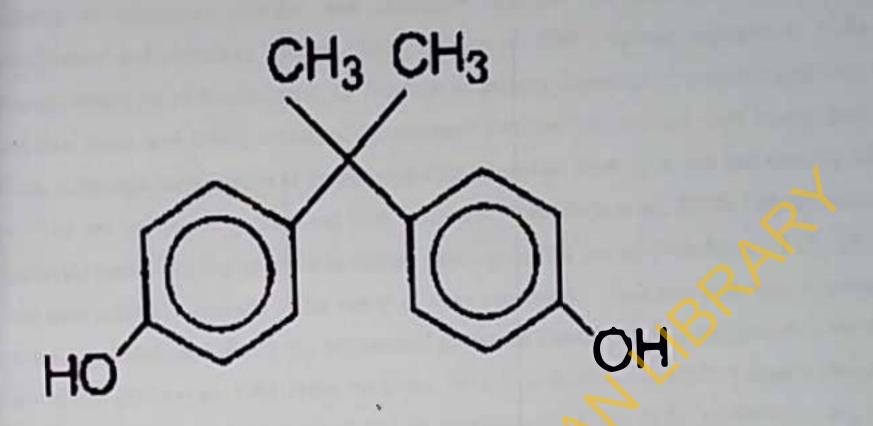
Figure 2.2: The chemical structure of bisphenol-A (Nieminen, 2002).

2.27 Polychlorinated Biphenyls (PCBs)

Polychlorinated biphenyls are members of a chemical family that were widely used in the past in industry as lubricants, coatings and insulation materials for dielectric equipment like transformers and capacitors (Iyengar, 2005; Gray et al. 2009). Human exposure to PCBs is through inhalation of contaminated air (outdoor or indoor), ingestion of contaminated food or non-food items, and dermal contact of contaminated surfaces. The primary route of exposure to PCBs is through consumption of contaminated lipid-enriched foods (e.g. fish and cooking oils) as PCBs can accumulate in these and other foodstuffs (Van-Emon et al., 2013). Polychlorinated biphenyls were classified as probable human carcinogens (2A group) (Van-Emon et al., 2013). The semi-volatile chemically stable nature of these compounds, combined with their resistance to bio-degradation and photolysis, has resulted in "global distillation" and redistribution via the atmosphere (Atlas et al., 1986; Atlas and Giam, 1998; Van-Emon et al., 2013). Concern over the harmful ecological and human effects and the persistence of PCBs in the environment led the United States Congress to ban their domestic production in 1977. Polychlorinated biphenyls are still detected in various micro-environments (e.g., air, soil, dust, sediment, food, tissue) either as Aroctors or as individual congeners (Wilson et al., 2003; Kim et al., 2004; Sapozhnikova et al., 2004; Martinez et al., 2010). There is paucity of information on the serum level of PCBs in non-occupationally exposed women in Nigeria.

MATERIALS AND METHODS

3.1 Study Design

The study was a prospective case-control study conducted in the Surgical Oncology Clinic of the Department of Surgery, University College Hospital, Ibadan. The study protocol was approved by the University of Ibadan and University College Hospital Health Review Committee (UI/EC/10/0193, Appendix 1). Informed consent was obtained from the participants before recruitment into the study. Women with breast cancer were recruited between April, 2011 and March, 2012.

3.2 Study Participants

One hundred and seventy women aged 28-80 years were consecutively recruited for this study. Eighty-five were histologically confirmed breast cancer patients who had not commenced treatment (Cases). They were recruited from the Surgical Oncology Clinic of the Department of Surgery, University College Hospital, Ibadan, by a Consultant Surgical Oncologist. Eighty-five non-pregnant, apparently healthy women aged 28-80 years were recruited as controls. The controls were recruited at three Primary Health Clinics (PHC) in Ibadan North Local Government Area of Oyo state (PHC, Idi Odundun, Agodi, PHC, Agbowo and Elderly Women/Widows Clinic, Agodi-gate). Their breasts were examined by trained nurses for the presence of any breast lump. They were asked if they felt any pain or had any discomfort in their breasts. Those that complained of pain, discomfort and/or had lump in their breasts were excluded from the study. One of the controls was excluded from the study due to incomplete data on questionnaire and insufficient blood sample.

Each of the cases was matched for age and menstrual phases (follicular, luteal and postmenopausal) with the controls. Participants were reported as postmenopausal if they had stopped menstruating over the last twelve months (Wang et al., 2009). Participants that had bilateral oophorectomy were also considered postmenopausal.

3.2.1 Inclusion Criteria

Non pregnant, non hypertensive participants with histologically confirmed breast cancer who had not commenced treatment and gave informed consent.

3.2.2 Exclusion Criteria

Pregnant women and those who reported being on hormonal drugs (i.e. contraceptives), had other types of cancers and/or chronic diseases were excluded from the study. Postmenopausal women on hormone replacement therapy were also excluded.

3.3 Demographic, Social, Dietary and Reproductive History

Semi-structured pre-test questionnaire was administered to each participant to obtain data on demography, social, diet and reproductive history (Appendix 3).

3.4 Anthropometric Indices

Anthropometric indices were weight, height, BMI, waist circumference, hip circumference, waist hip ratio, waist height ratio.

3.4.1 Weight

This was taken with a bathroom weighing scale placed on a flat surface. The participants while wearing light clothing and without shoes were made to stand on the scale with the indicator at zero. The reading was recorded to the nearest 0.5kg.

3.4.2 Height

This was measured against a pre-graduated flat, vertical surface with the participants standing bare footed in an upright position without any head gear on, without raising the heels from the ground and the feet kept together. Measurements were taken with a sliding headpiece brought to the vertex of the participant's head. The reading at this level was taken to the nearest 0.1 cm.

3.4.3 Body Mass Index

This was calculated from the body weight and height of the participants using the formula stated below.

BMI (kg/m^2) = weight (kg)/height (m^2).

3.4.4 Waist and Hip Circumferences

Waist circumference (in cm) was measured using a measuring tape placed at the navel level, while hip circumference (in cm) was measured at the widest circumference of the hip over light clothing using a non-stretchable measuring tape without any pressure on the body surface. Both indices were recorded to the nearest 0.1cm.

3.4.5 Waist Hip Ratio (WHR)

This was calculated as the ratio of the waist circumference to the hip circumference

WHR= Waist Circumference (cm)/ Hip Circumference (cm)

3.4.6 Waist Height Ratio (WHtR)

This was calculated as the ratio of waist circumference to height measurements using the formula

WHtR= Waist Circumference (cm)/ Height (cm)

3.5 Blood Pressure (BP) Measurement

Blood pressure was determined using a mercury sphygmomanometer and recorded to the nearest mmHg. Each of the participants was allowed to rest for about ten minutes and in a sitting position before the BP was taken. The rotocuff was tied around the forearm and was inflated to obstruct the brachial artery. A stethoscope was placed at the cubital fossa and the pressure released. As the blood flowed through the arm, the first and the second sound produced were systolic blood pressure and diastolic blood pressure respectively.

3.6 Sample Collection

Ten millilitres of venous blood samples were drawn into plain bottle from participants after diagnosis and histological confirmation of invasive ductal carcinoma. This was done by applying a tourniquet 10-15 cm above the intended puncture site to obstruct the return of venous blood to the heart and to distend the vein. The site of puncture, the medial cubital vein in the antecubital fossa was cleansed with alcohol swab.

For premenopausal participants, blood samples were drawn between days 5 and 9 of their menstrual cycle in follicular phase (forward dating) and 5 to 9 days before the anticipated start of their next menstrual cycle in the luteal phase (backward dating) i.e. days 19-23 (Wang, et al.,

2009). The blood was allowed to retract and centrifuged at 3500 rpm for 5 minutes. The resulting serum was aliquoted and stored at -20°C until analysis. Breast biopsy samples of the affected breast were obtained from women with breast cancer for the determination of oestrogen receptor (ER), progesterone receptor (PR) and HER 2.

3.7 Biochemical Investigations

The biochemical indices assayed in serum were hormones (oestradiol, progesterone, LH, FSH, TSH, FT_3 and FT_4), and endocrine disruptors (lead, cadmium, arsenic, bisphenol-A and polychlorinated biphenyls). The expression of oestrogen receptor, progesterone receptor and HER 2 were determined by immunohistochemistry.

3.7.1 Determination of Progesterone

Serum progesterone was analysed by Enzyme Immuno Assay (EIA) on TOSOH AIA System Analyzers (Tosoh Corporation, Tokyo 105-8623, Japan).

The Principle of Test

The ST AIA-PACK Progesterone is a competitive enzyme immunoassay which was performed within the AIA-PACK test cups. Progesterone present in the test sample competed with enzyme-labelled progesterone for a limited number of binding sites on a progesterone-specific antibody immobilized on magnetic beads. The beads were washed to remove the unbound enzyme-labelled progesterone and were then incubated with a fluorogenic substrate, 4-methylumbelliferyl phosphate (4MUP). The amount of enzyme-labelled progesterone bound to the beads is inversely proportional to the progesterone concentration in the test sample. A standard curve using a range of known standard concentration was constructed and unknown progesterone concentrations were calculated using the curve.

Material and Reagents

Plastic test cups containing:

(1) Lyophilized twelve magnetic beads with anti-progesterone rabbit polyclonal antibody

56

(2) 75μL of progesterone conjugated to bovine alkaline phosphatase with sodium azide as a preservative.

Assay Procedure

The substrate solution, wash solution and diluents were poured into their respective containers provided by the manufacturer and placed in their respective position on the analyzer.

75μL of serum was pipetted into the analyzer's test cups and loaded into the analyzer for the determination of progesterone.

The controls were pipetted into their respective cups, thereafter, the analyzer was instructed to commence analysis by pressing the START icon on the operational menu on the analyzer's screen. The TOSOH AIA System Analyzers performed all reagent and sample handling operations automatically (i.e. immunoextraction of hormone, washing, labelled hormone-antibody reaction and colour development).

Calculation

The system analyzer read the rate of fluorescence produced by the reaction and automatically converts the rate to progesterone concentration.

3.7.2 Determination of Oestradiol (E₂)

Serum oestradiol was analysed by Enzyme Immuno Assay (EIA) on TOSOH AIA System Analyzers. (Tosoh Corporation, Tokyo 105-8623, Japan).

The Principle of Test

The ST AIA-PACK E_2 is a competitive enzyme immunoassay which was performed within the AIA-PACK test cups. Oestradiol present in the test sample competed with enzyme-labelled E_2 for a limited number of binding sites on an Anti-E_2 monoclonal antibody immobilized on magnetic beads. The magnetic beads were washed to remove the unbound enzyme-labelled E_2 and were then incubated with a fluorogenic substrate, 4-methylumbelliferyl phosphate (4MUP) The amount of enzyme-labelled E_2 that was bound to the beads was inversely proportional to the

E_2 concentration in the test sample. A standard curve using a range of known standard concentration was constructed and unknown E_2 concentrations were calculated using the curve.

Material and Reagents

Plastic test cups containing:

(1) Lyophilized twelve magnetic beads with anti- E_2 rabbit polyclonal antibody.

(2) $50\mu L$ of E_2 conjugated to bovine alkaline phosphatase with sodium azide as a preservative.

Assay Procedure

The substrate solution, wash solution and diluents were poured into their respective containers provided by the manufacturer and placed in their respective position on the analyzer.

$75\mu l$ of serum was pipeued into the analyzer's test cups and loaded into the analyzer for the determination of E_2.

The controls were pipetted into their respective cups, thereafter, the analyzer was instructed to commence analysis by pressing the START icon on the operational menu on the analyzer's screen. The TOSOH AIA System Analyzers performed all reagent and sample handling operations automatically (i.e. immunoextraction of hormone, washing, labelled hormone-antibody reaction and colour development).

Calculation

The system analyzer read the rate of fluorescence produced by the reaction and automatically converts the rate to E_2 concentration.

3.7.3 Determination of FSH

Serum (FSH) was analysed by Enzyme Immuno Assay (EIA) on TOSOH AIA System Analyzers. (Tosoh Corporation, Tokyo 105-8623, Japan).

The Principle of Test

The ST AIA-PACK FSH is a two site immunoenzymometric assay which was performed within the AIA-PACK test cups. FSH present in the test sample was bound with monoclonal antibody immobilized on a magnetic solid phase and enzyme-labelled monoclonal antibody in the AIA-PACK test cups. The magnetic beads were washed to remove unbound enzyme-labelled monoclonal antibody and were then incubated with a fluorogenic substrate, 4-methylumbelliferyl phosphate (4MUP). The amount of enzyme-labelled monoclonal antibody that was bound to the beads was directly proportional to the FSH concentration in the test sample. A standard curve using a range of known standard concentration was constructed and unknown sample concentrations were calculated using the curve.

Material and Reagents

Plastic test cups containing:

(1) Lyophilized twelve magnetic beads with anti-FSH mouse monoclonal antibody.

(2) 100µL of anti-FSH mouse monoclonal antibody (to human FSH) conjugated to bovine alkaline phosphatase with sodium azide as a preservative.

Assay Procedure

The substrate solution, wash solution and diluents were poured into their respective containers provided by the manufacturer and placed in their respective position on the analyzer.

50µL of serum was pipetted into the analyzer's test cups and loaded into the analyzer for the determination of FSH.

The controls were pipetted into their respective cups, thereafter, the analyzer was instructed to commence analysis by pressing the START icon on the operational menu on the analyzer's screen. The TOSOH AIA System Analyzers performed all reagent and sample handling operations automatically (i.e. immunoextraction of hormone, washing, labelled hormone-antibody reaction and colour development)

Calculation

The system analyzer read the rate of fluorescence produced by the reaction and automatically converts the rate to FSH concentration.

3.7.4 Determination of LH

Serum LH was analysed by Enzyme Immuno Assay (EIA) on TOSOH AIA System Analyzers. (Tosoh Corporation, Tokyo 105-8623, Japan).

The Principle of Test

The ST AIA-PACK Progesterone is a two-site immunoenzymometric assay which was performed within the AIA-PACK test cups. LH present in the test sample was bound with monoclonal antibody immobilized on a magnetic solid phase and enzyme-labelled monoclonal antibody in the AIA PACK CUPS. The magnetic beads were washed to remove unbound enzyme-labelled monoclonal antibody and were then incubated with a fluorogenic substrate, 4-methylumbelliferyl phosphate (4MUP). The amount of enzyme-labelled monoclonal antibody that was bound to the beads was directly proportional to the LH concentration in the test sample. A standard curve using a range of known standard concentration was constructed and unknown sample concentrations were calculated using the curve.

Material and Reagents

Plastic test cups containing:

> (1) Lyophilized twelve magnetic beads coated with mouse anti-LH monoclonal antibody

> (2) 100µL of mouse anti-LH monoclonal antibody (to human LH) conjugated to bovine alkaline phosphatase with sodium azide as a preservative.

Assay Procedure

The substrate solution, wash solution and diluents were poured into their respective containers provided by the manufacturer and placed in their respective position on the analyzer.

40µL of serum was pipetted into the analyzer's test cups and loaded into the analyzer for the determination of LH.

The controls were pipetted into their respective cups, thereafter, the analyzer was instructed to commence analysis by pressing the START icon on the operational menu on the analyzer's screen. The TOSOH AIA System Analyzers performed all reagent and sample handling operations automatically (i.e. immunoextraction of hormone, washing, labelled hormone-antibody reaction and colour development).

Calculation

The system analyzer read the rate of fluorescence produced by the reaction and automatically converts the rate to LH concentration.

3.7.5 Determination of Free Thyroxine (FT$_4$)

Serum FT$_4$ was analysed by Enzyme Immuno Assay (EIA) on TOSOH AIA System Analyzer. (Tosoh Corporation, Tokyo 105-8623, Japan).

The Principle of Test

The ST AIA-PACK FT$_4$ is a competitive enzyme immunoassay which was performed within the AIA-PACK test cups. The thyroxine not bound to serum protein (free T$_4$) competed with enzymelabelled T$_4$ for a limited number of binding sites on a T$_4$-specific antibody immobilized on magnetic beads. After incubation, the beads were washed to remove the unbound enzyme-labelled FT$_4$ and were then incubated with a fluorogenic substrate, 4-methylumbelliferyl phosphate (4MUP). The amount of enzyme-labelled T$_4$ that was bound to the beads was inversely proportional to the FT$_4$ concentration in the test sample. A standard curve using a range of known standard concentration was constructed and unknown FT$_4$ concentrations were calculated using the curve.

Material and Reagents

Plastic test cups containing:

(1) lyophilized twelve magnetic beads with anti-thyroxine rabbit polyclonal antibody.
(2) 140µL of thyroxine conjugated to bovine alkaline phosphatase with sodium azide as a preservative.

The controls were pipetted into their respective cups, thereafter, the analyzer was instructed to commence analysis by pressing the START icon on the operational menu on the analyzer's screen. The TOSOH AIA System Analyzers performed all reagent and sample handling operations automatically (i.e. immunoextraction of hormone, washing, labelled hormone-antibody reaction and colour development).

Calculation

The system analyzer read the rate of fluorescence produced by the reaction and automatically converts the rate to LH concentration.

3.7.5 Determination of Free Thyroxine (FT$_4$)

Serum FT$_4$ was analysed by Enzyme Immuno Assay (EIA) on TOSOH AIA System Analyzer. (Tosoh Corporation, Tokyo 105-8623, Japan).

The Principle of Test

The ST AIA-PACK FT$_4$ is a competitive enzyme immunoassay which was performed within the AIA-PACK test cups. The thyroxine not bound to serum protein (free T$_4$) competed with enzyme-labelled T$_4$ for a limited number of binding sites on a T$_4$-specific antibody immobilized on magnetic beads. After incubation, the beads were washed to remove the unbound enzyme-labelled FT$_4$ and were then incubated with a fluorogenic substrate, 4-methylumbelliferyl phosphate (4MUP). The amount of enzyme-labelled T$_4$ that was bound to the beads was inversely proportional to the FT$_4$ concentration in the test sample. A standard curve using a range of known standard concentration was constructed and unknown FT$_4$ concentrations were calculated using the curve.

Material and Reagents

Plastic test cups containing:

(1) lyophilized twelve magnetic beads with anti-thyroxine rabbit polyclonal antibody.

(2) 140µL of thyroxine conjugated to bovine alkaline phosphatase with sodium azide as a preservative.

Assay Procedure

The substrate solution, wash solution and diluents were poured into their respective containers provided by the manufacturer and placed in their respective position on the analyzer.

10μl of serum was pipetted into the analyzer's test cups and loaded into the analyzer for the determination of FT_4

The controls were pipetted into their respective cups, thereafter, the analyzer was instructed to commence analysis by pressing the START icon on the operational menu on the analyzer's screen. The TOSOH AIA System Analyzers performed all reagent and sample handling operations automatically (i.e. immunoextraction of hormone, washing, labelled hormone-antibody reaction and colour development).

Calculation

The system analyzer read the rate of fluorescence produced by the reaction and automatically converts the rate to FT_4 concentration

3.7.6 Determination of Free Trliodothyronine (FT_3)

Serum FT_3 was analysed by Enzyme Immuno Assay (EIA) on TOSOH AIA System Analyzer. (Tosoh Corporation, Tokyo 105-8623, Japan).

The Principle of Test

The ST AIA-PACK FT_3 is a competitive enzyme immunoassay which was performed within the AIA-PACK FT_3 test cups. Free triiodothyronine present in the test sample competed with enzyme-labelled progesterone for a limited number of binding sites on a T_3-specific antibody immobilized on magnetic beads. The beads were washed to remove the unbound enzyme-labelled T_3 and were then incubated with a fluorogenic substrate, 4-methylumbelliferyl phosphate (4MUP). The amount of enzyme-labelled T_3 that was bound to the beads was inversely proportional to the T_3 concentration in the test sample. A standard curve using a range of known standard concentration was constructed and unknown T_3 concentrations were calculated using the curve.

Material and Reagents

Plastic test cups containing:

(1) Lyophilized twelve magnetic beads with anti-T_3 rabbit polyclonal antibody.

(2) 100μL of FT_3 conjugated to bovine alkaline phosphatase with sodium azide as a preservative.

Assay Procedure

The substrate solution, wash solution and diluents were poured into their respective containers provided by the manufacturer and placed in their respective position on the analyzer.

50μL of serum was pipetted into the analyzer's test cups and loaded into the analyzer for the determination of FT_3.

The controls were pipetted into their respective cups, thereafter, the analyzer was instructed to commence analysis by pressing the START icon on the operational menu on the analyzer's screen. The TOSOH AIA System Analyzers performed all reagent and sample handling operations automatically (i.e. immunoextraction of hormone, washing, labelled hormone-antibody reaction and colour development).

Calculation

The system analyzer read the rate of fluorescence produced by the reaction and automatically converts the rate to FT_3 concentration

3.7.7 Determination of Thyroid Stimulating Hormone (TSH)

Serum TSH was analysed by Enzyme Immuno Assay (EIA) on TOSOH AIA System analyzer (Tosoh Corporation, Tokyo 105-8623, Japan).

The Principle of Test

The ST AIA-PACK TSH was a two-site immunoenzymometric assay which was performed within the AIA-PACK test cups, TSH present in the test sample was bound with monoclonal antibody immobilized on magnetic beads and monoclonal antibody conjugated with bovine

alkaline phosphate in the AIA-PACK test cups. The beads were washed to remove the unbound enzyme-labelled monoclonal antibody and were then incubated with a fluorogenic substrate, 4-methylumbelliferyl phosphate (4MUP). The amount of enzyme conjugated with monoclonal antibody that binds to the beads was directly proportional to the TSH concentration in the test sample. A standard curve was constructed and unknown sample concentrations were calculated using this curve.

Material and Reagents

Plastic test cups containing:

(1) Lyophilized twelve magnetic beads coated with anti-TSH mouse monoclonal antibody

(2) 50µL of anti-TSH mouse monoclonal antibody (to human TSH) conjugated to bovine alkaline phosphatase with sodium azide as a preservative.

Assay Procedure

The substrate solution, wash solution and diluents were poured into their respective containers provided by the manufacturer and placed in their respective position on the analyzer.

100µL of serum was pipetted into the analyzer's test cups and loaded into the analyzer for the determination of TSH.

The controls were pipetted into their respective cups, thereafter, the analyzer was instructed to commence analysis by pressing the START icon on the operational menu on the analyzer's screen. The TOSOH AIA System Analyzers performed all reagent and sample handling operations automatically (i.e. immunoextraction of hormone, washing, labelled hormone-antibody reaction and colour development).

Calculation

The system analyzer read the rate of fluorescence produced by the reaction and automatically converts the rate to TSH concentration.

3.8 Determination of Serum Toxic Metals (Pb, Cd and As) using Flame Atomic Absorption Spectrophotometry (Buck Scientific, 210 / 211 VGP. Atomic absorption spectrophotometer. Connecticut, USA).

Serum lead, cadmium and arsenic were determined with atomic absorption spectrophotometer (AAS) based on the direct method described by Kaneko (1999).

Principle

Atomic absorption spectrophotometry is an accurate and sensitive analytical method for the determination of trace metals. The elements were not appreciably excited in the flame but merely dissociated from its bonds and placed in an unexcited or ground state. The atoms were at a low level in which they were capable of absorbing radiation at a very narrow band width corresponding to their own line spectrum. Hollow cathode lamps made of the materials analysed were used to produce a wavelength of specific for the kind of metal in the cathode.

3.8.1 Determination of Lead

Serum lead was determined using atomic absorption spectrophotometry (AAS). Samples were treated with Triton X-100. A beam of light from a hollow cathode lamp (coated with lead) was passed through a flame containing the vapourized metal to be determined. The amount of light absorbed by the metal was proportional to the concentration of Lead in the solution and was determined at 283.3nm (wavelength).

Reagents

Triton X-100 (TX) (an alkyl phenoxy polyethoxy)

Ethanol (BDH Chemicals Ltd., Poole, England)

Sample Preparation and Procedure of Analysis

A 1:2 dilution of the serum was made by mixing 1.0 ml each of triton X-100 solution

1. A two-fold dilution of the sample was made with 0.1% triton X-100 and was mixed thoroughly. 0.2ml of the digested sample was aspirated into AAS.

2. The burner was lit under flow conditions of air and acetylene. The acetylene flow was then reduced until the flame was blue.

3. The air flow was then adjusted to remove all traces of yellow from the flame.

4. The machine was then properly calibrated with the appropriate standard solutions before analyzing the test samples.

5. Serum lead was detected at a wavelength of 283.3nm.

6. The assay results were displayed on the instrument reader's screen in $\mu g/dL$.

7. Analytical quality control was performed by analyzing an aliquot of pooled serum several times during the assay.

3.8.2 Determination of Cadmium

Serum cadmium was determined by the methods of (Kaneko, 1999) using atomic absorption spectrophotometry.

Reagents

Triton X-100 (TX) (an alkyl phenoxy polyethoxy)

Ethanol (BDH Chemicals Ltd., Poole, England)

Sample Preparation and Procedure of Analysis

1. A two-fold dilution of the sample was made with 0.1% triton X-100 and was mixed thoroughly. 0.2ml of the digested sample was aspirated into AAS for analysis at a wavelength of 228.9nm

2. The prepared samples were analysed in Buck 210/211 VGP atomic absorption spectrophotometer (Buck Scientific, Inc. 58 Fort Point St. East Norwalk, Ct. 06855)

3. The burner was lit under flow conditions of air and acetylene. The acetylene flow was then reduced until the flame was blue

4. The air flow was then adjusted to remove all traces of yellow from the flame

5. The machine was then properly calibrated with the appropriate standard solutions before analyzing the test samples.

6. Serum cadmium was detected at a wavelength of 228.9 nm.

7. The assay results were displayed on the instrument reader's screen in $\mu g/dL$

66

8. Analytical quality control was performed by analyzing an aliquot of pooled serum several times during the assay.

3.8.3 Determination of Arsenic

Serum arsenic was determined by the methods of (Kaneko, 1999) using atomic absorption spectrophotometry.

Reagents

Triton X-100 (TX) (an alkyl phenoxy polyethoxy)

Ethanol (BDH Chemicals Ltd., Poole, England).

Reagents

Triton X-100 (TX) (an alkyl phenoxy polyethoxy)

Ethanol (BDH Chemicals Ltd., Poole, England)

Sample Preparation and procedure of Analysis

1. A two-fold dilution of the sample was made with 0.1% triton X-100 and was mixed thoroughly. 0.2ml aspirated into AAS for analysis at a wavelength of 193.7nm
2. The prepared samples were analysed in Buck 210/211 atomic absorption spectrophotometer (Buck Scientific, Inc. 58 Fort Point St, East Norwalk, Ct. 06855).
3. The burner was lit under flow conditions of air and acetylene. The acetylene flow was then reduced until the flame was blue.
4. The air flow was then adjusted to remove all traces of yellow from the flame.
5. The machine was then properly calibrated with the appropriate standard solutions before analyzing the test samples.
6. The assay results were displayed on the instrument reader's screen in μg/dL.
7. Analytical quality control was performed by analyzing an aliquot of pooled serum several times during the assay.

3.9 Determination of Bisphenol-A

Serum BPA was estimated by high performance liquid chromatography (ALLIANCE, e2695 Waters, USA).

Principle

This based on the separation of the solutes of a sample mixture by their differential distribution between stationary and mobile phases.

Reagents

Labelled bisphenol-A (50ng)

4-methylumbelliferone glucuronide (250 ng)

Ammonium acetate buffer (pH 6.5) (300µL)

β-glucuronidase (10µL) (Escherichia coli K12, Roche Biomedical).

Assay Procedure

Serum samples were fortified with 12.5 nanograms of isotopically labelled phthalate metabolites, 50 nanograms of labelled bisphenol-A, 250 nanograms of 4-methylumbelliferone glucuronide, 300 microlitres of ammonium acetate buffer (pH 6.5) and 10 microliters of β-glucuronidase (Escherichia coli K12, Roche Biomedical). The samples were mixed and incubated at 37°C overnight to allow for the deglucuronidation. Following enzymatic hydrolysis, a 20µL aliquot of the sample was added to 70µL of HPLC- grade water and 10ng of labelled 4-methylumbelliferone to determine deglucuronidation efficiency. The remaining sample was loaded on to Zymark rapid trace solution for automated solid phase extraction (SPE). The 60 milligram/3mL Oasis-HLB cartridges were conditioned with HPLC-grade methanol (2ml) and 0.1 M formic acid (2mL). The samples were diluted with 5 mL of 0.1 M formic acid and loaded on the SPE cartridge at a rate of 1.0ml/min. The cartridge was washed with water (1mL) and 10% methanol in water (2mL) at a flow rate of 1mL/min. The samples were eluted with 1.0mL of acetonitrile at a flow rate of 0.5 mL/min. The eluate was evaporated to dryness under a stream of dry nitrogen and the residue was re-suspended in 85% methanol in water (200 microliters) and

transferred to glass autosampler vials. Quality control of the analysis was maintained by analyzing a method blank (calf serum) and two spiked calf serum samples (20ng/mL). The detection limit (0.2ng/mL) was based upon a lower calibration standard (0.5ng/ml) which gave an instrument signal to noise response of 3:1

3.10 Determination of Polychlorinated Biphenyls

Serum polychlorinated biphenyl was determined by gas chromatography-electron capture detector (GC-ECD).

Principle

This is based on the separation of the solutes of a sample mixture by their differential distribution between stationary and mobile phases.

Reagents

Methanol

N-hexane-diethyl-ether

Concentrated sulphuric acid

Assay Procedure

The determination of serum PCBs consisted of three steps (1) extraction of PCBs from the serum by organic solvent (2) Clean up of PCBs from impurities on chromatographic columns (3) Quantitation by Gas chromatography with a suitable detector (electron capture detector).

Serum samples were mixed with methanol and a mixture of internal standards were added to correct for recovery and ensure quality control. The samples were then extracted three times with n-hexane-diethyl-ether (1:1 v/v). After evaporation of the solvents the fat content was determined gravimetrically. The fat was re-dissolved in n-hexane and treated with concentrated sulphuric acid. The PCBs were separated from the bulk of the chlorinated compounds by elution through a silica gel column (4.5g of 3% water-deactivated silica-gel). The first fraction, containing the PCBs was eluted with 30ml of n-hexane. The columns were of different polarity to ease identification of analytes which was based on retention times relative to internal

standards. Quantification was performed using multilevel calibration curves obtained by injection of standard solutions of at least three different concentrations. The limit of determination (LOD) was determined as three standard deviations (SD) above the value of the blank and varied between 1 and 7 pg/g serum (not lipid adjusted). Samples with concentrations of LODs three SD above the blank have a 99% probability of being non-zero. To increase this probability, the quantification limits (LOQ) were set at higher levels than the LODs. In this case the lowest standard concentration was used; 10pg/g serum. The reproducibility of the method was demonstrated by 21 replicate determinations using an in-house control serum sample included in the analytical batches during the course of the study.

3.11 Determination of the Expression of ER, PR and HER 2

Immunohistochemistry was performed on 79 breast tissue biopsy samples obtained from the breast cancer participants in this study. The tissue samples were collected in bottles containing 10% buffered formalin. This was followed by embedding of tissue in paraffin wax pending when analysis will be done.

Principle

Immunohistochemistry combines histological, immunological and biochemical techniques for the identification and localization of specific tissue components (localization of antigens in tissue section). This is by the use of labelled antibodies as specific reagents through antigen-antibody interactions that are visualized by a marker such as fluorescent dye, enzyme, radioactive element or colloidal gold. It is among the most sensitive and specific histochemical techniques.

Reagents

1. 70%, 90% and 100% (2 jars) ethanol solutions.
2. Epitope retrieval buffer (citrate buffer 6.0 or EDTA buffer 9.0).
3. Xylene (2 jars).
4. Wash Buffer (2 jars).
5. Mounting solution.
6. Hematoxylin stain.
7. Diaminobenzidine tetrahydrochloride (DAB) substrate/ Chromogen.

70

8. Antibody/ Antibody diluents.

9. Secondary antibody.

Materials

1. Humidified chamber.

2. Oven.

3. Water Bath.

4. Cover slip (22x32, 22x40, 22x22).

Method

(1) Sample Preparation (Deparaffination and Rehydration)

Paraffin specimens were cut into 4-µm sections using a microtome and mounted on positively charged slides. Slides containing the breast tissue section were incubated for 10 minutes at 70°C. This was followed by incubation for 5 minutes in xylene jar #1. The slides were incubated for 5 minutes in xylene jar #2. Slides were incubated in graded alcohols i.e. Slides were incubated in 100% ethanol jar #1 for 2 minutes. This was followed by incubation in 100% ethanol jar #2 for another 2 minutes. The slides were further incubated in 95% ethanol jar #1 for 2 minutes. This was followed by incubation in 95% ethanol jar #2 for another 2 minutes. The slides were then incubated in 70% ethanol for 2 minutes. The slides were thereafter transferred into wash buffer for 2 minutes. The above step was aimed at deparaffinising and rehydrating the tissue samples.

(2) Epitope Retrieval (Antigen Unmasking)

Slides were transferred into pre-heated retrieval solution at 95°C and were incubated for 20-30 minutes. The slides were immediately transferred into wash buffer for 2 minutes and were drained off.

(3) Blocking for Endogenous Enzymes

The tissue area on the slide was marked with a hydrophobic pen. Peroxidase block solution was applied drop-wise to cover the tissue. This was followed by incubation for 15 minutes at room temperature in a humidity chamber. The slides were thereafter transferred to and immersed in wash buffer jar for 5 minutes

71

(4) Immunoperoxidase Staining

Excess liquid was wiped off the slide while care was taken not to clean off the tissue from the slide. The slides were placed in the humidity chamber, diluted primary antibody was applied. This was followed by incubation for 1 hour. It was thereafter washed in TBSt (wash solution) 3x2 minutes. Excess liquid was wiped off and secondary antibody was applied. This was followed by incubation in humidity chamber for 30 minutes. It was washed in TBSt (wash solution) 3x2 minutes.

(5) Detection

3, 3 diaminobenzidine tetrahydrochloride (DAB) solution was prepared (1ml + a drop DAB). The DAB was applied to the tissue section and incubated for 7 minutes. The slides were thereafter washed with running tap water.

(6) Counterstaining

The slides were dipped in Gill's hematoxylin for 10 seconds.

(7) Dehydration

The slides were immersed in 70% ethanol for 1 minute and thereafter were immersed in 95% ethanol for 1 minute. The slides were immersed in 100% ethanol for 1 minute. The slides were immersed in 50% ethanol for 5 minutes.

(8) Clearing and Mounting

The slides were immersed in xylene for 5 minutes and thereafter in a mounting solution (DPX). The samples were covered immediately with a cover slip using mounting solution and air dried for approximately 30 minutes before being examined under a light microscope by a pathologist.

72

3.12 Statistical Analysis

Data obtained from the research participants were collated and analyzed using the statistical package for social scientists (SPSS 18.0) SPP, Inc., Richmond, CA.

For quantitative variables;

(a) Student t-test was used to test the significance of difference between mean values. Data were expressed in mean±SEM (standard error of mean).

(b) Multiple regression analysis was employed to determine interrelationships between variables.

For qualitative variables;

(a) Chi-square test was used for association of qualitative variables

A two sided probability value at $p < 0.05$ was considered statistically significant

CHAPTER FOUR

4.0 RESULTS

Table 4.1 shows the association of breast cancer stage, affected breast and hormone receptors in pre and postmenopausal women with breast cancer. There was significant difference in the hormone receptors (p<0.05).

Table 4.2 shows the association of age and demographic indices in women with and without breast cancer. There was an association in the occupation (p=0.006). No association was observed in age, marital status, educational status and ethnic group (p>0.05).

Table 4.3 shows the association of contraceptive use and history of breast cancer and tumour in women with and without breast cancer. No association was observed (p>0.05).

Table 4.4 shows the association of diet history in women with breast cancer and non breast cancer women. An association was observed in vegetable, fruit, red meat and diary product intake (p<0.05). There was no association in beans and beans product, refined carbohydrates and refined carbohydrate type intake (p>0.05).

Table 4.5 shows the reproductive history in women with breast cancer and non breast cancer women. Age at menarche was significantly higher in women with breast cancer when compared with non breast cancer women (p=0.033). Menstrual cycle was significantly lower in women with breast cancer when compared with non breast cancer women (p=0.003). Number of previous pregnancies was significantly higher in women with breast cancer (p=0.009). Number of induced abortion was significantly higher in women with breast cancer (p<0.001).

Table 4.1 Breast Cancer Stages, Sites of Affected Breast and Hormone Receptors in Premenopausal and Postmenopausal Women with Breast Cancer

Variable	Premeno-HCBCa (n=54)	Postmeno-HCBCa (n=31)	Total 85 (100%)	χ^2	p
Breast Cancer Stage				3.394	0.335
1	5(9.3%)	1(3.2%)			
2	3(5.6%)	5(16.1%)			
3	24(44.4%)	14(45.2%)			
4	22(40.7%)	11(35.5%)			
Breast Site	(n=54)	(n=31)		2.236	0.135
Right Breast	30 (55.6%)	12 (38.7%)			
Left Breast	24 (44.4%)	19 (61.3%)			
Receptors	n=52	n=27	n=79(100%)		
ER				22.050	<0.001 •
Positive	0	10(37.0%)			
Negative	52(100%)	17(63.0%)			
PR				17.143	<0.001 •
Positive	0	8(29.6%)			
Negative	52(100%)	19(70.4%)			
HER2				5.488	0.019 •
Positive	6(11.5%)	9(33.3%)			
Negative	46(88.5%)	18(66.7%)			

n=number of participants, χ =Chi-Squared test, Fishers=Fishers Exact ratio. p=Probability value. • significant at p<0.05. ER=Oestradiol Receptor. PR=Progesterone Receptor. HER2=Human epithelial receptor 2, Premeno-HCBCa=premenopausal women with histologically confirmed breast cancer, Postmeno-HCBCa= postmenopausal women with histologically confirmed breast cancer,

Table 4.2 Demographic Indices of Women with and without Breast Cancer.

Variable	HCBCa (n=85) %	AHWB (n=84)%	χ^2	p
Age	48.3±1.3	48.45±1.27	t=-0.07	0.941
Marital Status			7.795	0.050
Married	63(74.1%)	55(65.5%)		
Single	2(2.4%)	11(13.1%)		
Widow	19(22.4%)	18(21.4%)		
Divorced/separated	1(1.2%)	0		
Educational Status			4.400	0.221
None	16(18.8%)	19(22.6%)		
Primary	21(24.7%)	13(15.5%)		
Secondary	24(28.2%)	20(23.8%)		
Tertiary	24(28.2%)	32(38.1%)		
Occupation			12.432	0.006*
Trading	60(70.6%)	41(48.8%)		
Civil Servants	14(16.5%)	26(31.0%)		
Unemployed (House wife)	5(5.9%)	14(16.7%)		
Others (farmers, clergy)	6(7.1%)	3(3.6%)		
Ethnic Group			14.704	0.070
Igbo	14(16.5%)	2(2.4%)		
Ebira	2(2.4%)	0		
Hausa	1(1.2%)	2(2.4%)		
Isan	2(2.4%)	2(2.4%)		
Isoko	2(2.4%)	1(1.2%)		
Tiv	1(1.2%)	0		
Urhobo	2(2.4%)	1(1.2%)		
Yoruba	61(71.8%)	76(90.5%)		

n=number of participants, χ^2=Chi-Squared test, p=probability value, *= significant at p<0.05,

t=Student's t-test, HCBCa=Women with histologically confirmed breast cancer.

AHWB=Apparently healthy women without breast cancer

Table 4.3 Contraceptive use and History of Breast Cancer and Tumour in Women with and without Breast Cancer.

Variable	HCBCa(n=85) %	AHWB (n=84) %	χ^2	p
Contraceptive Use			1.201	0.273
Yes	31(36.5%)	24(28.6%)		
No	54(63.5%)	60(71.4%)		
Contraceptive Type			6.658	0.471
Pills	11(12.9%)	6(7.1%)		
Injectibles	5(5.9%)	3(3.6%)		
Barrier	11(12.9%)	12(14.3%)		
Pills and injectible	2(2.4%)	1(1.2%)		
Pills, iojectible and barrier	0	1(1.2%)		
Barrier and injectible	0	1(1.2%)		
Implant	2(2.4%)	0		
Nil	54(63.5%)	60(71.4%)		
Family History of Breast Cancer			0.137	0.711
Yes	4(4.7%)	3(3.6%)		
No	81(95.3%)	81(96.4%)		
Relative with B. Cancer			4.994	0.288
Mother	1(1.2%)	1(1.2%)		
Aunty	0	2(2.4%)		
Half Sister	1(1.2%)	0		
Sister	2(2.4%)	0		
Nil	81(95.3%)	81(96.4%)		
Personal History of Breast Tumour			2.983	0.084
Yes	3(3.5%)	0		
No	82(96.5%)	84(100%)		
Breast Tumour Mgt.			3.018	0.080
Surgery	3(3.5%)	0		
Nil	82(96.5%)	84(100%)		

n=number of participants. χ^2 =Chi-Squared test, p=probability value. *=significant at p<0.05. ^{t}= Student's t-test value LMC=Length of menstrual cycle. DMM=Duration of monthly menstruation. NPP=Number of previous pregnancies. NIA=Number of induced abortion. HCBCa=Women with histologically confirmed breast cancer. AHWB=Apparently healthy women without breast cancer

77

Table 4.4 Diet History in Women with and without Breast Cancer.

Variable	HCBCa (n=85)	AHWB (n=84)	χ^2	p
Beans/Beans Product Intake			0.491	0.782
Daily	10(11.8%)	13(15.5%)		
Weekly	20(23.5%)	18(21.4%)		
Occasionally	53(62.4%)	53(63.1%)		
Nil	2(2.4%)	0		
Vegetable Intake			6.933	0.031*
Daily	23(27.1%)	23(27.4%)		
Weekly	43(50.6%)	28(33.3%)		
Occasionally	19(22.4%)	33(39.3%)		
Fruit Intake			6.824	0.033*
Daily	17(20.0%)	20(23.8%)		
Weekly	32(37.6%)	45(53.6%)		
Occasionally	36(42.4%)	19(22.6%)		
Red Meat Intake			56.869	<0.001*
Daily	70(82.4%)	22(26.2%)		
Weekly	4(4.7%)	26(31.0%)		
Occasionally	10(11.8%)	36(42.9%)		
Nil	1(1.2%)	0		
Dairy Product Intake			11.438	0.010*
Daily	2(2.4%)	2(2.4%)		
Weekly	7(8.2%)	23(27.4%)		
Occasionally	75(88.2%)	59(70.3%)		
Nil	1(1.2%)	0		
Refined Carbohydrate Intake			0.080	0.778
Yes	58(68.2%)	59(70.2%)		
No	27(31.8%)	25(29.8%)		
Refined Carbohydrate Type			4.272	0.370
Any	54(63.5%)	59(70.2%)		
Indomie	2(2.4%)	0		
Indomie/spaghetti	1(1.25)	0		
Spaghetti	1(1.2%)	0		
Nil	27(31.8%)	25(29.8%)		

n=number of participants. χ^2 =Chi-Squared test. p=Probability value. * significant at p<0.05.

HCBCa =Women with histologically confirmed breast cancer. AHWB=Apparently healthy women without breast cancer

Table 4.5 Reproductive History in women with Breast Cancer and without Breast Cancer

Variable	HCBCa (n=85)%	AHWB (n=84)%	t	p
Menstrual History				
Age at Menarche (Years)	15.4±0.2	14.7±0.2	2.154	0.033*
LMC (days)	27.8±0.1	28.3±0.1	-2.988	0.003*
DMM(days)	4.5±0.1	4.6±0.0	-1.026	0.306
NPP	5.1±0.3	4.0±0.3	2.632	0.009*
Number of Live births	3.6±0.2	3.1±0.2	1.664	0.098
NIA	0.9±0.1	0.3±0.1	3.816	<0.001*
Number of Miscarriage(s)	0.1±0.1	0.3±0.1	-1.635	0.104

Student's t-test value LMC=Length of menstrual cycle, DMM=Duration of monthly menstruation. NPP=Number of previous pregnancies, NIA=Number of induced abortion. HCBCa=Women with histologically confirmed breast cancer. AHWB=Apparently healthy women without breast cancer

Table 4.6 shows the mean blood pressure and anthropometric indices (WC, HC, weight, height, BMI, WHR, WHtR) and blood pressure of women with breast cancer compared with controls. WC, HC, weight, height, WHR, WHtR and SBP were significantly higher in women with breast cancer compared with controls (p<0.05). No significant differences were observed in the mean body mass index and diastolic blood pressure (p>0.05).

Table 4.7 shows the serum levels of hormones (progesterone, oestradiol, LH, FSH, FT_4, FT_3 and TSH) and endocrine disruptors (Pb, Cd, As, BPA and PCBs). FT_4, Pb, Cd, As, BPA and PCBs were significantly higher in women with breast cancer compared with controls (p<0.05). No significant differences were observed in other indices (p>0.05).

Table 4.6 Blood Pressure and Anthropometric Indices in Women with and without Breast Cancer.

Variable	HCBCa (n=85)	AHWB (n=84)	t	p
Blood Pressure				
Systolic BP(mmHg)	122.7±1.1	119.4±1.0	2.215	0.028*
Diastolic BP (mmHg)	81.7±0.9	80.7±0.8	0.811	0.418
Anthropometric Indices				
WC(cm)	89.9±1.1	82.6±1.2	4.535	<0.001*
HC (cm)	101.8±1.1	98.5±1.0	2.212	0.028*
Body Weight (kg)	69.2±1.4	62.1±1.1	3.975	<0.001*
Height (m)	1.63±0.0	1.58±0.0	4.882	<0.001*
Body Mass Index	26.1±0.5	24.9±0.4	1.826	0.070
WHR	0.9±0.0	0.8±0.0	4.856	<0.001*
WHtR	55.3±0.7	52.4±0.7	2.809	0.006*

Values are in mean±SEM (Standard error of mean), n=number of participants, t=Student's t-test, p=Probability value, *=significant at p<0.05, WC=Waist circumference, HC=Hip circumference, WHR=Waist hip ratio, WHtR=Waist height ratio, BP=Blood pressure. HCBCa=Women with histologically confirmed breast cancer; AHWB=Apparently healthy women without breast cancer.

Table 4.7 Serum Hormones and Endocrine Disruptors in Women with and without Breast Cancer.

Variable	HCBCa (n=85)	AHWB (n=84)	t	p
Hormones				
Progesterone (nmol/L)	8.6±1.8	5.9±1.4	1.167	0.245
Oestradiol (pmol/L)	344.8±31.9	307.8±34.7	0.786	0.433
LH (IU/L)	14.5±1.5	14.6±1.4	-0.038	0.970
FSH (IU/L)	26.8±3.7	32.9±4.2	-1.101	0.273
FT_3(pmol/L)	3.4±0.3	3.4±0.1	-0.045	0.964
FT_4 (pmol/L)	17.8±0.4	14.7±0.3	6.373	<0.001 *
TSH (mIU/L)	1.7±0.1	1.4±0.1	1.880	0.062
Endocrine Disruptors				
Lead (µg/dL)	5.5±0.2	1.8±0.0	24.167	<0.001 *
Cadmium (µg/dL)	0.04±0.0	0.01±0.0	24.602	<0.001 *
Arsenic (µg/dL)	0.3±0.0	0.04±0.0	23.209	<0.001 *
BPA(mg/dL)	0.8±0.7	0.4±0.0	6.81	<0.001 *
PCBs(µg/dL)	0.8±0.5	0.3±0.0	10.12	<0.001 *

Values are mean±SEM (Standard error of mean), n=number of participants, t=Student's t-test, p=Probability value, *=significant at. p<0.05, LH=Luteinizing hormone, FSH=Follicle-stimulating hormone, FT_3=Free triiodothyronine, FT_4=Thyroxine, TSH=Thyroid-stimulating hormone, Pb=Lead, Cd=Cadmium, As=Arsenic, BPA=Bisphenol-A, PCBs=Polychlorinated biphenyls. nmol/L=nanomole per liter, IU/L=mili international units per mililitre, pmol/L=picomol per litre, mIU/L=milliinternational unit per litre, µg/dL=micrograms per decilitre. HCBCa=Women with histologically confirmed breast cancer, AHWB=Apparently healthy women without breast cancer.

Table 4.8 shows the mean age, age at menarche, age at menopause (postmenopausal women only), blood pressure and anthropometric indices (WC, HC, weight, height, BMI, WHR, WHtR) of pre and postmenopausal women with breast cancer compared with their respective controls.

Premenopausal women

Age at menarche, WC, HC, weight, height, WHR, WHtR and SBP were significantly higher in premenopausal women with breast cancer (p<0.05). No significant differences were observed in the mean BMI and diastolic blood pressure in both premenopausal cases and control (p>0.05)

Postmenopausal women

Weight, height were significantly higher in postmenopausal women with breast cancer compared with controls (p<0.05). No significant differences were observed in the mean age, age at menarche, age at menopause other anthropometric indices and blood pressure measurements (p>0.05)

Table 4.9 shows the serum levels of sex hormones (progesterone and oestradiol, LH, FSH, FT₄, FT₃ and TSH) and endocrine disruptors (Pb, Cd, As, BPA and PCBs) in pre and postmenopausal women with breast cancer and their respective controls.

Premenopausal women:

LH, FSH, FT₄, Pb, Cd, As, BPA and PCBs were significantly higher in premenopausal women with breast cancer when compared with controls (p<0.05). No significant differences were observed in levels of progesterone, oestradiol, FT₃ and TSH (p>0.05)

Postmenopausal women:

Progestreone, oestradiol, FT₄, Pb, Cd, As, BPA and PCBs were significantly higher while FSH was significantly lower in postmenopausal women with breast cancer than controls (p<0.05). No significant difference was observed in LH, FT₃ and TSH (p>0.05)

Table 4.8 Age, Reproductive History, Blood Pressure and Anthropometric Indices in Women with and Without Breast Cancer in Pre and Postmenopausal Groups.

Variable	Premeno- HCBCa (n=54)	Premeno- AHWB (n=53)	t	p	Postmeno -HCBCa (n=31)	Postmeno- AHWB (n=31)	t	p
Age (years)	40.9±0.7	40.7±0.6	0.187	0.852	61.2±1.5	61.6±1.5	-0.199	0.843
AM.1 (years)	15.3±0.3	14.5±0.3	2.081	0.040*	15.6±0.39	15.1±0.38	0.840	0.404
AM.2 (years)	n/a	n/a	-	-	51±0.7	50±0.9	0.255	0.799
BP								
SBP(mmHg)	123.0±1.4	119.0±1.2	2.062	0.042*	122.3±1.8	120.0±1.6	0.925	0.360
DBP (mmHg)	82.4±1.1	80.9±1.0	0.967	0.336	80.3±1.3	80.3±1.2	0.000	1.000
AI								
WC (cm)	88.5±1.4	78.3±1.3	5.321	<0.001*	92.2±1.7	89.8±1.5	0.968	0.337
HC (cm)	100.5±1.5	95.9±1.0	2.512	0.014*	103.9±1.7	102.7±1.7	0.500	0.619
Wt (Kg)	68.0±1.9	60.1±1.3	3.435	0.001*	71.4±2.2	65.6±1.7	2.103	0.010*
Ht (m)	1.63±0.0	1.57±0.0	4.345	<0.001*	1.63±0.0	1.59±0.0	2.340	0.023*
BMI(Kg/m^2)	25.7±0.7	24.5±0.5	1.401	0.164	26.8±0.7	25.7±0.7	1.048	0.217
WHR	0.9±0.0	0.8±0.0	6.073	<0.001*	0.89±0.0	0.88±0.0	0.716	0.480
WHtR	54.6±1.0	49.9±0.9	3.516	0.001*	56.6±1.2	56.5±0.9	0.093	0.930

Values are mean±SEM (Standard error of mean), n=number of participants, t=Student's t-test, n/a=not applicable, p=Probability value, *=significant at p<0.05, Premeno. HCBCa=Premenopausal women with histologically confirmed breast cancer, Postmeno-HCBCa=Postmenopausal women with histologically confirmed breast cancer. SBP=Systolic blood pressure, DBP=Diastolic blood pressure. AM.1=Age at menarche, AM.2=Age at menopause. WC=Waist circumference, HC=Hip circumference, Wt=Body weight, Ht=Height, BMI=Body mass index, WHR=Waist hip ratio, WHtR=Waist height ratio, Systolic=Systolic blood pressure, Diastolic=Diastolic blood pressure. AI=Anthropometric Indices.

Table 4.8 Age, Reproductive History, Blood Pressure and Anthropometric Indices in Women with and Without Breast Cancer in Pre and Postmenopausal Groups.

Variable	Premeno-HCBCa (n=54)	Premeno-AHWB (n=53)	t	p	Postmeno-HCBCa (n=31)	Postmeno-AHWB (n=31)	t	p
Age (years)	40.9±0.7	40.7±0.6	0.187	0.852	61.2±1.5	61.6±1.5	-0.199	0.843
AM.1 (years)	15.3±0.3	14.5±0.3	2.081	0.040*	15.6±0.39	15.1±0.38	0.840	0.404
AM.2 (years)	n/a	n/a	-	-	51±0.7	50±0.9	0.255	0.799
BP								
SBP (mmHg)	123.0±1.4	119.0±1.2	2.062	0.042*	122.3±1.8	120.0±1.6	0.925	0.360
DBP (mmHg)	82.4±1.1	80.9±1.0	0.967	0.336	80.3±1.3	80.3±1.2	0.000	1.000
AI								
WC (cm)	88.5±1.4	78.3±1.3	5.321	<0.001*	92.2±1.7	89.8±1.5	0.968	0.337
HC (cm)	100.5±1.5	95.9±1.0	2.512	0.014*	103.9±1.7	102.7±1.7	0.500	0.619
Wt (Kg)	68.0±1.9	60.1±1.3	3.435	0.001*	71.4±2.2	65.6±1.7	2.103	0.010*
Ht (m)	1.63±0.0	1.57±0.0	4.345	<0.001*	1.63±0.0	1.59±0.0	2.340	0.023*
BMI (Kg/m²)	25.7±0.7	24.5±0.5	1.401	0.164	26.8±0.7	25.7±0.7	1.048	0.217
WHR	0.9±0.0	0.8±0.0	6.073	<0.001*	0.89±0.0	0.88±0.0	0.716	0.480
WHtR	54.6±1.0	49.9±0.9	3.516	0.001*	56.6±1.2	56.5±0.9	0.093	0.930

Values are mean±SEM (Standard error of mean), n=number of participants, t=Student's t-test, n/a=not applicable, p=Probability value, *=significant at p<0.05, Premeno-HCBCa=Premenopausal women with histologically confirmed breast cancer, Postmeno-HCBCa=Postmenopausal women with histologically confirmed breast cancer. SBP=Systolic blood pressure, DBP=Diastolic blood pressure. AM.1=Age at menarche, AM.2=Age at menopause, WC=Waist circumference, HC=Hip circumference, Wt=Body weight, Ht=Height, BMI=Body mass index, WHR=Waist hip ratio, WHtR=Waist height ratio, Systolic=Systolic blood pressure, Diastolic=Diastolic blood pressure, AI=Anthropometric Indices.

Table 4.9 Hormones and Endocrine Disruptors in Women with and without Breast Cancer in Pre and Postmenopausal Groups

Variable	Premeno-HCBCa (n=54)	Premeno-AHWB (n=53)	t	p	Postmeno-HCBCa (n=31)	Postmeno-AHWB (n=31)	t	p
Hormones								
Progest (nmol/L)	12.3±2.6	8.8±2.2	1.023	0.309	2.1±0.4	1.0±0.1	2.919	0.005
Oestradiol (pmol/L)	452.8±43.3	430.8±46.5	0.347	0.729	156.5±12.4	90.4±3.6	5.036	<0.00
LH (IU/L)	7.7±0.7	5.8±0.5	2.298	0.024*	26.4±2.9	29.7±1.1	-1.061	0.290
FSH (IU/L)	7.2±0.6	5.6±0.4	2.183	0.031*	60.9±6.4	79.6±4.1	-2.455	0.020
FT$_3$(pmol/L)	3.6±0.4	3.5±0.1	0.249	0.804	3.1±0.1	3.3±0.1	-1.372	0.175
FT$_4$ (pmol/L)	17.8±0.6	14.9±0.3	4.507	<0.001*	17.7±0.6	14.3±0.4	4.785	<0.00
TSH (mIU/L)	1.8±0.17	1.5±0.1	1.360	0.178	1.6±0.2	1.3±0.1	1.355	0.181
Endocrine Disruptors								
Lead (µg/dL)	5.4±0.2	1.8±0.1	18.349	<0.001*	5.8±0.2	1.8±0.1	15.975	<0.00
Cadmium (µg/dL)	0.04±0.0	0.01±0.0	18.788	<0.001*	0.05±0.0	0.01±0.0	15.993	<0.00
Arsenic (µg/dL)	0.3±0.0	0.04±0.0	17.413	<0.001*	0.3±0.0	0.04±0.0	15.219	<0.00
PCBs (µg/dL)	0.8±0.14	0.4±0.04	4.515	<0.001*	0.8±0.14	0.3±0.04	5.178	<0.00
BPA (mg/dL)	0.8±0.14	0.3±0.04	6.910	<0.001*	0.8±0.14	0.3±0.04	7.338	<0.00

Values are mean±SEM (Standard error of mean), n=number of participants, t=Student's t-test, p=Probability value, *=significant at p<0.05. Premeno=Premenopausal, Postmeno=Postmenopausal, Progest=Progesterone. LH=Luteinizing hormone, FSH=Follicle stimulating hormone, FT$_3$=Free triiodothyronine, FT$_4$=Thyroxine, TSH=Thyroid-stimulating hormone, Pb=Lead, Cd=Cadmium, As=Arsenic, BPA=bisphenol-A, PCBs=polychlorinated biphenyls. nmol/L=nanomole per liter, IU/L=mili international units per mililitre, Pmol/L=picomol per litre, mIU/L=milliinternational unit per litre, µg/dl=micrograms per decilitre. + =n for PCBs and BPA in premenopausal cases and controls are 22 and 23 respectively, while n for PCBs and BPA in postmenopausal cases and controls are 18 and 17. Premeno-HCBCo=Premenopausal women with histologically confirmed breast cancer, Postmeno-HCBCo=Postmenopausal women with histologically confirmed breast cancer. Premeno-AHWB=controls, Postmeno-AHWB=Postmenopausal controls

Table 4.10 shows the mean age, anthropometric indices (WC, HC, weight, height, BMI, WHR, WHtR) and blood pressure of pre and postmenopausal women with breast cancer. Age was significantly lower in premenopausal women with breast cancer than postmenopausal women with breast cancer (p<0.05). No significant differences were observed in the mean anthropometric indices and blood pressure measurements (p>0.05).

Table 4.11 shows the serum levels of sex hormones (progesterone, oestradiol, LH, FSH, FT₄, FT₃, TSH) and endocrine disruptors (Pb, Cd, As, BPA and PCBs) in pre and postmenopausal women with breast cancer. Progetsreone, oestardiol were significantly higher in premenopausal women with breast cancer than postmenopausal women with breast cancer (p<0.05). LH and FSH were significantly lower in premenopausal women with breast cancer than postmenopausal women with breast cancer (p<0.05). No significant differences were observed in the levels of FT₃, FT₄ TSH, Pb, Cd, As, BPA and PCBs (p>0.05).

Table 4.10 Age, Blood Pressure and Anthropometric Indices in Pre and Postmenopausal Women with Breast Cancer

Variable	Premeno-HCBCa (n=54)	Postmeno-HCBCa (n=31)	t	p
Age (years)	40.9±0.7	61.2±1.5	-14.223	<0.001 •
BP				
Systolic BP (mmHg)	123.0±1.4	122.3±1.8	0.299	0.765
Diastolic BP (mmHg)	82.4±1.1	80.32±1.3	1.182	0.241
Anthropometric Indices				
WC (cm)	88.5±1.4	92.2±1.7	-1.581	0.118
HC (cm)	100.5±1.5	103.9±1.7	-1.467	0.146
Wt (Kg)	70.0±1.9	71.4±2.2	-1.147	0.255
Ht (m)	1.62±0.0	1.63±0.0	-0.275	0.784
BMI (Kg/m^2)	25.7±0.7	26.8±0.7	-1.081	0.283
WHR	0.88±0.0	0.89±0.0	-0.510	0.611
WHtR	54.6±1.0	56.6±1.2	-1.348	0.181

Values are Mean±SEM (Standard error of mean), n=Number of subjects, t=Student's t-test, P=Probability value, •=significant at p<0.05, WC=Waist circumference, HC=Hip circumference, Wt=Body weight, Ht=Height, BMI=Body mass index, WHR=Waist hip ratio, WHtR=Waist height ratio, Systolic BP=Systolic blood pressure, Diastolic BP=Diastolic blood pressure, Premeno-HCBCa=Premenopausal women with histologically confirmed breast cancer, Postmeno-HCBCa=Postmenopausal women with histologically confirmed breast cancer,

Table 4.11 Scrum Hormones and Endocrine Disruptors in Pre and Postmenopausal Women with Breast Cancer

Variable	Premeno-HCBCa (n=54)	Postmeno-HCBCa (n=31)	t	p
Hormones				
Prog (nmol/L)	12.3±2.6	2.1±0.4	2.907	0.005*
E_2 (pmol/L)	452.8±43.3	156.5±12.4	5.100	<0.001*
LH (IU/L)	7.7±0.7	26.4±2.9	-7.711	<0.001*
FSH (IU/L)	7.2±0.6	60.9±6.4	-11.034	<0.001*
FT_3(Pmol/L)	3.6±0.4	3.1±0.1	0.868	0.388
FT_4 (pmol/L)	17.8±0.7	17.7±0.6	0.211	0.833
TSH (mIU/L)	1.8±0.2	1.6±0.2	0.492	0.624
Endocrine Disruptors				
Lead (µg/dL)	5.4±0.2	5.8±0.2	-1.293	0.200
Cadmium (µg/dL)	0.04±0.0	0.05±0.0	-1.049	0.297
Arsenic (µg/dL)	0.3±0.0	0.3±0.0	-0.294	0.769
PCB (µg/dL)	0.79±0.1ⱡ	0.8±0.1ⱡ	-0.206	0.838
BPA (mg/dL)	0.76±0.1ⱡ	0.8±0.1ⱡ	-0.529	0.600

Values are Mean±SEM (Standard error of mean), n=number of subjects, t=Student's t-test, p=Probability, *=significant at p<0.05, Prog=Progesterone, E_2=Oestradiol, LH=Luteinizing hormone, FSH=Follicle stimulating hormone, FT_3=Free triiodothyronine, FT_4=Thyroxine, TSH=Thyroid stimulating hormone, Pb=Lead, Cd=Cadmium, As=Arsenic. nmol/L=nanomole per liter, IU/L=mili international units per mililitre, pmol/L=picomol per litre, mIU/L=milliinternational unit per litre, µg/dL=micrograms per decilitre. ⱡ=n for PCBs and BPA in Premenopausal and postmenopausnl women with breast cancer are 22 and 18 respectively. Premeno-HCBCa=Premenopausal women with histologically confirmed breast cancer, Postmeno-HCBCa=Postmenopausal women with histologically confirmed breast cancer.

Table 4.11 Serum Hormones and Endocrine Disruptors in Pre and Postmenopausal Women with Breast Cancer

Variable	Premeno-HCBCa (n=54)	Postmeno-HCBCa (n=31)	t	p
Hormones				
Prog (nmol/L)	12.3±2.6	2.1±0.4	2.907	0.005*
E_2 (pmol/L)	452.8±43.3	156.5±12.4	5.100	<0.001*
LH (IU/L)	7.7±0.7	26.4±2.9	-7.711	<0.001*
FSH (IU/L)	7.2±0.6	60.9±6.4	-11.034	<0.001*
FT_3(pmol/L)	3.6±0.4	3.1±0.1	0.868	0.388
FT_4 (pmol/L)	17.8±0.7	17.7±0.6	0.211	0.833
TSH (mIU/L)	1.8±0.2	1.6±0.2	0.492	0.624
Endocrine Disruptors				
Lead (µg/dL)	5.4±0.2	5.8±0.2	-1.293	0.200
Cadmium (µg/dL)	0.04±0.0	0.05±0.0	-1.049	0.297
Arsenic (µg/dL)	0.3±0.0	0.3±0.0	-0.294	0.769
PCB (µg/dL)	0.79±0.1†	0.8±0.1†	-0.206	0.838
BPA (mg/dL)	0.76±0.1†	0.8±0.1 †	-0.529	0.600

Values are Mean±SEM (Standard error of mean), n=number of subjects, t=Student's t-test, P=Probability, *=significant at p<0.05, Prog=Progesterone, E_2=Oestradiol, LH=Luteinizing hormone, FSH=Follicle stimulating hormone, FT_3=Free triiodothyronine, FT_4=Thyroxine, TSH=Thyroid stimulating hormone, Pb=Lead, Cd=Cadmium, As=Arsenic, nmol/L=nanomole per liter, IU/L=mili international units per mililitre, pmol/L=picomol per litre, mIU/L=miliinternational unit per litre, µg/dL=micrograms per deci litre, †=n for PCBs and BPA in premenopausal and postmenopausal women with breast cancer are 22 and 18 respectively, Premeno-HCBCa=Premenopausal women with histologically confirmed breast cancer, Postmeno-HCBCa=Postmenopausal women with histologically confirmed breast cancer.

Table 4.12 shows the mean age, anthropometric indices (WC, HC, weight, height, BMI, WHR and WHtR) and blood pressure of pre and postmenopausal women without breast cancer. Age, waist circumference, hip circumference, weight, waist-hip ratio and waist-height ratio were significantly lower in premenopausal women without breast cancer than postmenopausal women without breast cancer (p<0.05). No significant differences were observed in height, weight, systolic and diastolic blood pressure (p>0.05)

Table 4.13 shows the serum levels of sex hormones (progesterone, oestradiol, LH, FSH, FT₃, FT₄ and TSH) and endocrine disruptors (Pb, Cd, As, BPA and PCBs) in pre and postmenopausal women without breast cancer. Progesterone, oestradiol were significantly higher in premenopausal women than postmenopausal women (p<0.05). Luteinizing hormone and FSH were significantly lower in premenopausal women compared with postmenopausal women. No significant differences were observed in the levels of the endocrine disruptors (p>0.05).

Table 4.12 Age, Blood Pressure and Anthropometric indices in Pre and Postmenopausal Women without Breast Cancer.

Variable	Premeno-AHWB (n=53)	Postmeno-AHWB (n=31)	t	p
Age (years)	40.7±0.6	61.7±1.5	-14.846	<0.001*
Blood Pressure				
Systolic BP (mmHg)	119.0±1.2	120.0±1.6	-0.475	0.636
Diastolic BP (mmHg)	80.9±1.0	80.3±1.2	0.386	0.701
Anthropometric Indices				
WC (cm)	78.3±1.3	89.9±1.5	-5.741	<0.001*
HC (cm)	95.9±1.0	102.7±1.7	-3.619	0.001*
Wt (Kg)	60.1±1.3	65.6±1.7	-2.566	0.012*
Ht (m)	1.57±0.0	1.59±0.0	-1.777	0.079
BMI (Kg/m^2)	24.5±0.5	25.9±0.7	-1.612	0.111
WHR	0.8±0.0	0.9±0.0	-5.154	<0.001*
WHtR	49.9±0.9	56.5±0.9	-4.840	<0.001*

Values are Mean±SEM (Standard error of mean), n=Number of subjects, t=Student's t-test, p=Probability value, *=significant at p<0.05. WC=Waist circumference, HC=Hip circumference, Wt=Body weight, Ht=Height, BMI=Body mass index, WHR=Waist hip ratio, WH$_t$R=Waist height ratio. Systolic=Systolic blood pressure, Diastolic=Diastolic blood pressure, Premeno-AHWB=apparently healthy premenopausal women, Postmeno-AHWB=apparently healthy postmenopausal women.

Table 4.13 Hormones and Endocrine Disruptors in Pre and Postmenopausal women without Breast Cancer.

Variable	Premeno-AHWB (n=53)	Postmeno-AHWB (n=31)	t	P
Hormones				
Prog (nmol/L)	8.8±2.2	1.0±0.1	2.756	0.007*
E$_2$ (pmol/L)	430.8±46.5	90.4±3.6	5.491	<0.001*
LH (IU/L)	5.8±0.5	29.8±1.1	-22.862	<0.001*
FSH (IU/L)	5.6±0.4	79.6±4.1	-23.147	<0.001*
FT$_3$(pmol/L)	3.5±0.1	3.3±0.1	1.218	0.227
FT$_4$ (pmol/L)	14.9±0.3	14.3±0.4	1.088	0.280
TSH (mIU/L)	1.5±0.1	1.3±0.1	0.833	0.407
Endocrine Disruptors				
Lead (µg/dL)	1.8±0.1	1.8±0.1	0.042	0.967
Cadmium (µg/dL)	0.01±0.0	0.01±0.0	-0.230	0.818
Arsenic (µg/dL)	0.39±0.0	0.04±0.0	-1.579	0.118
PCBs(µg/dL)	0.4±0.0†	0.3±0.0†	0.899	0.374
BPA (mg/dL)	0.3±0.0†	0.3±0.0†	1.397	0.171

Values are Mean±SEM (Standard error of mean), n=Number of subjects, t=Student's t-test, P=Probability value, *=significant at p<0.05, Prog=Progesterone, E$_2$=Oestradiol, LH=Luteinizing hormone, FSH=Follicle stimulating hormone, FT$_3$=Free triiodothyronine, FT$_4$=Thyroxine, TSH=Thyroid stimulating hormone,nmol/L=nanomole per liter, IU/L=mili international units per mililitre, pmol/L=picomol per litre, mIU/L=milliinternational unit per litre, µg/dL=micrograms per decilitre. †=n for PCBs and BPA in premenopausal and postmenopausal women without breast cancer are 23 and 17 respectively. Premeno-AHWB=apparently healthy premenopausal women. Postmeno-AHWB=apparently healthy postmenopausal women.

Figure 4.1: Photomicrograph of ER Positive (X400). Marked area indientes stained cells

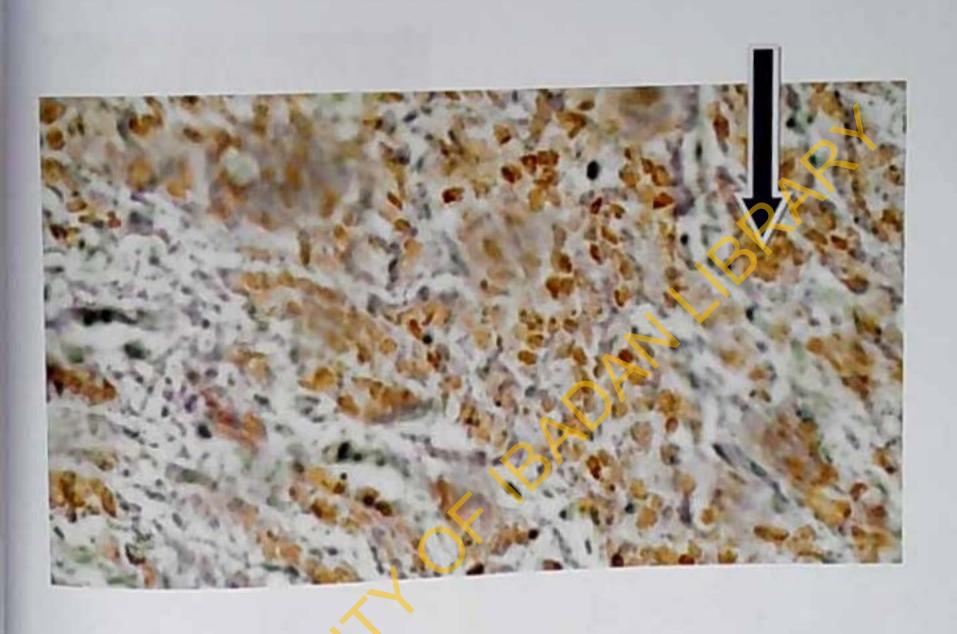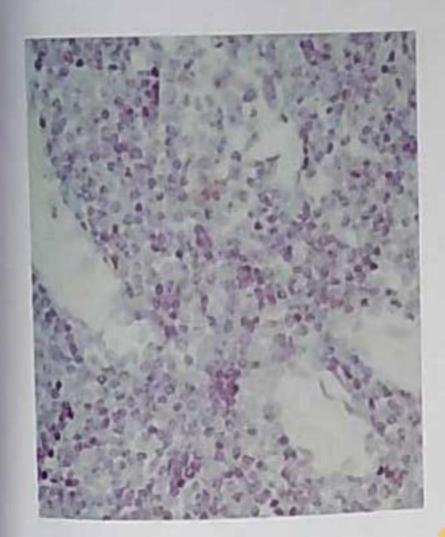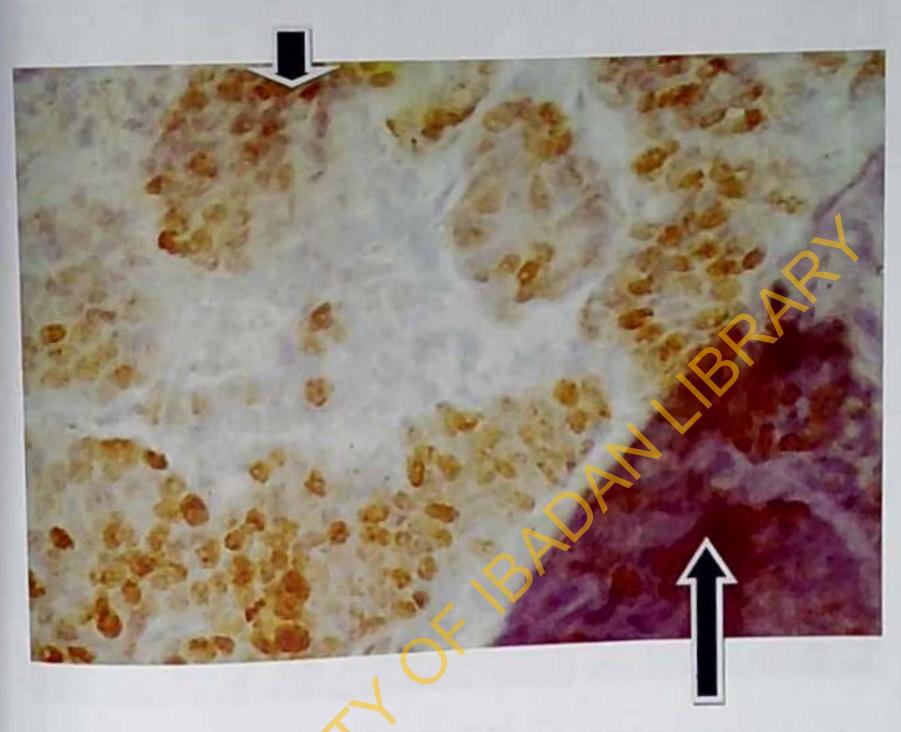Figure 4.2: Photomicrograph of ER Negative (X400)

Figure 4.3: Photomicrograph of PR Positive (X400). Marked area represents stained cells
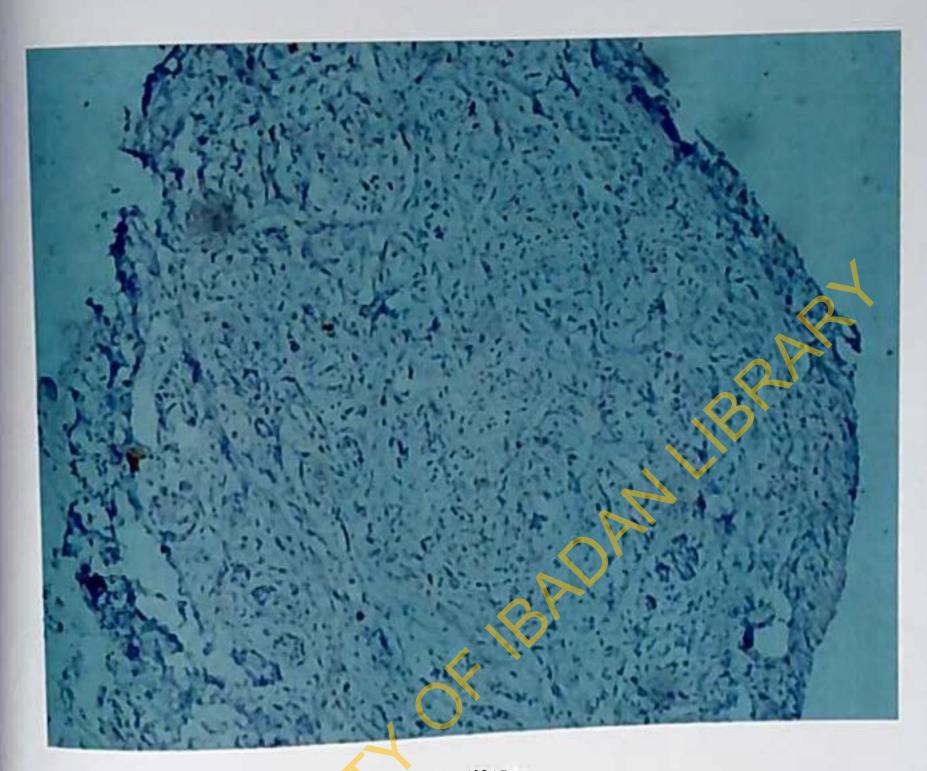
Figure 4.4: Photomicrograph of PR Negative (X400)
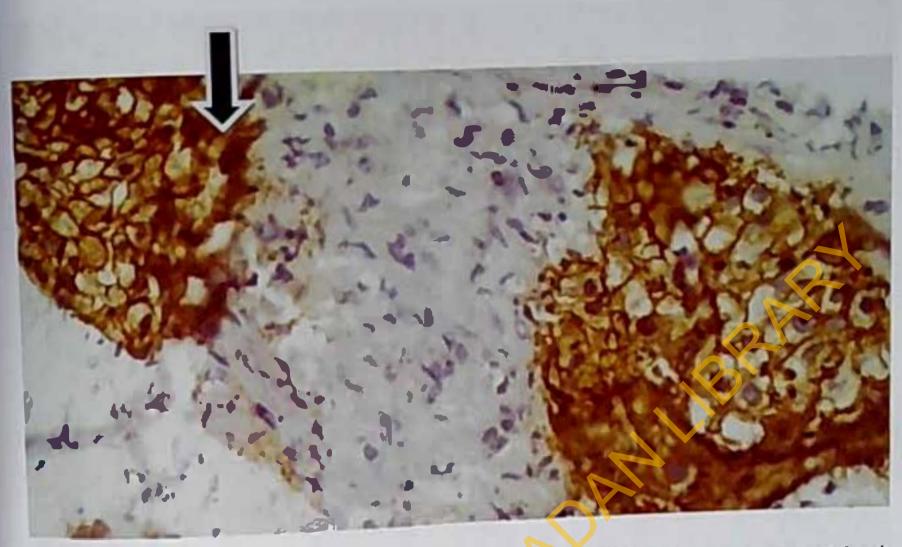
Figure 4.5: Photomicrograph of HER 2 Positive (X400). Marked area represents stained cells
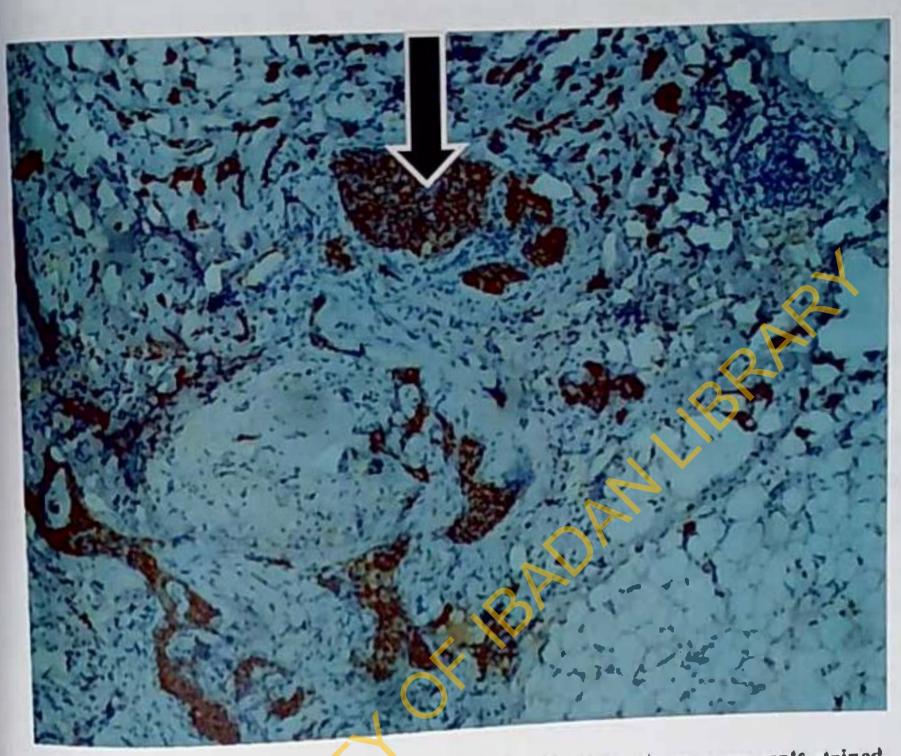
Figure 4.6: Photomicrograph of HER 2 Negative (X400). Marked area represents stained cells

Table 4.14 shows the expression pattern of oestrogen receptor (ER), progesterone receptor (PR), HER 2 and co-expression pattern of oestrogen receptor and progesterone receptors (ER/PR) of breast tumours. Participants with oestrogen receptor positive breast cancer were ten (12.7%) while sixty-nine participants (87.3%) had oestrogen receptor negative breast cancer. Eight (10.1%) had progesterone positive breast cancer, while seventy-one (89.9%) had progesterone receptor negative breast cancer. Fifteen participants (19.0%) had HER 2 positive breast cancer, while sixty-four (81.0%) had HER 2 negative breast cancer.

Table 4.15 shows the frequency and percentages of oestrogen, progesterone and HER 2 receptors in participants with breast cancer. Fifty-five (69.62%) had triple negative breast cancer i.e. negative expressions of the markers; ER, PR and HER 2. Two individuals (2.53%) were positive for the three receptors (triple positive breast cancer). Two (2.53%) were positive for oestrogen and progesterone receptors but negative for HER 2. Five (6.33%) were positive for oestrogen receptor but negative for progesterone receptor and HER 2. An individual (1.27%) was positive for oestrogen receptor and HER 2 but negative for progesterone receptor. Ten (12.66%) had negative oestrogen and progesterone receptors but positive HER 2. Two (2.53%) had negative oestrogen receptor and HER 2 but positive progesterone receptor. Two (2.53%) had negative oestrogen receptor but positive progesterone receptor and HER 2.

Table 4.14 Distribution of Hormone Receptor Positivity and Negativity in Women with Breast Cancer

Marker	Frequency(n)	Percentage (%)
Oestrogen Receptor		
ER+	10	12.7
ER-	69	87.3
Progesterone Receptor		
PR+	8	10.1
PR-	71	89.9
HER 2		
HER 2+	15	19.0
HER 2-	64	81.0

ER=Oestrogen receptor, PR= Progesterone receptor, HER 2=Human epithelial receptor 2

Table 4.15 Different Expression Patterns according to the Positivity and Negativity of ER, PR and HER2 in women with Breast Cancer

Number of Cases, n=79 (%)	ER	PR	HER 2
2 (2.53%)	+	+	+
2 (2.53%)	+	+	-
5 (6.33%)	+	-	-
1 (1.27%)	+	-	+
55 (69.62%)	-	-	-
10 (12.66%)	-	-	+
2 (2.53%)	-	+	-
2 (2.53%)	-	+	+
Premenopausal, n=52 (%)			
46 (88.5%)	-	-	+
6 (11.5%)	-	-	+
0 (0%)	+	+	+
Postmenopausal, n=27 (%)			
2 (7.4%)	+	+	+
2 (7.4%)	+	+	-
4 (14.8%)	+		+
1 (3.7%)	+	-	-
9 (33.3%)	-		+
4 (14.8%)	-	+	-
2 (7.4%)	-	+	+
3 (11.1%)	-		

ER=Oestrogen receptor, PR= Progesterone receptor, HER 2=Human epithelial receptor 2

Table 4.16 Multiple regression of endocrine disruptors with hormones in women with breast cancer. Oestradiol significantly predicted Pb (β=0.374, p=0.027), progesterone significantly predicted Cd (β=0.348, p=0.039) and FT_3 significantly predicted BPA (β=0.404, p=0.036). However, FT_4 inversely predicted As (β=-0.337, p=0.002).

Table 4.17 shows the multiple of endocrine disruptors and hormones with anthropometric indices and blood pressure and hormone receptors in women with breast cancer. WC significantly predicted Pb (β=5.830, p=0.031), Cd (β=5.855, p=0.029). Diastolic blood pressure significantly predicted Cd (β=0.299, p=0.021). Waist circumference significantly predicted As. (β=-7.074, p=0.010). Hip circumference and WHR positively predicted As (β=3.832, p=0.011; β=2.732, p=0.007 respectively). Waist height ratio and height positively and significantly predicted BPA (β=8.786, p=0.047; β=3.046, p=0.045, respectively).

Table 4.18 shows the multiple regression of endocrine disruptors with Hormones in premenopausal women with breast cancer. Oestradiol positively and significantly predicted Pb (β=0.464, p=0.022) and Cd (β=0.423, p=0.038). FT_4 and TSH inversely and significantly predicted As (β=-0.277, p=0.046; β=-0.323, p=0.036, respectively). Progesterone significantly predicted PCBs (β=1.106, P=0.019), FT_3 inversely predicted PCBs (β=-0.605, p=0.033).

Table 4.19 shows the multiple regression of endocrine disruptors with anthropometric indices and blood pressure in premenopausal women with breast cancer. Hip circumference, height and WHR significantly predicted As (β=6.848, p=0.000; β=0.620, p=0.038; β=4.195, p=0.000, respectively). Waist circumference and SBP inversely and significantly predicted As (β=-9.861, p=0.001; β=-0.361, p=0.012, respectively).

Table 4.16 Multiple Regression of Endocrine Disruptors with Hormones in Women with Breast Cancer (HCBCa)

Dependent	Predictors	Beta	t	P
Lead $R^2=0.103$, F=1.259, p=0.282	Progesterone	-0.240	-1.448	0.152
	Oestradiol	0.374	2.253	0.027*
	LH	0.150	1.008	0.317
	FSH	0.116	0.733	0.466
	FT_3	0.147	1.265	0.210
	FT_4	-0.083	-0.760	0.449
	TSH	-0.074	-0.642	0.523
Cadmium $R^2=0.106$, F=1.300, p=0.262	Progesterone	-0.240	-1.447	0.152
	Oestradiol	0.348	2.096	0.039*
	LH	0.226	1.514	0.134
	FSH	0.025	0.156	0.876
	FT_3	0.148	1.280	0.204
	FT_4	-0.072	-0.656	0.514
	TSH	-0.087	-0.754	0.453
Arsenic $R^2=0.154$, F=1.997, p=0.066	Progesterone	-0.230	-1.428	0.157
	Oestradiol	0.154	0.957	0.342
	LH	-0.091	-0.629	0.531
	FSH	0.079	0.515	0.608
	FT_3	0.004	0.032	0.974
	FT_4	-0.337	-3.162	0.002*
	TSH	-0.124	-1.108	0.271
BPA $R^2=0.371$, F=2.698, p=0.026	Progesterone	0.107	0.389	0.700
	Oestradiol	-0.080	-0.311	0.758
	LH	-0.301	-0.964	0.342
	FSH	0.019	0.058	0.954
	FT_3	0.404	2.192	0.036*
	FT_4	-0.054	-0.366	0.716
	TSH	-0.198	-1.338	0.190

*=significant at p<0.05, beta= Standardized coefficient, p=Probability value.

102

Table 4.17 Multiple Regression of Endocrine Disruptors with Anthropometric Indices, Blood Pressure and Hormone Receptors in Women with Breast Cancer (HCBCa)

Dependent	Predictors	Beta	t	P
Lead $R^2=0.223$, F=1.745, p=0.082	Waist Circumference	5.830	2.199	0.031*
	Hip Circumference	-1.574	-1.090	0.280
	Waist Hip Ratio	-0.858	-0.877	0.384
	Waist Height Ratio	-.4532	-1.900	0.062
	Systolic Blood Pressure	0.059	0.474	0.637
	Diastolic Blood Pressure	0.225	1.770	0.081
	Height	-1.502	-1.803	0.076
	Body weight	-0.177	-1.233	0.222
	BMI	0.163	0.105	0.917
	ER	-0.226	-1.794	0.077
	PR	-0.095	-0.733	0.466
	HER 2	0.144	1.260	0.210
Cadmium $R^2=0.235$, F=1.870, p=0.059	Waist Circumference	5.855	2.226	0.029*
	Hip Circumference	-1.833	-1.280	0.205
	Waist hip Ratio	-1.042	-1.073	0.287
	Waist Height Ratio	-4.225	-1.785	0.079
	Systolic Blood Pressure	-0.009	-0.075	0.941
	Diastolic Blood Pressure	0.299	2.371	0.021*
	Height	-1.440	-1.742	0.086
	Body weight	-0.190	-1.336	0.186
	BMI	0.953	0.592	0.557
	ER	-0.213	-1.705	0.093
	PR	-0.088	-0.685	0.495
	HER 2	0.160	1.419	0.160
Arsenic $R^2=0.208$, F=1.596, p=0.120	Waist Circumference	-7.074	-2.643	0.010*
	Hip Circumference	3.832	2.630	0.011*
	Waist Hip Ratio	2.732	2.765	0.007*
	Waist Height Ratio	2.751	1.142	0.257
	Systolic Blood Pressure	-0.092	-0.738	0.463
	Diastolic Blood Pressure	-0.118	-0.922	0.360
	Height	0.969	1.153	0.253
	Body weight	0.180	1.246	0.217
	BMI	-1.594	-1.072	0.290
	ER	0.095	0.747	0.458
	PR	-0.233	-1.776	0.080
	HER 2	0.149	1.299	0.199
BPA $R^2=0.322$, F=1.164, p=0.356	Waist Circumference	-5.251	-1.055	0.301
	Hip Circumference	-2.697	-1.032	0.311
	Waist Hip Ratio	-1.721	-0.889	0.382
	Waist Height Ratio	8.786	2.083	0.042*
	Systolic Blood Pressure	0.135	0.678	0.503
	Diastolic Blood Pressure	-0.172	-0.852	0.401
	Height	3.046	2.106	0.045*
	Body weight	0.245	1.209	0.237
	BMI	-3.668	-0.811	0.433
	ER	-0.057	-0.177	0.861
	PR	-0.018	-0.063	0.950
	HER 2	0.038	0.199	0.844

Table 4.17 Multiple Regression of Endocrine Disruptors with Anthropometric Indices, Blood Pressure and Hormone Receptors in Women with Breast Cancer.

*=significant at p<0.05, beta= Standardized coefficient, P=Probability value

103

Table 4.18 Multiple Regression of Endocrine Disruptors with Hormones and HER 2 in Premenopausal women with Breast Cancer (Premenopausal-HCBCa)

Dependent	Predictors	Beta	t	p
Lead R^2=0.190, F=1.150, p=0.350	Progesterone	-0.346	-1.692	0.097
	Oestradiol	0.464	2.379	0.022*
	LH	0.185	1.229	0.226
	FSH	0.049	0.347	0.730
	FT3	0.221	1.514	0.137
	FT4	-0.092	-0.675	0.503
	TSH	-0.045	-0.300	0.765
Cadmium R^2=0.158, F=1.234, p=0.304	Progesterone	-0.329	-1.587	0.119
	Oestradiol	0.423	2.136	0.038*
	LH	0.184	1.199	0.237
	FSH	0.052	0.358	0.722
	FT3	0.215	1.455	0.152
	FT4	-0.085	-0.620	0.538
	TSH	-0.058	-0.383	0.703
Arsenic R^2=0.192, F=1.565, p=0.170	Progesterone	-0.390	-1.921	0.061
	Oestradiol	0.268	1.385	0.173
	LH	0.022	0.145	0.885
	FSH	0.149	1.053	0.298
	FT3	0.026	0.178	0.859
	FT4	-0.277	-2.051	0.046*
	TSH	-0.323	-2.165	0.036*
PCBs R^2=0.462, F=1.718, p=0.184	Progesterone	1.106	2.658	0.019*
	Oestradiol	-0.628	-1.781	0.097
	LH	-0.280	-1.208	0.247
	FSH	-0.057	-0.267	0.793
	FT3	-0.605	-2.359	0.033*
	FT4	0.225	1.044	0.314
	TSH	0.112	0.405	0.690

*=significant at p<0.05, beta= Standardized coefficient, p=Probability value

Table 4.19 Multiple Regression of Endocrine Disruptors with Anthropometric indices and Blood Pressure in Premenopausal women with Breast Cancer (Premenopausal-HCBCa)

Dependent	Predictors	Beta	t	P
Arsenic $R^2=0.442$, F=3.873, p=0.001	WC	-9.861	-3.719	0.001*
	HC	6.848	4.118	0.000*
	Height	0.620	2.135	0.038*
	Body weight	0.278	0.167	0.868
	SBP	-0.361	-2.630	0.012*
	DBP	-0.113	-0.834	0.409
	BMI	0.112	0.071	0.944
	WHR	4.195	4.273	0.000*
	WHtR	2.103	1.004	0.321

*=significant at p<0.05, beta= Standardized coefficient. p=Probability value. BMI=Body mass index, WC=Waist circumference, HC=Hip circumference. WHR=Waist hip ratio, WHtR=Waist height ratio, SBP=Systolic blood pressure, DBP=Diastolic blood pressure, TSH=Thyroid stimulating hormone.

Table 4.20 shows multiple regression of endocrine disruptors with Hormones and PR in postmenopausal women with breast cancer. FT_4 inversely predicted As ($\beta = -0.484$, p=0.009).

Table 4.21 shows multiple regression of endocrine disruptors with anthropometric indices and blood pressure in postmenopausal women with breast cancer. Weight and WC significantly predicted Pb ($\beta = 4.993$, p=0.037; $\beta = 9.560$, p=0.027, respectively). BMI, height and WHtR inversely predicted Pb ($\beta = -4.183$, p=0.035; $\beta = -6.460$, p=0.005; $\beta = -8.326$, p=0.037, respectively). Waist circumference significantly predicted Cd ($\beta = 8.910$, p=0.042). Height inversely predicted Cd ($\beta = -5.651$; p=0.015).

Table 4.22 shows the multiple regression of endocrine disruptors with hormones in postmenopausal women without breast cancer. Progesterone significantly predicted Cd and PCBs ($\beta = 0.506$, p=0.031, $\beta = 0.818$, p=0.019, respectively). FSH and FT_3 significantly predicted PCBs ($\beta = 0.785$, p=0.030, $\beta = 0.724$, p=0.043, respectively).

Table 4.23 shows multiple regression of endocrine disruptors with anthropometric indices and blood pressure in postmenopausal women without breast cancer. BMI inversely predicted Pb ($\beta = -1.831$; p=0.013), body weight significantly predicted Pb ($\beta = 2.356$, p=0.007). Waist circumference and WHtR significantly predicted BPA ($\beta = 28.357$, p=0.024; $\beta = 21.638$, p=0.020, respectively). Waist circumference inversely predicted As ($\beta = -20.648$, p=0.034). Height inversely predicted BPA ($\beta = -8.205$, p=0.013).

Table 4.20 Multiple Regression of Endocrine Disruptors with Hormones and PR in Postmenopausal Women with Breast Cancer (Postmenopausal-HCBCa)

Dependent	Predictors	Beta	t	P
Arsenic $R^2=0.416$, F=2.336, p=0.056	Progesterone	-0.199	-1.071	0.295
	Oestradiol	-0.108	-0.581	0.567
	LH	0.034	0.177	0.861
	FSH	-0.084	-0.394	0.697
	FT$_3$	-0.086	-0.524	0.605
	FT$_4$	-0.484	-2.857	0.009*
	TSH	0.238	1.392	0.177

*=significant at $p<0.05$, beta= Standardized coefficient, p=Probability value.
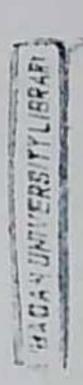
Table 4.21 Multiple Regression of Endocrine Disruptors with Anthropometric indices and Blood Pressure in Postmenopausal Women with Breast Cancer (Postmenopausal-HCBCa)

Dependent	Predictors	Beta	t	p
Lead				
R^2=0.453, F=1.934, p=0.102	BMI	-4.183	-2.259	0.035*
	Height	-6.460	-3.099	0.005*
	Body weight	4.993	2.221	0.037*
	WC	9.560	2.384	0.027*
	WHtR	-8.326	-2.221	0.037*
	HC	-1.726	-0.889	0.384
	WHR	-1.445	-0.875	0.391
	SBP	0.044	0.219	0.829
	DBP	0.298	1.529	0.141
Cadmium				
R^2=0.427, F=1.740, p=0.142	BMI	-3.501	-1.847	0.079
	Height	-5.651	-2.648	0.015*
	Body weight	4.183	1.818	0.083
	WC	8.910	2.170	0.042*
	WHtR	-7.336	-1.912	0.070
	HC	-1.935	-0.974	0.341
	WHR	-1.598	-0.946	0.355
	SBP	0.031	0.154	0.876
	DBP	0.322	1.616	0.121

*=significant at p<0.05, beta= Standardized coefficient, p=Probability value, BMI=Body mass index, WC=waist circumference, HC=Hip circumference, WHR=Waist hip ratio, WHtR=Waist height ratio, SBP=Systolic blood pressure, DBP=Diastolic blood pressure, LH=Luteinizing hormone, TSH=Thyroid stimulating hormone

Table 4.22 Multiple Regression of Endocrine Disruptors with Hormones in Postmenopausal Women without Breast Cancer (Postmenopausal-AHWB)

Dependent	Predictors	Beta	t	p
Cadmium $R^2=0.355$, F=1.727, p=0.154	Progesterone	0.506	2.305	0.031*
	Oestradiol	0.221	1.112	0.278
	LH	0.154	0.605	0.551
	FSH	0.171	0.695	0.494
	FT_3	0.331	1.556	0.134
	FT_4	0.049	0.278	0.783
	TSH	0.314	1.675	0.108
PCBs $R^2=0.700$, F=2.667, p=0.096	Progesterone	0.818	2.941	0.019*
	Oestradiol	-0.135	-0.449	0.665
	LH	-0.635	-1.910	0.092
	FSH	0.785	2.638	0.030*
	FT_3	0.724	2.397	0.043*
	FT_4	-0.102	-0.452	0.663
	TSH	0.137	0.585	0.574

*=significant at p<0.05, beta= Standardized coefficient, p= Probability value

109

Table 4.23 Multiple Regression of Endocrine Disruptors with Anthropometric Indices and Blood Pressure in Postmenopausal Women without Breast Cancer (Postmenopausal-AHWB)

Dependent	Predictors	Beta	t	p
Lead R^2=0.372, F=1.382, p=0.257	BMI	-1.831	-2.718	0.013*
	Height	-0.089	-0.038	0.970
	Body weight	2.356	2.959	0.007*
	WC	-8.611	-1.000	0.329
	WHtR	2.605	0.377	0.710
	HC	6.028	1.856	0.078
	WHR	3.676	1.859	0.077
	SBP	-0.021	-1.000	0.921
	DBP	-0.252	-1.104	0.282
Arsenic R^2=0.301, F=1.004, p=0.467	BMI	-0.682	-0.959	0.348
	Height	4.145	1.689	0.106
	Body weight	1.076	1.281	0.214
	WC	-20.648	-2.273	0.034*
	WHtR	13.995	1.921	0.068
	HC	6.390	1.865	0.076
	WHR	4.058	1.945	0.065
	SBP	-0.014	-0.064	0.949
	DBP	-0.072	-0.297	0.749
BPA R^2=0.820, F=3.546, p=0.055	BMI	0.702	1.007	0.347
	Height	-8.205	-3.284	0.013*
	Body weight	0.218	0.275	0.792
	WC	28.357	2.862	0.024*
	WHtR	21.638	3.011	0.020*
	HC	-6.274	-1.700	0.133
	WHR	-3.558	-1.792	0.116
	SBP	0.210	0.903	0.396
	DBP	0.294	1.451	0.190

*=significant at p<0.05, beta= Standardized coefficient   p=Probability value. BPA =Bisphenol-A, BMI=Body mass index, WC=Waist circumference, HC=Hip circumference. WHR=Waist hip ratio, WHtR=Waist height ratio, SBP=Systolic blood Pressure, DBP=Diastolic blood pressure.

110

CHAPTER FIVE

5.0 DISCUSSION

Breast cancer is the most common type of cancer among women worldwide with a noticeable fatality rate (Wang et al., 2009). An increase in premenopausal breast cancer accounting for between 57 and 67% of breast cancer has been reported. This represents a higher proportion of premenopausal than postmenopausal breast cancer. Postmenopausal breast cancer accounts for about 20% of breast cancer in indigenous African women (Okonofua, 1999; Adesunkanmi, 2006; Oluwatosin and Oladepo, 2006; Okobia et al., 2006; Abdulkareem, 2009; Kene, 2010; Sule, 2011). These observations are similar to the findings in this present study. Fifty four (63.5%) of the HCBCa were premenopausal while 31 (36.5%) were postmenopausal, illustrating the prominence of premenopausal breast cancer in Nigeria.

In this study, the mean age at presentation by HCBCa was 48.32 ± 1.3 years. This is consistent with other studies (Elumelu et al., 2011; Popoola et al., 2012). The reduced life expectancy in Nigeria and other developing countries has been attributed to young people constituting a large percentage of the population (Adebamowo and Ajayi, 2000). Late presentation in the clinic of advanced breast cancer in stages 3 and 4 is a peculiar feature that has been widely reported, particularly in indigenous Nigerian women (Ntekim et al., 2009; Elumelu et al., 2011). In this study, 83.5% participants presented at advanced stages of the disease (stages 3 and 4). This has been adduced to lack of adequate knowledge of the disease, fear of mastectomy and poverty (Ajekigbe, 1991; Oluwole et al., 2003; Elumelu et al., 2011).

Unilateral breast cancer is more frequent in the left breast than in the right (Tulinius et al., 1990). Contrarily, in this study, there was no association between left and right breast cancer site in both pre and postmenopausal-HCBCa. This confirms the study of Ohanaka (2007), who did not observe an association between the left and the right breasts of young women with breast cancer. Emerging information suggests that breast density rather than anatomical site is a strong quantitative risk factor for breast cancer (Hennessey et al., 2014). Breast density reflects fibro-glandular tissue which comprises of epithelial and stromal tissues in the breast (McCormack and DosSantos-Silva, 2006).

111

Observations in this study showed that reproductive factors such as number of previous pregnancies, number of live births and number of induced abortions were significantly higher in premenopausal-HCBCa compared with premenopausal-AHWB (p<0.05). Induced abortion was significantly associated with increased risk of breast cancer among Chinese females (Huang et al., 2014). Age at menarche was also significantly higher in premenopausal-HCBCa compared with premenopausal-AHWB (p<0.05). This is at variance with an earlier report of an association between early age at menarche and increased risk of breast cancer attributed to increased exposure to oestrogens (Orgeas et al., 2008).

Endogenous sex steroid hormones have been reported to play a major role in the aetiology of breast cancer (Bernstein and Ross, 1993; Clemons and Goss, 2001). Both premenopausal and postmenopausal women secrete steroid hormones throughout their lives with a difference in the pattern of secretion. The hormones are mainly regulated by the ovary in premenopause while they are regulated in postmenopause by the adrenal gland. In the development of breast cancer, the tumour grows within a hormonal milieu which has a decisive influence on its growth. (Harnandez et al., 2005). In spite of the multiple epidemiological studies that have investigated the association of serum sex hormones and premenopausal breast cancer risk, the results have been inconsistent (Wysowski et al., 1987; Key and Pike, 1988; Helzlsouer et al., 1994; Rosenberg et al., 1994; Thomas et al., 1997; Kabuto et al., 2000; Haslarn et al., 2002; Yu et al., 2003; Micheli et al., 2004; Missimer et al., 2004; Kaaks et al., 2005; Eliassen et al., 2006; Ho et al., 2009).

Comparison of E_2 level between premenopausal-HCBCa and premenopausal-AHWB in this study showed no significant difference (p>0.05). These findings have been reported by others (Sturgeon et al., 2004; Ho et al., 2009). However, E_2 and progesterone levels were higher in postmenopausal-HCBCa compared with postmenopausal-AHWB (p<0.05) in this present study. Positive association of E_2 with breast cancer risk in postmenopausal women has previously been observed (Hankinson et al., 1998). The underlying mechanisms of action of E_2 in the aetiology of breast cancer include the alkylation of cellular molecules, generation of active radicals and genotoxicity of oestrogen metabolites which are involved in initiation, promotion and progression of breast cancer (Nandi et al., 1995; Clemons and Goss, 2001; Yager and Davidson, 2006; Drabsch et al., 2007). Wang et al. (2009) also observed high levels of progesterone in

112

postmenopausal breast cancer. Increased postmenopausal progesterone levels have also been implicated in dementia, with unknown reasons but may relate to small subclinical cerebral thrombosis (Yaffe, 2003; Zhu and Brinton, 2012). It is uncertain if the elevated progesterone in the postmenopausal-HCBCa in this study is related to menopause or breast cancer.

Follicle stimulating hormone controls E_2 level by negative feedback mechanism in premenopausal women (Fabian et al., 2015). Serum LH and FSH were significantly higher in premenopausal-HCBCa compared with premenopausal-AHWB (p<0.05). High serum LH and FSH were reported to be associated with a significantly worse breast cancer prognosis in premenopausal breast cancer patients (Pujol et al., 2001). The ability of FSH to activate adenylyl cyclase thereby resulting in increased cAMP levels could be associated with its ability to induce breast cancer cell proliferation, differentiation and metastasis (Tunizicker-Dunn and Maizels, 2006; Zreik et al., 2006, Zhou et al., 2013). These findings implicate gonadotropin exposure in premenopausal breast carcinogenesis. Serum FSH level was significantly lower in postmenopausal-HCBCa compared with postmenopausal-AHWB (p<0.05) in this study. Although, the reasons are not clear, low FSH level has also been observed in postmenopausal women with ovarian cancer (Arslan et al., 2003; McSorley et al., 2009). However, breast and ovarian cancers are hormone-dependent cancers with genomic similarities (CGAN, 2012). Mechanisms involving FSH reduction and increased E_2 may underlie postmenopausal breast cancer in this study.

Oestrogen receptor, PR and HER 2 play important roles in the growth and differentiation of breast cancers making them important prognostic markers (Patel et al., 2013; Mohamed et al., 2015; Deepti et al., 2015). Women with ER+ breast cancer can benefit from endocrine therapy explaining their better survival outcomes (Makanjuola et al., 2014). In this study ER-, PR- and HER 2- were observed in 69 (87.3%), 71 (89.9%), 64 (81.0%) HCBCa respectively. Huo et al. (2009) reported the predominance of hormone receptor negative breast cancer in indigenous African women. Oestrogen receptor negative and PR- were observed in all premenopausal-HCBCa. These findings suggest the involvement of genetics in the aetiology of breast cancer. Young women are diagnosed with breast cancer with more aggressive tumour and are associated with higher mortality, shorter disease-free survival and more likely to recur after treatment both

loco regionally and at distant sites than in older women (Nixon *et al.*, 1994; Gajdose *et al.*, 2000; Foxcroft *et al.*, 2004; Ntekim *et al.*, 2009).

Hormone receptor positive expressions were however observed in less than 20% of postmenopausal-HCBCa in this study. These findings were similar to other studies in Africans (Huo *et al.*, 2009; Stark *et al.*, 2010). Contrarily, observations in blacks residing in the United States of America and United Kingdom showed a higher proportion of positive receptors expressions (Chu and Anderson, 2002; Bowen *et al.*, 2008; Ahmed *et al.*, 2011; Ali *et al.*, 2012). These reports implicate geographic or environmental factors beyond genetics. Most evidence regarding the prognostic role of PR is based on the assumption that PR expression indicates a functioning ER pathway (Ravdin *et al.*, 1992). Hence, PR+ and ER- tumours have a better response to endocrine therapy than ER+ and PR- (Payne *et al.*, 2008).

Triple negative breast cancers are poorly differentiated and are characterized by an aggressive clinical history. No specific treatment guidelines are currently available for this breast cancer sub-type. However, they are managed with standard treatment, which leaves them with a high rate of local and systemic relapse (Cleator *et al.*, 2007). In this study, 55 (69.62%) HCBCa were triple negative. Forty six (88.5 %) HCBCa were premenopausal while 9 (11.5 %) were postmenopausal. Stark *et al.* (2010) and Makanjuola *et al.* (2014) reported a high prevalence of triple negative hormone receptors in their different studies in indigenous African women.

Thyroid hormones may be critical in the pathogenesis and progression of diseases due to their regulatory role on cell maturation (Mourouzis *et al.*, 2013; Mourouzis *et al.*, 2015). Thyroid signalling may be altered in cancer as a result of the activation of growth kinase signaling which may be of physiological relevance (Pallud *et al.*, 1999; Casula and Bianco, 2012). Several studies which compared levels of peripheral thyroid hormones in women with breast cancer and women without breast cancer are inconclusive regarding associations between thyroid hormones and breast cancer risk (Goldman, 1990; Smyth, 1997; Sarlis *et al.*, 2002, Tosovic *et al.*, 2012).

In this present study, there was an association between FT_4 and HCBCa (pre and postmenopausal). Guigon *et al.* (2011) reported an association between FT_4 and breast cancer. Thyroid hormones appear to stimulate lobular development, contributing to the differentiation of breast tissue (Neville *et al.*, 2002). It is postulated that the thyroid gland interacts with the breast

tissues based on the common property of the mammary and thyroid epithelial cells to concentrate iodine by a membrane active transport mechanism. Additionally, TSH receptors in fatty tissues which are abundant in the mammary gland have been reported to be a possible reason for this interaction (Turken et al., 2003; Ali et al., 2011). However, serum levels of the thyroid hormones in the study participants were within the normal reference interval (FT3, 3.2-6.0pmol/L; FT4, 10.6-21.0 pmol/L; TSH, 0.38-4.31mIU/L). Emerging reports show that changes in thyroid hormone levels within normal range may be associated with proliferative activity of breast tumours in euthyroid patients with breast cancer (Milionis and Milionis, 2013).

The increase in breast cancer incidence in women has been related to industrialization consequent upon the widespread contamination of the soil, air and water by the toxic metals (Jarup and Akesson, 2009; Julin et al., 2012; Ragab et al., 2014). Breast cancer is a multistep process involving both genetic and epigenetic changes (Lustberg and Ramaswamy, 2009) such as differential DNA methylation and altered histone modifications. Hypermethylation blocks the promoter region of a gene and results in gene silencing. Identification of epigenetic changes and their correlation with other factors could lead to improvements in cancer diagnosis and treatment (Sunami et al., 2008). Metals act as catalyst in the oxidative deterioration of biological macromolecules, induce reactive oxygen species, which accumulate and induce epigenetic factors (Hou et al., 2012).

In this present study, there was an association between Cd and breast cancer (pre and postmenopausal). The ability of Cd to induce cell proliferation, differentiation, apoptosis and signal transduction by enhancement of protein phosphorylation, activation of transcription and translation factors suggests its ability to induce breast cancer (Joseph et al., 2001; Jin et al., 2003; Shih et al., 2004; Martinez-campa et al., 2006; Sun et al., 2007; Templeton and Liu, 2010; Yu et al., 2010; Siewt et al., 2010). Hypermethylation and repression of DNA repair genes appear to be an early signature of cadmium-induced cancer and may constitute part of the mechanisms by which the toxicant induces tumorigenesis (Zhou et al., 2008). Additionally, Cd has the potential to disrupt endocrine function by behaving like sex hormones (Enmark and Gustafsson, 1999; Stoica et al., 2000b; Thomas and Dong, 2006). At low concentrations, the metal mimics the effects of E2 and binds with high affinity to the hormone-binding domain of ERα. This binding involves several amino acids, suggesting that Cd activates the receptor

through the formation of a complex with specific residues in the hormone-binding domain (Johnson et al., 2003; Benbrahim-Tallaa, 2009).

Lead is of concern due to its wide use (Florea and Busselberg, 2011). However, results of epidemiologic studies investigating the association of Pb with cancers are inconsistent and vary according to the type of cancers reported (Steenland et al., 1992; Wong and Harris, 2000). Direct DNA damage as a result of oxidative stress, clastogenicity, inhibition of DNA synthesis or repair has been reported as the mechanisms of Pb carcinogenicity (Martin et al., 2003; Ragab et al., 2014). In this present study, Pb was associated with pre and postmenopausal breast cancer. This is consistent with the findings of Siddiqui et al. (2006) in which blood Pb level was significantly higher in breast cancer patients than their controls. Lead adversely affects steroidogenesis by substituting for zinc in the DNA binding zinc (Zn^{2+})-finger motif of steroidogenic enzymes, resulting in their decreased expression. These enzymes are steroidogenic acute regulatory protein (StAR), cytochrome P450 side chain cleavage enzyme (CYP450cc) and 3 beta hydroxysteroid dehydrogenase (3β HSD), (Lutzen et al., 2004).

Arsenic exposure constitutes one of the most wide-spread environmental carcinogens and is associated with increased risk of different types of cancers (Florea et al., 2007; Florea and Busselberg, 2008; Ying et al., 2009). However, few studies have focused on the association of environmental exposure to As and breast cancer risk. Information on the association of As with breast cancer in sub-Saharan Africa is sparse. In this present study, As was associated with pre and postmenopausal breast cancer. Low dose As represses tumour suppressor genes (Li et al., 2010). Transcription factors in human MDA-MB-435 breast cancer and rat H411E hepatoma cells were reportedly sensitive to low dose As (Kaltreider et al., 1999; Stoica et al., 2000a). Arsenic is thought to induce carcinogenicity by inducing DNA hypomethylation leading to aberrant gene expression (Zhao et al., 1997; Verma and Srivastava, 2002) or by DNA methylation silencing genes associated with controlling tumourigenesis (Vaissiere et al., 2008). Arsenic competes with DNA methyl transferase genes (DNMT) for S adenosylmethionine (SAM), potentially limiting the availability of SAM to be used by DNMT to catalyze methylation of CpG. This could result in hypomethylation and reactivation of silenced tumour suppressor genes (Vo An and Millis, 2012; Pogribny and Rusyn, 2013). Altered histone modification associated with arsenic-induced gene expression in carcinogenesis has been suggested (Zhou et al., 2008).

Humans are exposed daily to a variety of compounds. It is thus likely that the combination or mixture of chemicals may become dangerous even when none of the chemicals reaches an effective level. These chemicals enter the food chain and accumulate in animals and eventually humans (Lubrano et al., 2013). Bisphenol-A, a breakdown product of coatings in food and beverage containers, may act as oestrogen receptor agonist (Meerts et al., 2001; Fernandez and Russo, 2010). Bisphenol-A promotes the proliferation of both ER+ and ER- breast cancer cells (Song et al., 2015). Recent report suggests that BPA enhances the growth of triple negative breast cancer cells via oestrogen related receptor gamma (ERRγ) and matrix metalloproteinases (MMPs). This involves the activation of extracellular signal regulated kinases and protein kinases B (Akt) which inhibit apoptosis (Zhang et al., 2016). In this present study, BPA was associated with pre and postmenopausal breast cancer.

Environmental exposure to PCBs has been suggested as potential causes of breast cancer (Davis et al., 1993; Wolff et al., 1993). Polychlorinated biphenyls are weak oestrogens in vitro, have tumour promoting ability and are able to induce metabolic enzymes (Norback and Weltman, 1985; McKinney and Waller, 1994). In this study, an association was observed between PCBs and breast cancer (pre and postmenopausal). Polychlorinated biphenyls exposure may lead to formation of DNA adduct through a pathway involving cytochrome P450 1A1 (CYP1A1) (Oakley et al., 1996). Cytochrome P450 1A1 is important in the metabolism of potentially genotoxic chemicals (Pelkonen and Nebert, 1982). The interaction of PCBs with CYP1A1 polymorphisms in the aetiology of breast cancer is therefore suggested. Polychlorinated biphenyls activate aryl hydrocarbon receptor (AhR). The mechanisms through which AhR regulates energy metabolism are not clearly established, although ER has been implicated (Lubrano et al., 2013). Emerging evidence indicates that BPA and PCBs can affect mitochondrial function and cause pro-oxidative conditions leading to pathological conditions like cancer (Valavinides et al., 2006; Albers et al., 2010; Farohat et al., 2011). Oxidative stress may thus be one of the the mechanisms, whereby environmental toxicants cause breast cancer.

Cadmium has the potential of disrupting endocrine function by behaving like sex hormones (Yu et al., 2003; Siewt et al., 2010). Cadmium and Pb were positively related with E₂ in premenopausal-HCBCa in this study. There was also a positive relationship between PCBs and progesterone in premenopausal-HCBCa. Polychlorinated biphenyls activate aryl hydrocarbon

receptor (AhR) which has been associated with ER (Lubrano et al., 2013). Polychlorinated biphenyls are also associated with ER-negative tumours which have a faster rate of progression (Carpenter et al., 2005).

Arsenic was inversely related with FT₄ in pre and postmenopausal-HCBCa. An inverse relationship was also observed between As and TSH as well as PCBs and FT₃ in premenopausal-HCBCa. These observations suggest the possible interference of thyroid hormones by As and PCBs in women with breast cancer. This could be due to the binding of As to the thyroid hormone receptors which blocks the binding of the thyroid hormones (Davey et al., 2008). Bisphenol-A was positively related with FT₃ in HCBCa. In vitro studies demonstrate that BPA binds to thyroid receptors with relatively low affinity and mediate growth stimulatory effect via T₃-receptors (Meerts et al., 2001).

Adiposity is a prognostic factor of breast cancer as well as an independent risk factor of postmenopausal breast cancer (Chan and Norat, 2015). Waist circumference, waist hip ratio and waist height ratio are indicators of visceral adiposity. Body weight and BMI are indicators of general adiposity while hip circumference is an indicator of subcutaneous adiposity (Charles-Davies et al., 2012; Amadou et al., 2013). Although the women in this present study were matched for age and menstrual status, increased adiposity (waist circumference, hip circumference, body weight, height, waist hip ratio and waist height ratio) was observed in premenopausal-HCBCa compared with premenopausal-AHWB. Fagherazzi et al. (2012) showed an association between hip circumference and premenopausal breast cancer. Increased adiposity (body weight and height) was also significantly higher in postmenopausal-HCBCa compared with postmenopausal-AHWB in this study.

Increased visceral adiposity and insulin resistance characterise metabolic syndrome (MS), which predisposes individuals to chronic diseases-cancer, cardiovascular diseases and type 2 diabetes mellitus. It is associated with the female gender and is prevalent in 44.5% of apparently healthy women in Ibadan. Increased visceral adiposity, a strong metabolic risk factor was the most frequent component in these women while reduced high density lipoprotein cholesterol was the most frequent component in males (Charles-Davies et al., 2014; Chan and Norat, 2015).

Sex hormones-testosterone and oestrogen are synthesised from cholesterol. Increased conversion of testosterone to oestradiol by aromatase in increased adipose tissue has been reported in pre-menopausal women with MS (Fabian et al., 2015). Thus, increased adiposity alone may not underlie the aetiology of breast cancer. Ogundiran et al. (2010) showed no association between body weight and the risk of breast cancer in indigenous African women with breast cancer irrespective of their menstrual status.

However, endocrine disruptors are known to accumulate in adipose tissue (Grun and Blumberg, 2009). It thus appears that increased adiposity may enhance the accumulation of endocrine disruptors in the pathology of breast cancer (Ajayi et al., 2014). This hypothesis is corroborated in this study as height had a positive relationship with As in premenopausal-HCBCa. However, menopause may define the role of endocrine disruptors in increased adipose tissue, in breast cancer as body weight had a positive relationship with Pb in both postmenopausal-HCBCa and postmenopausal-AHWB. In postmenopausal women, Pb from prolonged environmental exposure may accumulate in adipose tissue without causing breast cancer. Height had a negative relationship with Cd and Pb in postmenopausal-HCBCa.

Mechanisms in the pathogenesis of breast cancer may differ between pre and postmenopause and may involve different endocrine disruptors and fat depots. Previous studies showed increased height in apparently healthy premenopausal women with metabolic syndrome than without metabolic syndrome (Charles-Davies et al., 2012). Short term exposure to As rather than increased height may be a breast cancer risk factor in pre-menopause. Long term exposure to Pb and Cd may be involved in breast cancer pathogenesis without the contribution of height in postmenopause.

Body mass index was inversely related with Pb in both postmenopausal-HCBCa and postmenopausal-AHWB. This suggests that BMI and Pb may not be important as breast cancer risk factors in postmenopause. Moreover, waist height ratio (a strong index of visceral obesity) was inversely related with Pb in postmenopausal-HCBCa. Although, visceral adiposity appeared not important in postmenopausal breast cancer, waist circumference was positively related with Cd and Pb in HCBCa and postmenopausal-HCBCa. These findings suggest that Cd and Pb may accumulate in increased abdominal adiposity in postmenopausal women with breast cancer. In HCBCa in this study, waist hip ratio was positively related with As while an inverse relationship

existed between waist circumference and As in HCBCa. Hip circumference was positively related with As in HCBCa and premenopausal-HCBCa. Arsenic is lipophilic and probably has preference for subcutaneous fat (Ying *et al.*, 2009).

In this present study, mean values of SBP and DBP in the HCBCa and AHWB reflect normal blood pressure. However, SBP was significantly higher in HCBCa and premenopausal-HCBCa than their respective controls. This might reflect the mild increase in visceral obesity in premenopausal-HCBCa compared with premenopausal-AHWB. Hypertension was associated with metabolic syndrome and the female gender (Fabian *et al.*, 2015). Experimental studies indicate that As exposure may be involved in the development of hypertension through the activation of stress response transcription factors including activator protein and nuclear factor – kappa B (Aposhian *et al.*, 2003; Balakumar *et al.*, 2008). In vitro arsenite altered vascular tone in blood vessels by suppressing vasorelaxation and increased the expression of cyclooxygenase-2 in endothelial cells (Lee *et al.*, 2003). In this study, SBP was inversely related with As in premenopausal-HCBCa. This reason for this observation is unclear, it is hypothesized that the influence of As on blood pressure in women with breast cancer could be menstrual phase specific. Diastolic blood pressure was positively related with Cd in HCBCa in this study. Tellez-Plaza *et al.* (2008) reported an association between blood pressure and DBP via these mechanisms; partial agonism for calcium channels, direct vasoconstrictor action, activation of the sympathetic nervous system and inhibition of vasodilator substances such as nitric oxide (Bilgen *et al.*, 2003; Varoni *et al.*, 2003).

In HCBCa, BPA was positively related with waist height ratio and height. In postmenopausal-AHWB, BPA was positively related with waist circumference and waist height ratio, while it was inversely related with height. These observations suggest that BPA could be involved in adiposity. Studies have shown that exposure to BPA could suppress the release of adiponectin, an adipocyte-specific hormone that increases insulin sensitivity, this could lead to insulin resistance and increased susceptibility to obesity and metabolic syndromes which have been implicated in breast cancer (Hugo *et al.*, 2008; Li *et al.*, 2013).

An association between diets and the risk of breast cancer has been observed. This is because environmental toxicants are present in the food chain (Reuben, 2010). Regular consumption of fruits and vegetables are associated with decrease risk of many cancers, however, results for

breast cancer risk are not conclusive (Riboli and Norat, 2003; Guadet *et al.*, 2004; Hirose *et al.*, 2005; WCRF, 2007; de Lima *et al.*, 2008). In this present study, HCBCa consumed more vegetables but less fruits weekly. Nutrient loss occurs in the preparation and cooking processes of vegetables, particularly in Nigeria, resulting in the reduction of bio-available phytochemicals (including antioxidant vitamins) and other anticarcinogenic compounds capable of protecting against cancer (Toiwo and Akanbi, 1997; Cavagnaro and Galinarini, 2012; Czarnowska and Gujska, 2012).

Meat and dairy products contain fat with a high proportion of saturated fatty acids which have been associated with increased breast cancer risk. They may also contain insulin-like growth factor-1 (IGF-1) which has been reported to promote breast cancer cell growth and pesticides that are potentially carcinogenic (Moormar and Terry, 2004). Consumption of red meat has been associated with increased risk of breast cancer in some studies, while the association of dairy product intake with breast cancer risk is inconclusive (Zheng *et al.*, 1998; Moormar and Terry, 2004; Taylor *et al.*, 2007). In this present study, daily consumption of red meat was associated with HCBCa while weekly consumption of diary product was associated with AHWB. This suggests that red meat consumption may be involved in breast carcinogenesis

CHAPTER SIX

6.0 SUMMARY, CONCLUSIONS AND RECOMMENDATION

6.1 Summary and Conclusions

Breast cancer is the most common type of cancer among women worldwide with a noticeable fatality rate. Fifty four (63.5%) of the HCBCa were premenopausal while 31 (36.5%) were postmenopausal, illustrating the prominence of premenopausal breast cancer in Nigeria. The reduced mean age at presentation by HCBCa was 48.32 ±1.3 years reflects the reduced life expectancy in Nigeria and other developing countries. Late presentation of 83.5% of HCBCa in the clinic of advanced breast cancer in stages 3 and 4 was a peculiar feature in this study. This has been adduced to lack of adequate knowledge of the disease, fear of mastectomy and poverty.

Reproductive factors-increased number of previous pregnancies, increased number of live births and increased number of induced abortions were associated with premenopausal breast cancer. These findings corroborate earlier reports. The association of increased age at menarche with premenopausal breast cancer risk in this present study is contrary to reports by others on early age at menarche and increased risk of breast cancer which was attributed to increased exposure to oestrogens.

Endogenous sex steroid hormones have been reported to play a major role in the aetiology of breast cancer. Thyroid hormones appear to stimulate lobular development, contributing to the differentiation of breast tissue. Elevated FT₄ level was associated premenopausal breast cancer while elevated oestradiol and progesterone levels and FT₄ levels were associated with postmenopausal breast cancer in this study, also corroborating previous findings. Although the serum levels of the thyroid hormones in the study participants were within the normal reference interval, emerging reports show that changes in thyroid hormone levels within normal range may be associated with proliferative activity of breast tumours in euthyroid patients with breast cancer. It is however uncertain if the elevated progesterone in the postmenopausal with breast cancer in this study is related to menopause or breast cancer.

The role of gonadotropins in the aetiology of breast cancer is increasingly gaining attention. increased levels of serum gonadotropins-LH and FSH were associated with premenopausal

breast cancer in this study probably reflecting worse breast cancer prognosis in premenopausal breast cancer patients. However, reduced serum FSH level was associated with postmenopausal breast cancer in this study similar to previous studies with unclear reasons.

Oestrogen receptor, PR and HER 2 play important roles in the growth and differentiation of breast cancers making them important prognostic markers. In this study, 52 (100%) and 46 (88.5%) of premenopausal HCBCa had ER/PR negative and triple negative expressions respectively. Hormone receptor positive expressions were observed in less than 20% of postmenopausal breast cancer in this study. This corroborates the predominance of hormone receptor negative and aggressive cancer breast cancer with high mortality particularly in younger indigenous African women contrary to observations in the Caucasians. These findings implicate geographic or environmental factors beyond genetics.

Environmental toxicants studied-Cd, Pb, As, BPA and PCBs may be breast cancer risk factors Hypermethylation and repression of DNA repair genes, disruption of endocrine function through interaction and mimicry of specific steroid hormones and their receptors, induction of metabolic enzymes and pro-oxidative conditions may underlie mechanisms that lead to breast cancer. Thus identification of epigenetic changes and their correlation with other factors could lead to improvements in cancer diagnosis and treatment. Lead adversely affects steroidogenesis by substituting for zinc in the DNA-binding zinc (Zn^{2+})-finger motif of steroidogenic enzymes resulting in their decreased expression. Arsenic is thought to induce carcinogenicity by inducing DNA hypomethylation leading to aberrant gene expression

Adiposity has been implicated is a prognostic and independent risk factor of postmenopausal breast cancer. Increased adiposity was also associated with pre and postmenopausal breast cancer in this study. However, findings suggest that endocrine disruptors are the actual culprits as some accumulate in adipose tissue to exert their deleterious effects.

Identification of hormone receptor expression, appropriate diet rich in antioxidants, low fat and reduced red meat; physical activity and reduction of environmental pollution may be beneficial in the prevention and management of breast cancer.

6.2 Recommendation

Determination of hormone receptors status may assist in the proper management of breast cancer. Reduction of environmental pollution by appropriate government legislation, safety regulations on the use of toxic substances will reduce the incidence of breast cancer. Intake of diet rich in antioxidants will protect against oxidative stress which is involved in breast carcinogenesis. Moreover, diet low in fat, reduced consumption of red meat as well as regular physical exercise may be of benefit to the women diagnosed with breast cancer. Routine screening for thyroid status is also recommended. Overall, maintenance of a healthy lifestyle is key in the prevention and management of breast cancer.

Contributions to Knowledge in the Discipline

i. The endocrine disrupting ability of known environmental toxicants-cadmium, lead, arsenic, bisphenol-A and polychlorinated biphenyls may result in development of breast cancer.

ii. The accumulation of these endocrine disruptors in adipose tissue and their interaction with oestradiol, progesterone and thyroid hormones may be underlie mechanisms in breast cancer aetiology in Nigeria.

iii. All premenopausal breast cancer are ER/PR receptor negative. Receptor triple negative expressions (ER, PR and HER 2) are the predominant in breast cancer particularly in premenopause in Nigeria suggesting that mechanisms involved in breast cancer development may be different between receptor negative premenopausal breast cancer and receptor positive post menopausal breast cancer.

iv. Elevated FT_4 level was associated premenopausal breast cancer while elevated E_2, progesterone and FT_4 levels were associated with postmenopausal breast cancer.

v. Increased levels of serum gonadotropins-LH and FSH were associated with premenopausal breast cancer while reduced serum FSH level was associated with postmenopausal breast cancer in this study.

REFERENCE

Aaltomaa, S., Lipponen, P., Eskelinen, M., Kosma, V.M., Marin, S., Alhava, E. and Syrjanen, K. 1991. Hormone receptors as prognostic factors in female breast cancer. *Annals of Medicine* 23:643-648.

Abdulkareem, F. 2009. Epidemiology and incidence of common cancers in Nigeria. *A presentation at Cancer Registration and Epidemiology workshop.* 1-58.

Adachi, K. and Tainosho, Y. 2004. Characterization of heavy metal particles embedded in tire dust. *Environment International* 30.8:1009-1017.

Adami, H. O., Signorello, L. B. and Trichopoulos, D. 1998. Towards an understanding of breast cancer etiology. *Seminars in Cancer Biology* 8.4:255-262.

Adams, J., White, M. and Forman, D. 2004. Are there socioeconomic gradients in stage and grade of breast cancer at diagnosis? Cross sectional analysis of UK cancer registry data. *British Medical Journal* 329.7458: 142.

Adebamowo, C. A, Cho, E., Sampson, L., Katan, M. B., Spiegelman, D., Willett, W. C. and Holmes, M. D. 2005. Dietary flavonols and flavonol-rich foods intake and the risk of breast cancer. *International Journal of Cancer* 114.4:628-633.

Adebamowo, C. A. 2007. Cancer in Nigeria. American Society of Clinical Oncology (ASCO) News and Forum. Retrieved Oct, 15 2014, from http://www.ascocancerfoundation.org/anf/Past+Issues/April+2007/Cancer+in+Nigeria?cp sextcurrchannel.

Adebamowo, C. A. and Adekunle, O.O. 1999. Case controlled study of epidemiological risk factors of breast cancer in Nigeria. *British Journal of Surgery* 86:665-668.

Adebamowo, C. A. and Ajayi, O. O. 2000. Breast Cancer in Nigeria. *West African Journal of Medicine* 19:179-191.

Adebamowo, C. A., Ogundiran, T. O., Adenipekun, A. A., Oyesegun, R. A., Campbell, O. B., Olopade, O. I. and Akang, C. N. 2003. Waist-hip ratio and breast cancer risk in urbanized Nigerian women. *Breast Cancer Research* 5:18-24.

Adesunkanmi, A. R., Lawal, O. O., Adelusola, K. A. and Durosimi, M. A. 2006. The severity, outcome and challenges of breast cancer in Nigeria. *Breast.* 15.3: 399-409.

Adetifa, F. A. and Ojikutu, R. K. 2009. Prevalence and Trends in Breast Cancer in Lagos State, Nigeria. *African Research Review.* 3.5:1-15.

REFERENCE

Aaltomaa, S., Lipponen, P., Eskelinen, M., Kosma, V.M., Marin, S., Alhava, E. and Syrjanen, K. 1991. Hormone receptors as prognostic factors in female breast cancer. *Annals of Medicine* 23:643-648.

Abdulkareem, F. 2009. Epidemiology and incidence of common cancers in Nigeria. *A presentation at Cancer Registration and Epidemiology workshop.* 1-58.

Adachi, K. and Tainosho, Y. 2004. Characterization of heavy metal particles embedded in tire dust. *Environment International* 30.8:1009-1017.

Adami, H. O., Signorello, L. B. and Trichopoulos, D. 1998. Towards an understanding of breast cancer etiology. *Seminars in Cancer Biology* 8.4:255–262.

Adams, J., White, M. and Forman, D. 2004. Are there socioeconomic gradients in stage and grade of breast cancer at diagnosis? Cross sectional analysis of UK cancer registry data. *British Medical Journal* 329.7458: 142.

Adebamowo, C. A, Cho, E., Sampson, L., Katan, M. B., Spiegelman, D., Willett, W. C. and Holmes, M. D. 2005. Dietary flavonols and flavonol-rich foods intake and the risk of breast cancer. *International Journal of Cancer* 114.4:628-633.

Adebamowo, C. A. 2007. Cancer in Nigeria. American Society of Clinical Oncology (ASCO) News and Forum. Retrieved Oct, 15 2014, from http://www.ascocancerfoundation.org/an/Past+Issues/April+2007/Cancer+in+Nigeria?cp sextcurrchannel.

Adebamowo, C. A. and Adekunle, O.O. 1999. Case controlled study of epidemiological risk factors of breast cancer in Nigeria. *British Journal of Surgery* 86:665-668.

Adebamowo, C. A. and Ajayi, O. O. 2000. Breast Cancer in Nigeria. *West African Journal of Medicine* 19:179-191.

Adebamowo, C. A., Ogundiran, T. O., Adenipekun, A. A., Oyesegun, R. A., Campbell, O. B., Olopade, O. I. and Akang, C. N. 2003. Waist-hip ratio and breast cancer risk in urbanized Nigerian women. *Breast Cancer Research* 5:18-24.

Adesunkanmi, A. R., Lawal, O. O., Adelusola, K. A. and Durosimi, M. A. 2006. The severity, outcome and challenges of breast cancer in Nigeria. *Breast.* 15.3: 399-409.

Adetifa, F. A. and Ojikutu, R. K. 2009. Prevalence and Trends in Breast Cancer in Lagos State, Nigeria. *African Research Review.* 3.5:1-15.

Agency for Toxic Substances and Disease Registry (ATSDR), Toxicological Profile for Lead-Update. 1996. US Department of Health and Human Services, Atlanta, pp. 205–208.

Agency for Toxic Substances and Disease Registry (ATSDR), Toxicological Profile for Arsenic-ATSDR. 2005. U.S. Department of Health and Human Services, Public Health Services, ATSDR, Atlanta, Georgia.

Ahmed, H. G, Al-Adhraei, M. A and Al-Thobhani, A. K. 2011. Correlations of Hormone Receptors (ER and PR), Her2/neu and p53 Expression in Breast Ductal Carcinoma among Yemeni Women. *The Open Cancer Immunology Journal* 4: 1-9.

Ajayi, O. O., Charles-Davies, M. A., Anetor, J. L and Ademola, Y. 2014. Serum polychlorinated biphenyls and bisphenol-A levels in Nigerian women with breast cancer. *Archive of Basic and Applied Medicine.* 2014; 2:71-75.

Ajekigbe, A.T. 1991. Fear of mastectomy: The most common factor responsible for late presentation of carcinoma of the breast in Nigeria. *Clinical Oncology* 3.2: 78-80.

Akande, E. O. and Hockaday, T. D. 1972. Plasma oestrogen and luteinizing hormone concentrations in thyrotoxic menstrual disturbance. *Proceedings of Reproductive and Social Medicine* 65:789–790.

Akande, E.O. 1974. The effect of oestrogen on plasma levels of luteinizing hormone in euthyroid and thyrotoxic postmenopausal women. *Journal of Obstetrics and Gynaecology of British Commonwealth* 81:795–803.

Al-Asmakh. M. 2007. Reproductive functions of progesterone. *Middle East Fertility Society Journal* 12.3: 1-7.

Albers, G., Echteld, M. A, de Vetite, Onwuteaka-Philipsen, B. D., van der Linden, M. H. and Deliens, L. 2010. Evaluation of quality of life measures for use in palliative care: A systematic review. *Palliative Medicine* 24:17-37.

Ali Al-Ahmed, A.H. and Jumaah, N. S. 2012. Evaluation of hormone receptors status (oestrogen & progesterone) and human epidermal growth factor receptor R-2(HER2) in Breast cancer in Basrah. *The Medical Journal of Basrah University* 30. 2: 133-142.

Ali, A., Mir, M. R., Bashir, S. and Hassan, T. 2011. Impact of Serum Thyroid Hormones and Estrogen Status on the Risk of Breast Cancer in Kashmiri Women. *Journal of Cell Science and Therapy* 2.4:113-115.

AL-Khafaji., A. H., Fadhil, A. Y. A. and Hameed, M. A. 2014. Immunohistochemical Study of

Estrogen, Progesterone Receptor and Her-2neu Oncogene with Her-2neu Biomarker Estimation by ELISA Technique in Primary Breast Cancer before Chemical Therapy. *Iraqi Journal of Science* 55.1: 132-144.

Alonso-Magdalena, P., Laribi, O., Ropero, A. B., Fuentes, E., Ripoll, C., Soria, B. and Nadal, A. 2005. Low doses of bisphenol-A and diethylstilbestrol impair Ca^{2+} signals in pancreatic alpha-cells through a nonclassical membrane estrogen receptor within intact islets of Langerhans. *Environmental Health Perspectives* 113:969-977.

Altmannsberger, M., Dirk, T., Droese, M., Weber, K. and Osborn, M. 1986. Keratin polypeptide distribution in benign and malignant breast tumors: subdivision of ductal carcinomas using monoclonal antibodies. *Virchows Archive of Biology Cell Pathology Including Molecular Pathology* 51: 265–275.

American Association of Prolife Obstetricians and gynaecologists-AAPLOG. 2008. Induced abortion and subsequent breast cancer risk; An overview.

Amzal, B., Julin, B., Vahter, M., Wolk, A., Johanson. G. and Akesson. A. 2009. Population toxicokinetic modeling of cadmium for health risk assessment. *Environmental Health Perspectives* 117: 1293-1301.

Anderson, W.F., Chu, K.C, Chatterjee, N., Brawle y, O. and Brinton, L.A. 2001. Tumor variants by hormone receptor expression in white patients with node-negative breast cancer from the surveillance, epidemiology, and end results database. *Journal of Clinical Oncology* 19:18-27.

Anetor, J.I., Akingbola, T.S., Adeniyi, F.A.A. and Taylor, G.O. 2005. Decreased total and ionized calcium levels and haematological indices in occupational lead exposure as evidence of the endocrine disruptive effect of lead. *Indian Journal of Occupational and Environmental Medicine* 9. 1:15-21

Ansell, J. E. 1996. The blood in the hypothyroidism. In: Braveman LE, Utiger RD. eds. Werner and Ingbar's the thyroid-a fundamental and clinical text. 7th ed. Philadelphia: Lippincott-Raven: 821–825.

Antila, E., Mussalo-Rauhamaa, H., Kantola, M., Atroshi, F. and Westermarck, T. 1996. Association of cadmium with human breast cancer. *Science of the Total Environment* 186.3:251-6.

Aposhian, H.V., Zakharyam, R.A., Avram, M.D., Kopplin, M.J and Wollenberg. M.L. 2003.

Oxidation and detoxification of trivalent arsenic species. *Toxicology and Applied Pharmacology* 193: 1-8.

Arslan, A. A., Zeleniuch-Jacquotte, A., Lukanove, A., Rinaldi, S., Kaaks, R. and Toniolo, P. 2003. Reliability of follicle-stimulating hormone measurements in serum. *Reproductive Biology and Endocrinology* 1; 49.

Ashwell, M., Cole, T. J. and Dixon, A. K. 1996. Ratio of waist circumference to height is strong predictor of intra-abdominal fat. *British Medical Journal* 313.7056: 559-560.

Ashwell, M. and Hsieh, S. D. 2005. Six reasons why the waist-to-height ratio is a rapid and effective global indicator for health risks of obesity and how its use could simplify the international public health message on obesity. *International Journal of Food Science and Nutrition* 56: 303-307.

Atlas, E. L., Bidleman, T. F. and Giam, C. S. 1986. Atmospheric transport of PCB to the oceans. In: Waid JS (ed.) PCB and the environment. CRC Press, Boca Raton, FL, pp 79-100.

Atlas, E. L. and Giam, C. S. 1998. Ambient concentration and precipitation scavenging of atmospheric organic pollutants. *Water, Air and Soil Pollution* 38:19-36.

Autier, P., Hery, C., Haukka, J., Boniol, M. and Byrnes, G. J. 2009. Advanced breast cancer and breast cancer mortality in randomized controlled trials on mammography screening. *Journal of Clinical Oncology* 27: 5919-5923.

Bagga, D., Ashley, J.M., Geffrey, S.P., Wang, H.J., Barnard, R.J., Korenman, S and Heber D. 1995. Effects of a very low fat, high fiber diet on serum hormones and menstrual function. Implications for breast cancer prevention. *Cancer* 76.12:2491-2946.

Bagnardi, V., Blangiardo, M., La Vecchia, C. and Corrao, G. 2001. A meta-analysis of alcohol drinking and cancer risk. *British Journal of Cancer* 85: 1700-1705.

Balakumar, P., Kaur, T. and Singh, M. 2008. Potential target sites to modulate vascular endothelial dysfunction: current perspectives and future direction. *Toxicology* 245:49-64.

Balasch, J. 2003. Sex steroids and bone: current perspectives. *Human Reproduction Update* 9: 207-222.

Bani, D., Riva, A., Bigazzi, M. and Bani-Sacchi, T. 1994. Differentiation of breast cancer cells in vitro is promoted by the concurrent influence of myoepithelial cells and relaxin. *British Journal of Cancer* 70:900-904.

Barclay, A.W, Petocz, P., McMillan-Price, J., Flood, V.M., Prvan, T., Mitchell, P., Brand-Miller,

Oxidation and detoxification of trivalent arsenic species. *Toxicology and Applied Pharmacology* 193: 1-8.

Arslan, A. A., Zeleniuch-Jacquotte, A., Lukanove, A., Rinaldi, S., Kaaks, R. and Toniolo, P. 2003. Reliability of follicle-stimulating hormone measurements in serum. *Reproductive Biology and Endocrinology* 1; 49.

Ashwell, M., Cole, T. J. and Dixon, A. K. 1996. Ratio of waist circumference to height is strong predictor of intra-abdominal fat. *British Medical Journal* 313.7056: 559-560.

Ashwell, M. and Hsieh, S. D. 2005. Six reasons why the waist-to-height ratio is a rapid and effective global indicator for health risks of obesity and how its use could simplify the international public health message on obesity. *International Journal of Food Science and Nutrition* 56: 303-307.

Atlas, E. L., Bidleman. T. F. and Giam, C. S. 1986. Atmospheric transport of PCB to the oceans. In: Waid JS (ed.) PCB and the environment. CRC Press, Boca Raton, FL, pp 79–100.

Atlas, E. L. and Giam, C. S. 1998. Ambient concentration and precipitation scavenging of atmospheric organic pollutants. *Water, Air and Soil Pollution* 38:19–36.

Autier, P., Hery, C., Haukka, J., Boniol, M. and Byrnes, G. J. 2009. Advanced breast cancer and breast cancer mortality in randomized controlled trials on mammography screening. *Journal of Clinical Oncology* 27: 5919-5923.

Bagga, D., Ashley, J.M., Geffrey, S.P., Wang, H.J., Barnard, R.J., Korenman. S and Heber D. 1995. Effects of a very low fat, high fiber diet on serum hormones and menstrual function. Implications for breast cancer prevention. *Cancer* 76.12:2491-2946.

Bagnardi, V., Blangiardo, M., La Vecchia, C. and Corrao, G. 2001. A meta-analysis of alcohol drinking and cancer risk. *British Journal of Cancer* 85: 1700–1705.

Balakumar, P., Kaur, T. and Singh, M. 2008. Potential target sites to modulate vascular endothelial dysfunction: current perspectives and future direction. *Toxicology* 245:49-64.

Balasch, J. 2003. Sex steroids and bone: current perspectives. *Human Reproduction Update* 9: 207-222.

Bani, D., Riva, A., Bigazzi, M. and Bani-Sacchi, T. 1994. Differentiation of breast cancer cells in vitro is promoted by the concurrent influence of myoepithelial cells and relaxin. *British Journal of Cancer* 70:900–904.

Barclay, A.W., Petocz, P., McMillan-Price, J., Flood, V.M., Prvan, T., Mitchell, P., Brand-Miller,

J. C. 2008. Glycemic index, glycemic load, and chronic disease risk-a meta-analysis of observational studies. *American Journal of Clinical Nutrition* 87.3:627-637.

Bardou, V.J., Arpino, G., Elledge, R.M., Osborne, C.K. and Clark, G.M. 2003. Progesterone receptor status significantly improves outcome prediction over estrogen receptor status alone for adjuvant endocrine therapy in two large breast cancer databases. *Journal of Clinical Oncology* 21:1973-1979.

Bartholomew, L. L. and Grimes, D. A. 1998. The alleged association between induced abortion and risk of breast cancer: biology or bias? *Obstetrics and Gynecology Survey* 53:708-714.

Bartolome, B., Cordoba, S., Nieto, S., Fernandez-Herrera, J. and Garcia-Diez, A. 1999. Acute arsenic poisoning, clinical and histopathologic features. *British Journal of Dermatology* 141:1106-1109.

Barton, M., Harris, R. and Fletcher, S.W. 1999. Does this patient have breast cancer? The screening clinical breast examination: should it be done? How? *Journal of American Medical Association* 282.13: 1270-1280.

Bauer, K. R., Brown, M., Cress, R. D., Parise, C. A.and Caggiano V. 2007. Descriptive analysis of oestrogen receptor (ER)-negative, progesterone receptor (PR)-negative and HER2-negative invasive breast cancer, the so- called triple negative phenotype: A population-based study from the California cancer registry. *Cancer* 109:1721-1728.

Bax, C. M., Chatzaki, E., Chard, T. and Illes, R. K. 2000. Regulation of endometrial cancer cell growth by luteinizing hormone and follicle-stimulating hormone. *British Journal of Cancer* 83: 1730-1734.

Beato, M. and Sanchez-Pacheco, A. 1996. Interaction of steroid hormone receptors with the transcription initiation complex. *Endocrinology Reviews* 17.6: 587.

Beckmann, M.W., Niederacher, D., Schnurch, H.G., Gusterson, B.A and Bender, H.G. 1997. Multistep carcinogenesis of breast cancer and tumour heterogeneity. *Journal of Molecular Medicine* 75: 429-439.

Deiler, J.S., Zhu, K., Hunter, S., Payne-Wilks, K., Roland, C.L. and Chinchilli. V.M. 2003. A case-control study of the menstrual factors in relation to breast cancer risk in African-american women. *Journal of National Medical Association* 95:930-938.

Bello, F. and Bakari, A. G. 2012. Hypothyroidism in adults: A review and recent advances in

management. *Journal of Diabetes and Endocrinology.* 3.5:57-69.

Ben-Jonathan, N., Hugo, E. R. and Brandebourg, T. D. 2009. Effects of bisphenol-A on adipokine release from human adipose tissue: Implications for the metabolic syndrome. *Molecular and Cellular Endocrinology* 304:49-54.

Ben Josef, E., Yang, S. Y., Ji, T. H., Bidart, J. M., Garde, S. V., Chopra, D.P., Porter, A. T., Tang, D. G. 1999. Hormone-refractory prostate cancer cells express functional follicle-stimulating hormone. *Journal of Urology* 161:970-976.

Benbrahim-Tallaa, L., Tokar, E. J., Diwan, B. A., Dill, A. L., Coppin, J. and Waalkes, M. P. 2009. Cadmium malignantly transforms normal human breast epithelial cells into basal-like phenotype. *Environmental Health Perspectives.* 117:1847-1852.

Bensyl, D. M., Iuliano, D. A., Carter, M., Santelli, J. and Gilbert, B. C. 2005. Contraceptive use-United States and territories, Behavioral Risk Factor Surveillance System. *Morbidity and Mortality Weekly Report Surveillance Summaries.* 54.6:1-72.

Beral, V. 2003. Breast cancer and hormone-replacement therapy in the Million Women Study. *Lancet.* 362:419-427.

Berclaz, G., Li, S., Price, K. N, Coates, A. S., Castiglione-Gertsch, M., Rudenstam, C. M., Holmberg, S. B., Lindtner, J., Erien, D. Collins, J., Snyder, R., Thurlimann, B., Fey, M. M., Mendiola, C., Dudley Werner, I., Simoncini, E., Crivellan, D., Gelbel, R. D. and Goldhirch, A. 2004. Body mass index as a prognostic feature in operable breast cancer: the International Breast Cancer Study Group experience. *Annals of Oncology* 15.6:875-884.

Bergeron, C. 2000. Morphological changes and protein secretion induced by progesterone in the endometrium during the luteal phase in preparation for nidation. *Human Reproduction* 15: 119-128.

Bergman, A., Heindel, J.J., Jobling, S., Kidd, K. A. and Zoeller, T. R. 2013. State of the science of endocrine disrupting chemicals, 2012: Summary for decision-makers. Geneva, Switzerland.1-38 Retrieved Aug. 6, 2014 from www.unep.org/hazardoussubstances/dti/1554/cc.

Bernstein, L., Yuan, J. M, Ross, R. K, Pike, M. C., Hanisch, R., Lobo, R.Stanczyk, F., Gao, Y-T

and Henderson, B. E. 1990. Serum hormone levels in pre-menopausal Chinese women in Shangbai and white women in Los Angeles: results from two breast cancer case-control studies. *Cancer Causes Control* 1:51-58.

Bernstein, L. and Ross, R. K. 1993. Endogenous hormones and breast cancer risk. *Epidemiology Review* 15:48-65.

Betancourt, A. M., Wang, J., Sarah Jenkins, S., Mobley, J., Russo, J. and Lamartiniere C. A. 2012. Altered Carcinogenesis and Proteome in Mammary Glands of Rats after Prepubertal Exposures to the Hormonally Active Chemicals Bisphenol A and Genistein. *Journal of Nutrition* 142: 7 1382S-1388S.

Bilgen, I., Oner, G., Edremitlioglu, M., Alkan, Z.and Cirrik, S. 2003. Involvement of cholinoceptors in cadmium-induced endothelial dysfunction. *Journal of Basic Clinical Physiology and Pharmacology* 14:55-76.

Bocker, W., Bier, B., Freytag, G., Brommelkamp, B., Jarasch, E. D., Edel, G., Dockhorn-Dwomiczak, B. and Schmid, K.W. 1992. An immunohistochemical study of the breast using antibodies to basal and luminal keratins, alpha-smooth muscle actin, vimentin, collagen IV and laminin. Part II; epitheliosis and ductal carcinoma in situ. *Virchows Archiv A. Pathological Anatomy and Histopathology* 421.4: 315-322.

Bose, K. and Mascie-Taylor, C. G. N. 1998. Conicity index and waist-to-hip ratio and their relationship with total cholesterol and blood pressure in middle aged Europeans and migrant Pakistani men. *Annals of Human Biology* 25:11-16.

Bowen, R.L., Duffy, S.W., Ryan, D.A., Hart IR. and Jones JL. 2008. Early onset of breast cancer in a group of British black women. *British Journal of Cancer* 98:277-281.

Bowlin, S. J., Leske, M. C., Varma, A., Nasca, P. and Weinstein, A. 1997. Breast cancer risk and alcohol consumption: results from a large case-control study. *International Journal of Epidemiology* 26: 915-923.

Bray, F., McCarron, P. and Parkin, D. M. 2004. The changing global patterns of female breast cancer incidence and mortality. *Breast Cancer Research* 6:229-239.

Brind, J., Chinchilli, V.M., Severs, W.B., Summy-Long, J. 1996. Induced abortion as an independent risk factor for breast cancer: a comprehensive review and meta-analysis. *Journal of Epidemiology and Community Health* 50:481-496.

Brinton, L. A., Benichou, J., Gammon, M. D., Brogan, D. R. and Coates, R. 1997. Ethnicity and

variation in breast cancer incidence. *International Journal of Cancer* 73: 349–355.

Brisken, C. 2008. Endocrine disruptors and breast cancer. *Chimia* 62.5:406–409.

Brody, J. G., Moysich, K. B., Humblet, O., Attfield, K. R., Beehler, G. P., Rudel, R. A. 2007. Environmental pollutants and breast cancer: epidemiologic studies. *Cancer* 109. 12 Suppl: 2667-2711.

Brosens, J. J., Tullet, J., Varshochi, R. and Lam, E.W. 2004. Steroid receptor action. *Best Practice and Research in Clinical Obstetrics and Gynaecology* 18: 265-283.

Butt, Z., Arif, S., Ashfaq, U., Shahbaz, U., Haider, S.F. and Bukhari, M.H. 2012. Breast cancer risk factors: a comparison between pre-menopausal and post-menopausal women. *Journal of Pakistan Medical Association* 62:120.

Cade, J. E., Burley, V. J. and Greenwood, D. C. 2007. UK Women's Cohort Study Steering Group. Dietary fibre and risk of breast cancer in the UK Women's Cohort Study. *International Journal of Epidemiology* 36.2:431-438.

Calafat, A. M, Koch-Holger, M., Swan, S. H., Hauser, R., Goldman, L. R., Lanphear, B. P., Longnecker, M. P., Rudel, R. A., Teitelbaum, S. L, Whyatt, R. M. and Wolff, M. S. 2013. Misuse of blood serum to assess exposure to bisphenol A and phthalates. *Breast Cancer Research* 15.5:403.

Calafat, A. M., Kuklenyik, Z., Reidy, J. A., Caudill, S. P., Ekong, J. and Need-ham, L. L. 2005. Urinary concentrations of bisphenol A and 4-nonylphenol in a human reference population. *Environmental Health Perspectives* 113.4:391–395.

Calle, E.E., Rodriguez, C., Walker-Thurmond, K. and Thun, M. J. 2003. Overweight, obesity and mortality from cancer in a prospectively studied cohort of US adults. *New England Journal of Medicine* 348:1625-1638.

Cameron, I. T., Irvine, G. and Norman, J. E. 1996. Menstruation. In: Scientific essentials of reproductive Medicine, Eds SG Hillier, HC Kitchener and JP Neilson. London: W.B. Saunders.

Cancello, G., Maisonneuve, P., Rotmensz, N., Viale, G., Mastropasqua, M. G., Pruneri, G., Montagna, E., Iorfida, M., Mazza, M., Balduzzi, A., Veronesi, P., Luini, A., Intra, M., Goldhirsch, A. and Colleoni M. 2013. Progesterone receptor loss identifies luminal B breast cancer subgroups at higher risk of relapse *Annals of Oncology* 24.661-668.

Cancer Genome Atlas Network (CGAN). 2012. *Nature* 490: 51.70.

Carl, A. B. and Edwards, R. 2001. Tietz Fundamentals of Clinical Chemistry. 5th Ed. An imprint of Elsevier. 883.

Carpenter, C. L., Ross, R. K., Paganini-Hill, A. and Bernstein, L. 2003. Effect of family history, obesity and exercise on breast cancer risk among postmenopausal women. *International Journal of Cancer* 106.1:96-102.

Carpenter, D. O., DeCaprio, A. P., O'Hehir, D., Akhtar, F., Jonson, G., Scrudato, R. J., Apatiki L., Kava, J., Gologergen, J., Miller, P. K. and Eckstein, L. 2005. Polychlorinated biphenyls in the serum of the Siberian Yupik people from St Lawrence Island, Alaska. *International Journal of Circumpolar Health* 64.4:322-335.

Carey, L. A., Perou, C. M., Livasy, C. A., Dressler, L. G., Cowan, D., Conway, K., Karaca, G., Troester, M. A., Tse, C. K., Edmiston, S., Deming, S. L., Geradts, J., Cheang, M. C.,Nielsen, T. O., Moorman, P. G., Earp, H. S., Millikan, R. C. 2006. Race, breast cancer subtypes and survival in the Carolina Breast Study. *Journal of American Medical Association* 295:2492-2502.

Carr, B.R. 1998. Disorders of ovary and female reproductive tract In: Williams, R. H., Foster, D. W., Kronenberg, H. M., Larsen, P. R., Wilson, J. D eds. Williams text book of endocrinology. 9th ed.Philadelphia: WB Saunders.751-817.

Caserta, D., L. Maranghi, L., Mantovani, A., Marci, R., Maranghi, F. and Moscarini, M. 2008 Impact of endocrine disruptor chemicals in gynaecology. *Human Reproductive Update* 14.1: 59-72.

Casey, M. P., Cerhan, J. R. and Pruthi, S. 2008. Oral Contraceptive Use and the Risk of Breast Cancer. *Mayo Clinical Proceedings* 83.1:86-91.

Casula S and Bianco A.C. 2012. Thyroid hormone deiodinases and cancer. *Frontiers in Endocrinology*. 74.3.doi; 10.3389/fendo.2012.00074

Cavagnaro, P.F. and Galmarini, C. R. 2012. Effect of processing and cooking conditions on onion (Allium cepa L) induced antiplatelet activity and thiosulphate content. *Journal of Agriculture and Food Chemistry.* 60.35:8731-8737.

Cengiz, O., Bozkurt, B., Unal, B., Yildirim, O., Karabeyoglu, M., Eroglu, A., Kocer, B. and Ulas, M. 2004. The relationship between prognostic factors of breast cancer and thyroid disorders in Turkish women. *Journal of Surgical Oncology* 870: 19.25.

Centers for Disease Control and Prevention (CDC). 2005. Third national report on human

exposure to environmental chemicals. Atlanta (GA): Centers for Disease Control and Prevention.

Chaffin, C. L. and Stouffer, R. L. 1999. Expression of matrix metalloproteinases and their tissue inhibitor messenger ribonucleic acids in macaque periovulatory granulosa cells: time course and steroid regulation. *Biology of Reproduction* 61: 14-21.

Chaffin, C. L., Hess, D. L. and Stouffer, R. L 1999. Dynamics of periovulatory steroidogenesis in the rhesus monkey follicle after controlled ovarian stimulation. *Human Reproduction* 14: 642-649.

Chakrabarti, S. K, Bai, C. and Subramanian, K. S. 2001. DNA-protein cross links induced by nickel compounds in isolated rat lymphocytes, role of reactive oxygen species and specific amino acids. *Toxicology and Applied Pharmacology* 170:153-165.

Chakraborty, R. and Bose, K. 2009. Central adiposity, body mass index and percent body fat among Bengalee Hindu Male slum dwellers of dumdum, West Bengal, India. *The open Obesity Journal* 1:32-37.

Challier, B., Perarnau, J. M. and Viel, J. F 1998. Garlic, onion and cereal fibre as protective factors for breast cancer: a French case-control study. *European Journal of Epidemiology* 14: 737-747.

Chan, D.S. and Norat, T. 2015. Obesity and breast cancer, not only a risk factor of the disease. *Current Treatment Options in Oncology* 16.5:22. Doi: 10.1007/s11864-015-0341-9

Chandran, U., Zirpoli, G., Ciupak, G., McCann, S. E. and Gong, Z. 2013. Does alcohol increase breast cancer risk in African-American women? Findings from a case-control study. *British Journal of Cancer* 109:1945-1953.

Chariyalerstak, S., Chariyalerstak, A. and Ruangvej, V. P. 1996. Immunohistochemical detection of Estrogen and Progestrone receptors in primary breast cancer. *Asian Pacific Journal of Cancer* 16:161-166.

Charles-Davies, M.A., Arinola, O.G., Fasanmade, A.A., Olaniyi, J.A., Oyewole, O.E., Owolabi, M.O., Hassan, O.O., Ajobo, M.T., Adigun, K., Akinlade, K.S., Adebusuyi, J.R., Ebesunun, M.O., Popoola, O.O., Okunbolade, W., Fabian, U.A., Rahamon, S.K., Ogunlakin, M.A, and Agbedana. E.O. 2012. Indices of metabolic syndrome in 534 apparently healthy Nigerian traders. *Journal of US-China Medical Science* 9.2:91-100.

Chen, F. C., Oskayozcelik, G., Buhling, K. J., Kopstein, K., Mentze, M., Lichtenegger, W.,

Sehouli, J. 2009. Prognostic values of serum and ascites level of oestradiol, luteinizing hormone, follicle-stimulating hormone and prolactin in ovarian cancer. *Anticancer Research* 29: 1575-1578.

Chiedozie, C. 1985. Breast Cancer in Nigeria. *Cancer* 55:653-657.

Cho, E., Spiegelman, D., Hunter, D. J, Chen, W.Y, Colditz, G. A. and Willett, W. C. 2003. Premenopausal dietary carbohydrate, glycemic index, glycemic load, and fiber in relation to risk of breast cancer. *Cancer Epidemiology Biomarkers Preview* 12.11 Part 1:1153-1158.

Chu, K. C.and Anderson, W. F. 2002. Rates for breast cancer characteristics by estrogen and progesterone receptor status in the major racial/ethnic groups. *Breast Cancer Research and Treatment* 74:199–211.

CIA. 2009. World fact book

Ciocca, D. R., Gago, F. E., Fanelli, M. A. and Calderwood, S. K. 2006. Co-expression of steroid receptors (estrogen receptor alpha and/or progesterone receptors and Her-2/neu): Clinical implications. *Journal of Steroid Biochemistry and Molecular Biology* 102:32-40.

Clark, S. C., Adebamowo, C.A., Roda, S. and Adebamowo, E.O. 2007. Lead content of dried films of domestic paints currently sold in Nigeria. *Science and Total Environment* 388.1-3:116-120.

Clark, C. L. and Sutherland, R. L. 1990. Progestin regulation of cellular proliferation. *Endocrine Reviews* 11: 266-301.

Cleator, S., Heller, W. and Coombes, R. C. 2007. Triple-negative breast cancer: therapeutic options. *Lancet Oncology* 8:235-244.

Clemons, M. and Goss, P. 2001. Estrogen and the risk of breast cancer. *New England Journal of Medicine* 344: 276-285.

Cobleigh, M. A., Vogel, C. L., Tripathy, D., Robert, N. J., Scholl, S., Fehrenbacher, L., Wolter, J. M., Paton, V., Shak, S., Lieberman, G and Slamon, D. J. 1999. Multinational study of the efficacy and safety of humanized anti-HER2 monoclonal antibody in women who have HER2-overexpressing metastatic breast cancer that has progressed after chemotherapy for metastatic disease. *Journal of Clinical Oncology* 9: 2639-2648.

CoCritchley, O. D. and Healy, L. 1998. Collaborative reanalysis of individual data from 47

epidemiological studies in 30 countries, including 50,302 women with breast cancer and 96973 women without the disease. *Lancet* 360:187–195.

Cogliano, V., Grosse, Y., Baan, R., Straif, K., Secretan, B., ElGhissassi, F. and WHO international agency for research on cancer. 2005. Carcinogenicity of combined oestrogen-progestagen contraceptives and menopausal treatment. *Lancet Oncology* 6.8:552-553.

Colditz, G. A. and Frazier, A. L. 1995. Models of breast cancer show that risk is set by events of early life, prevention efforts must shift focus. *Cancer Epidemiology Biomarkers Preview* 4: 567-71.

Colditz, G. A., Rosner, B. A., Chen, W. Y., Holmes, M. D. and Hankinson, S. E. 2004. Risk factors for breast cancer according to estrogen and progesterone receptor status. *Journal of National Cancer Institute* 96:218-228.

Collaborative Group on Hormonal Factors in Breast Cancer (CGHFBC). 1996. Breast cancer and hormonal contraceptives: collaborative reanalysis of individual data on 53 297 women with breast cancer and 100 239 women without breast cancer from 54 epidemiological studies. *Lancet* 347:1713-1727.

Colozza, M., Larsimont, D. and Piccart, M. J. 2005. Progesterone receptor testing: not the right time to be buried. *Journal of Clinical Oncology* 23:3867-3868. author reply 3869-3870.

Conde, I., Paniagua, R., Zamora, J., Blanquez, M. J., Fraile, B., Ruiz, A. and Arenas, M. I. 2006. Influence of thyroid hormone receptors on breast cancer cell proliferation. *Annals of Oncology* 17:60-64.

Conneely, O. M., Mulac-Jericevic, B., DeMayo, F., Lydon, J. P. and O'Malley, B. W. 2002. Reproductive function of progesterone receptors. *Recent Progress in Hormone Research* 57: 339- 355.

Cousins, I. T., Staples, C. A., Klecka, G. M. and Mackay, D. 2002. A multimedia assessment of the environmental fate of bisphenol A. *Human Ecology Risk Assessment* 8: 1107-1135.

Cowley, S. M., Hoare, S., Mosselman, S. and Parker, M. G. 1997. Estrogen receptors alpha and beta form heterodimers on DNA. *Journal of Biological Chemistry* 272. 19858–19862.

Crain, D.A., Eriksen, M., Iguchi, T., Jobling, S., Laufer, H., LeBlanc, G.A. and Guillette, L.J.,

2007. An ecological assessment of bisphenol-A: evidence from comparative biology. *Reproductive Toxicology* 24: 225-239.

Critchley, O. D and Healy, D. L. 1998. Effects of estrogens and progesterone on the endometrium. In: Estrogens and progestogens in clinical practice, pp 145-161. Ed Ian S. Fraser. London, UK: Churchill Livingstone.

Crowe, J. P. Jr, Gordon, N. H., Hubay, C. A., Shenk, R. R., Zollinger, R. M., Brumberg, D. J., McGuire, W. L. and Shuck, J. M. 1991. Estrogen receptor determination and long term survival of patients with carcinoma of the breast. *Surgical Gynecology and Obstetrics* 173:273-278.

Curry, T. E. Jr and Osteen, K. G. 2003. The matrix metalloproteinase system: changes, regulation, and impact throughout the ovarian and uterine reproductive cycle. *Endocrine Review* 24: 428-65.

Czarnowska, M. and Gujska, E. 2012. Effect of freezing technology and storage conditions on folate content in selected vegetables. *Plant Foods for Human Nutrition*.67.4:401-406

Dalunoun, M., Boman, K., Cajander, S., Westin, P. and Backstroni, T. 1999. Apoptosis, proliferation, and sex hormone receptors in superficial parts of human endometrium at the end of the secretory phase. *The Journal of Clinical Endocrinology and Metabolism* 84: 1737-1743.

Dairkee, S. H., Puell, L. and Hackett, A. J. 1988. Expression of basal and luminal epithelium-specific keratins in normal, benign and malignant breast tissue. *Journal of the National Cancer Institute* 80: 691-695.

Daling, R. J, Malone, K. E., Voigt, L. F., White, E. and Weiss, N. S. 1994. Risk of breast cancer among young women: relationship to induced abortion. *Journal of National Cancer Institute* 86:1584-921.

Danilova, N. 2006. The evolution of immune mechanisms. *Journal of Experimental Zoology, Molecular Biology, Development and Evolution* 306.6:496-520.

Davey, J.C., Nomikos, A.P., Wungjiranirun, M., Sherman, J.R., Ingram. L., Batki, C., Lariviere, J.P. and Hamilton, J.W. 2008. Arsenic as an endocrine disruptor: arsenic disrupts retinoic acid receptor and thyroid hormone receptor-mediated gene regulation and thyroid hormone mediated amphibian tail metamorphosis. *Environmental Health Perspectives* 116.2:165-172.

Davies, C., Godwin, J., Gray, R., Clarke, M., Cutter D, Darby, S., McGale, P., Pan. H. C., Taylor, C., Wang, Y. C., Dowsett, M., Ingle, J, Peto, R. and Early Breast Cancer Trialists' Collaborative Group (EBCTCG). 2011. Relevance of breast cancer hormone receptors and other factors to the efficacy of adjuvant tamoxifen: patient-level meta-analysis of randomised trials. *Lancet* 378:771-784.

Davis, D.L., Bradlow, H.L., Wolff, M., Woodruff, T., Hoel, D.G and Anton-Culver, H. 1993. Medical hypothesis: xenoestrogens as preventable causes of breast cancer. *Environmental Health Perspectives* 101:372-377

De Bont, R. and van Larebeke, N. 2004. Endogenous DNA damage in humans: a review of quantitative data. *Mutagenesis* 19.3:169-185.

Deepti, G., Veena, G., Nisha, M., Meenu, G., Sumiti, G., Gopal, G., Promil, J., and Rajeev, S. 2015. Correlation of Hormone Receptor Expression with Histologic Parameters in Benign and Malignant Breast Tumors. *Iranian Journal of Pathology* 10.1: 23–34.

de Lima, F. E., do Rosário Dias de Oliveira Latorre M, de Carvalho Costa M. J. and Fisberg, R. M. 2008. Diet and cancer in Northeast Brazil: evaluation of eating habits and food group consumption in relation to breast cancer. *Cad Saude Publica* 244:820-828.

De Maeyer, L., Van Limbergen, E., De Nys, K., Moerman, P., Pochet, N., Hendrickx, W., Wildiers, H., Paridaens, R., Smeets, A., Christiaens, M. R., Vergote, I., Leunen, K., Amant, F. and Neven, P. 2008. Does estrogen receptor negative/progesterone receptor positive breast carcinoma exist? *Journal of Clinical Oncology* 26:335-336. author reply 336-338.

De Stefani, E., Correa, P., Ronco, A., Mendilaharsu, M., Guidobono, M. and Deneo-Pellegrini, H. 1997. Dietary fiber and risk of breast cancer: a case-control study in Uruguay. *Nutrition and Cancer* 28:14-19.

Deng, G., Lu, Y., Zlotnikov, G., Thor, A. D and Smith, H. S 1996. Loss of heterozygosity in normal tissue adjacent to breast carcinomas. *Science* 274: 2057–2059.

Depress, J. P. and Lemieux, I. 2006. Abdominal obesity and metabolic syndrome. *Nature* 444:881-887.

Denner, O.C., 1977. Bisphenol-A. In: McKetta, J.J. (Ed.), Encyclopedia of Chemical Processing and Design. Marcel Dekker, Inc., New York, USA.

Di Pietro, P. F., Medeiros, N. I., Vieira, F. G., Fausto, M. A. and Belló-Klein, A. 2007. Breast

cancer in Southern Brazil: association with past dietary intake. Nutr Hosp. 22.5:565-572.

Dickerman, Z. and De Vries, L. 1997. Prepubertal and pubertal growth, timing and duration of puberty and attained adult height in patients with congenital hypothyroidism (CH) detected by the neonatal screening programme for CH. A longitudinal study. Clinical Endocrinology 47: 649-654

Dickerson, L. M, Shrader, S. P. and Diaz, V. A. 2008. Chapter 8: Contraception". In Wells BG, DiPiro, J. T, Talbert, R. L, Yee, G.C., Matzke, G. R. Pharmacotherapy: a pathophysiologic approach. McGraw-Hill Medical. 1313–1328.

Dignam, J. J, Wieand, K., Johnson, K. A, Raich, P., Anderson, S. J. and Somkin, C. 2005. Effects of obesity and race on prognosis in lymph node-negative, estrogen receptor-negative breast cancer. *Breast Cancer Research and Treatment* 1-10.

Dinda, S., Sanchez, A. and Moudgil, V. 2002. Estrogen-like effects of thyroid hormone on the regulation of tumor suppressor proteins, p53 and retinoblastoma, in breast cancer cells. *Oncogene* 21:761-768.

Distiller, L. A, Sagel, J., Morley, J. E., Oxenham, E. 1975. Assessment of pituitary gonadotropin reserve using luteinizing hormone-releasing hormone (LRH) in states of altered thyroid function. *Journal of Clinical Endocrinol Metabolism* 40:512–515.

Do, M. H., Lee, S. S. Kim, J. Y., Jung, P. J. and Lee, M. H 2007. Fruits, vegetables, soy foods and breast cancer in pre- and postmenopausal Korean women: a case-control study. *International Journal of Vitamins and Nutrition Research* 77.2:130-141.

Dodds, E.C., Goldberg, L., Lawson, W. and Robinson, R., 1938. Oestrogenic activity of certain synthetic compounds. *Nature* 141: 247-248.

Dodds, E.C. and Lawson, W., 1936. Synthetic oestrogenic agents without the phenan-threne nucleus. *Nature* 137: 996.

Donohoe, C. L., Doyle, S. L. and Reynolds, J. V. 2011. Visceral adiposity, insulin resistance and cancer risk. *Diabetology and Metabolic Syndrome* 3: 12. PMC 3145556.

Dotzlaw, H., Leygue, E., Watson P. H. and Murphy, L. C. 1997. Expression of estrogen receptor-beta in human breast tumour. *Journal of Clinical Endocrinology and Metabolism* 82: 2371-2374.

Drabsch, Y., Hugo, H., Zheng, R., Dowhan, D. H., Miao, Y. R., Gewirtz, A. M., Barry, S. C.,

Ramsay, R.G., Gonda, T. J. 2007. Mechanism of and requirement for estrogen-regulated MYB expression in estrogen-receptor positive breast cancer cells. *Proceedings of National Academy of Science USA* 104:1376-1377.

Dumitrescu, R. G.dand Shields, P. G 2005. The etiology of alcohol-induced breast cancer. *Alcohol* 35: 213–225.

Dunnwald, L. K., Rossing, M. A. and Li C. 1. 2007. Hormone receptor status, tumor characteristics, and prognosis: a prospective cohort of breast cancer patients. *Breast Cancer Research* 9:R6.

Durosinmi, M. A. 2008. Cancer control in an economically disadvantaged setting: Nigeria. INCTR Newsletter. Retrieved Aug., 9, 2014 from www.inctr.org/publications/2004_v05-n01_s02.shtml.

Dvorak, H. F. 1986. Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing. *New England Journal of Medicine* 315:1650-1659.

Egbe, A. E. 2007. Evaluation of some breast cancer risk factors in overweight and obese women. M.Sc. Dissertation. Dept. of Chemical Pathology, University of Ibadan. xi +111pp.

Eliassen, A.H., Missimer, S. A., Tworoger, S. S., Spiegelman, D., Barbieri, R. L., Dowsett, M. and Hankinson, S. E. 2006. Endogenous steroid hormone concentration and risk of breast cancer among premenopausal women. *Journal of National Cancer Institute* 98:1406-1415.

Elumelu, T. N., Adenipekun, A.A., Abdus-salam A.A. and Bojude A.D. 2011. Pattern of breast cancer metastasis at the radiotherapy clinic, Ibadan-A ten year review. *Journal of American Science* 7.7:906-912.

Enmark E, and Gustafsson J.A. 1999. Oestrogen receptors-an overview. Journal of Internal Medicine. 246.2:133-138

Environment Canada, 2008. Screening Assessment for the Challenge: Phenol, 4.4 '-(1-Methylethylidene) bis-Bisphenol A. Chemical Abstracts Service Registry Number 80-05-7. Gatineau, Quebec, Retrieved on January 20, 2012, from http://www.ec.gc.ca/esc-ces/default.asp? lang¼En&n¼3C756383-1#a9.

Environmental Protection Agency (EPA), 1987. Cadmium. Washington (DC): Environmental Protection Agency.

European Food Safety Authority Scientific (EFSA).Opinion.2009. Cadmium in food.

The European Food Safety Authority Journal 980:1-139.

Evans, R. M. 1988. The steroid and thyroid hormone receptor super family. *Science* 240.4854:889-895.

Fabian, U. A., Charles-Davies, M. A., Fasanmade, A. A., Olaniyi, J. A., Oyewole, O. E., Owolabi, M. O., Adebusuyi, J. R., Hassan, O., Ajobo, B. M., Ebesunun, M. O., Adigun, K., Akinlade, K. S., Arinola, O. G., Agbedana, E. O. 2015. Sex hormones and their relationship with leptin and cardiovascular risk factors in pre and post menopausal Nigerian women with metabolic syndrome. *Cardiology and Angiology: An International Journal*. 3.3:149-156.

Fagherazzi, G., Chabbert-Buffet, N., Fabre, A., Guillas, G., Boutron-Ruault, M-C., Mesrine, S. and Clavel-Chapelon, F. 2012. Hip circumference is associated with the risk of premenopausal ER-/PR- breast cancer. *International Journal Obesity (Lond)*. 36.3:431-439.

Fan, H. Y., Cheng, X. and Richards, J. S. 2007. FSH induces multiple signaling cascades: evidence that activation of Rous sarcoma oncogene, RAS and epidermal growth factor receptor are critical for differentiation. *Molecular Endocrinology* 21:1940-1957.

Farahat, F. M, Ellison, C. A., Bonner, M. R., McGarriagle, B. P., Crane, A. L. and Fenske, R. A 2011. Biomarkers of chlorpyrifos exposure and effect in Egyptian cotton field workers. *Environmental Health Perspectives* 119:801-806.

Feigelson, H. S., Jonas, C. R., Teras, L. R., Thun, M. J. and Calle, E. E. 2004. Weight gain, body mass index, hormone replacement therapy, and postmenopausal breast cancer in a large prospective study. *Cancer Epidemiology Biomarkers Preview* 13.2:220-224.

Ferlay, J., Bray, F., Pisani, P. and Parkin, D. M 2001: GLOBOCAN 2000: *Cancer Incidence, Mortality and Prevalence Worldwide. IARC Cancer Base No. 5. [1.0]*. Lyon, France: IARC.

Fernandez, S. V. and Russo, J. 2010. Estrogen and xenoestrogens in breast cancer. *Toxicology and Pathology* 38.1:110-122.

Ferrero-Pous, M., Trassard, M. and Le Doussal, V. et al. 2001. Comparison of enzyme

immunoassay and immunohistochemical measurements of estrogen and progesterone receptors in breast cancer patients. *Applied Immunohistochemistry and Molecular Morphology* 9: 267-75.

Fisher, B., Jeong, J. H., Bryant, J., Anderson, S., Dignam, J., Fisher, E. R. and Wolmark, N 2004. Treatment of lymph-node-negative, oestrogen-receptor-positive breast cancer: long-term findings from National Surgical Adjuvant Breast and Bowel Project randomised clinical trials. *Lancet* 364:858-868.

Fisher, B., Redmond, C., Fisher, E. R. and Caplan, R. 1988. Relative worth of estrogen or progesterone receptor and pathologic characteristics of differentiation as indicators of prognosis in node negative breast cancer patients: findings from National Surgical Adjuvant Breast and Bowel Project Protocol B-06. *Journal of Clinical Oncology* 6:1076-1087.

Fitzpatrick, S. L., Carlone, D. L., Robker, R. L. and Richards, J. 1997. Expression of aromatase in the ovary: Down-regulation of mRNA by the ovulatory luteinizing hormone surge. *Streoids* 62:197-206.

Flint, S., Markle, T., Thompson, S. and Wallace E. 2012. Bisphenol-A exposure, effects and policy; a wildlife perspective. *Journal of Environmental Management* 104:19-34.

Florea, A.M. and Busselberg, D. 2008. Arsenic trioxide in environmentally and clinically relevant concentrations interacts with calcium homeostasis and induces cell type specific cell death in tumour and non tumour cells. *Toxicology Letters* 179.1:34-42.

Florea, A. M. and Busselberg, D. 2011. Metals and breast cancer: risk factors or healing agents? *Journal of Toxicology* 1-8.

Florea, A. M., Splettstoesser, F. and Busselberg, D. 2007. Arsenic trioxide induced calcium signals and cytotoxicity in two human cell lines: SY-5Y neuroblastoma and 293 embryionic kidney (HEK). *Toxicology and Applied Pharmacology* 220.3:292-301.

Foidart, J. M., Colin, C., Denoo, X., Desreux J., Beliard A., Fournier S. and de Ligueres B 1998. Estradiol and progesterone regulate the proliferation of human breast epithelial cells. *Fertility and Sterility* 69:963-969.

Fowler, P. A., Sorsa-Leslie, T., Harris, W. and Mason, H. D. 2003. Ovarian gonadotrophin

surge-attenuating factor (GnSAF): where are we after 20 years of research? *Reproduction* 126.6: 689-699.

Fowler, P. B., McIvor, J., Sykes, L. and Macrae, K. D. 1996. The effect of long-term thyroxine on bone mineral density and serum cholesterol. *Journal of Royal College of Physicians London* 30:527-532.

Foxcroft, L. M., Evans, E. B. and Porter, A. J. 2004. The diagnosis of breast cancer in women younger than 40 years. *Breast* 13:297-306.

Francis, J. M., Grosskurth, H., Changalucha, J., Kapiga, S. H. and Weiss, H. A. 2014. Systematic review and meta-analysis: prevalence of alcohol use among young people in eastern Africa. *Tropical Medicine and International Health* 19.4:476–488.

Frenkel, K. 1992. Carcinogen-mediated oxidant formation and oxidative DNA damage. *Pharmacology Therapy* 53:127-166.

Freudenheim, J. L., Marshall, J. R., Vena, J. E., Laughlin., R. Brasure, J. R., Swanson, M. K., Nemoto, T. and Graham, S. 1996. Premenopausal breast cancer risk and intake of vegetables, fruits, and related nutrients. *Journal of National Cancer Institute* 88 .6:340-348.

Friedenreich, C. M. 2001. Review of anthropometric factors and breast cancer risk. *European Journal of Cancer Preview* 10.1:15–32.

Frumklin, M. C., Wang, J. and Stetz, T. A. 2001. Structure of the replicating complex of the pol alpha family DNA polymerase. *Cell* 105.5: 657-667.

Fujishiro, H., Yano, Y., Takada, Y., Tanihara, M. and Himeno, S. 2012. Roles of ZIP8, ZIP14, and DMT1 in transport of cadmium and manganese in mouse kidney proximal tubule cells. *Metallomics* 4: 700-708.

Fuqua, S. A., Cui, Y., Lee, A. V., Osborne, C. K. and Horwitz, K. B. 2005. Insights into the role of progesterone receptors in breast cancer. *Journal of Clinical Oncology* 23:931-932. Author's reply 932–933.

Gago, F. E., Fanelli, M. A. and Ciocca, D. R. 2006. Co-expression of steroid hormone receptors (estrogen receptor alpha and/or progesterone receptor) and HER2/neu (c-erbB-2) in breast cancer: Clinical outcome following tamoxifen based adjuvant therapy. *Journal of Steroid Biochemistry and Molecular Biology* 98:36-40.

Gajdose, C., Tartter, P. L. and Bleweiss, I. J. 2000. Stage 0 to 3 breast cancer in young women.

Journal of American College of Surgeons 190:525-529.

Gakwaya, A., Kigula-Mugambe, J. B., Kavuma, A., Luwaga, A., Fualal, J., Jombwe, J., Galukande, M. and Kanyike, D. 2008. Cancer of the breast: 5-year survival in a tertiary hospital in Uganda. *British Journal of Cancer* 99: 63-67.

Galic, S., Oakill, J. S., Steinberg, G. R. 2010. Adipose tissue as an endocrine organ. *Molecular Cell and Endocrinology* 316: 129-139.

Gallagher, T. F., Fukushima, D. K., Noguchi, S., Fishman, J., Bradlow, H. L., Cassouto, J., Zumoff, B. and Hellman, L. 1966. Recent studies in steroid hormone metabolism in man. *Recent Progress in Hormone Research* 22:283–303.

Gammon, M. D., Neugut, A. I., Santella, R. M., Teitelbaum, S.L., Britton, J. A., Terry, M. B., Eng, S. M., Wolff, M. S., Stellman, S. D., Kabat, G. C., Levin, B., Bradlow, H. L., Hatch, M., Beyea, J., Camann, D., Trent, M., Senie, R. T., Garbowski, G. C., Maffeo, C., Montalvan, P., Berkowitz, G.S., Kemeny, M., Citron, M., Schnabe, F., Schuss, A., Hajdu, S., Vineguerra, V., Collman, G. W and Obrams, G. I. 2002. The Long Island breast cancer study project, description of a multi-institutional collaboration to identify environmental risk factors for breast cancer. *Breast Cancer Research and Treatment* 74:235–254.

Garcia-Morales, P., Saceda, M., Kenney, N., Kim, N., Salomon, D. S., Gottardis, M. M., Solomon, H. B., Sholler, P. F., Jordan, V. C and Martin, M. B. 1994. Effect of cadmium on estrogen receptor levels and estrogen-induced responses in human breast cancer cells. *Journal of Biological Chemistry* 269 24:16896-16901

Gaub MP, Bellard M, Scheuer I, Chambon P. and Sassone-Corsi P.1990 Activation of the ovalbumin gene by the estrogen receptor involves the fos-jun complex. *Cell* 63:1267–1276.

Gaudet, M. M., Britton J. A., Kabat, G. C., Steck-Scott, S., Eng, S. M., Teitelbaum, S. L., Terry, M. B., Neugut, A. I. and Gammon, M. D. 2004. Fruits, vegetables, and micronutrients in relation to breast cancer modified by menopause and hormone receptor status. *Cancer Epidemiology Biomarkers Preview* 13.9:1485-1494.

Gellersen, B. and Brosens, J. J. 2003. Cyclic AMP and progesterone receptor cross-talk in human endometrium: a decidualizing affair. *The Journal of Endocrinology* 178: 357-372.

Genazzani, A. R, Stomati, M., Morittu, A., Bernardi, F., Monteleone, P., Casarosa, E., Gallo, R.,

144

Salvestroni, C. and Luisi, M. 2000. Progesterone, progestagens and the central nervous system *Human Reproduction* 15:14-27.

Gill, T., Chittlebourough, C. and Taylor, A. 2003. Body mass index, waist hip ratio and waist circumference; which measure to classify obesity? *Soz Preventivemed* 48:191-200.

Gingras, S., Cote, S. and Simard, J. 2001. Multiple signal transduction pathways mediate interleukin-4 induced 3-beta-hydroxysteroids dehydrogenase/delta 5-delta 4 isomerase in normal and tumour target tissues. *Journal of Steroid Biochemistry and Molecular Biology* 76:213-225.

Girotti, A. W. and Thomas, J. P. 1984. Damaging effects of oxygen radicals on resealed erythrocyte ghosts. *Journal of Biological Chemistry* 259.3:17441752.

Giustarini, E., Pincheta, A., Fierabracci, P., Roncella, M., Fustaino, L., Mammoli, C.and Giani, C. 2006. Thyroid autoimmunity in patients with malignant and benign breast diseases before surgery. *European Journal of Endocrinology* 154:645-649.

Glass, C. K., Rose, D. W. and Rosenfeld, M. G. 1996. Nuclear receptor coactivators. *Current Opinion in Cell Biology* 9.222–232.

Gobbi, H., Rocha, R. M. and Nunes. C. B. 2008. Predictive factors of breast cancer evaluated by immunohistochemistry. *Journal of Brasilian Pathology and Medical Laboratory* 44.2: 131-140.

Gogas, J., Kouskos, E., Tseleni-Balafouta, S., Markopoulos, C., Revenas, K., Gogas, G. and Kostakis, A. 2001. Autoimmune thyroid disease in women with breast carcinoma. *European Journal of Surgical Oncology* 27: 626-630.

Goldhirsch, A., Wood, W. C, Gelber, R. D., Coates, A. S. Thurlimann, B. and Senn, H. J. 2003. Meeting highlights; updated international expert consensus on the primary therapy of early breast cancer. *Journal of Clinical Oncology* 21:3357-3365.

Goldin, B. R., Adlercreutz, H., Gorbach, S. L., Warram, J. H., Dwyer, J. T., Swenson L. and Woods, M. N. 1982. Estrogen excretion patterns and plasma levels in vegetarian and omnivorous women. *New England Journal of Medicine* 307:1542-1547.

Goldman, M. B. 1990. Thyroid diseases and breast cancer. *Epidemiologic Reviews* 12: 16-28.

Gonullu, G., Ersoy, C., Ersoy, A., Evrensel, T., Basturk, B., Kurt, E., Oral, K., Gokgos, S and

Manavoglu, O. 2005. Relation between insulin resistance and serum concentrations of IL-6 and TNF-alpha in overweight or obese women with early stage breast cancer. *Cytokine* 31.4:264-269.

Goodwin, P. J., Ennis, M., Pritchard, K. I., Trudeau, M. E., Koo, J., Madamas, Y., Hartwick, Y., Hoffman, B. and Hood, N. 2002. Fasting insulin and outcome in early-stage breast cancer: results of a prospective cohort study. *Journal of Clinical Oncology* 20:42-51.

Gordon, G. G. and Southren, A. L. 1977. Thyroid-hormone effects on steroid-hormone metabolism. *Bull New York Academy of Medicine* 53:241‾259.

Gould, M. N. 1993. Cellular and molecular aspects of the multistage progression of mammary carcinogenesis in humans and rats. *Seminars in Cancer Biology* 4:161–169.

Gown, A. M. 2008. Current issues in ER and HER-2 testing by IHC in breast cancer. *Modern Pathology* 21:S8-S15.

Graham, J. D. and Clarke, C. L. 1997. Physiological Action of Progesterone in Target Tissues. *Endocrine Reviews* 18: 502-519.

Grann, V. R., Troxel, A. B., Zojwalla, N. J., Jacobson, J. S., Hershman, D. and Neugut, A. I. 2005. Hormone receptor status and survival in a population-based cohort of patients with breast carcinoma. *Cancer* 103:2241-2251.

Gray, J., Evans, N., Taylor, B., Rizzo, J. and Walker, M. 2009. State of the evidence: the connection between breast cancer and the environment. *International Journal of Occupational and Environmental Health* 15:43-78.

Green, S., Walter, P., Greene, G., Krust, A., Goffin, C., Jensen, E., Scrace, G., Waterfield, M. and Chambon, P. 1986. Cloning of the human oestrogen receptor cDNA. *Journal of Steroid Biochemistry* 24: 77–83.

Greene, G. L., Gilna, P., Waterfield, M., Baker, A., Hort, Y. and Shine, J. 1986. Sequence and expression of human estrogen receptor complementary DNA. *Science* 231:1150-1154.

Greenwald, G. and Roy, S. 1994. Follicular development and its control in: Knobil E., Neill J., editors. The physiology of reproduction. New York. Ravern Press. 629-724.

Greiner, E., Kaelin, T. and Nakamura, K. 2007. Bisphenol A. CEH Report by SRI Consulting

Grun, F. and Blumberg B. 2009. Endocrine disruptors as obesogens. *Molecular and Cellular Endocrinology* 304.1-2; 19-29

Guelstein, V. I., Tchypysheva, T. A., Ermilova, V. D., Litvinova, L. V., Troyanovsky, S. M. and Bannikov, G. A. 1988. Monoclonal antibody mapping of keratins 8 and 17 and of vimentin in normal human mammary gland, benign tumors, dysplasias and breast cancer. *International Journal of Cancer* 42:147–153.

Guigon, C. J., Kim, D. W., Willingham, M. C. and Cheng, S.Y. 2011. Mutation of thyroid receptor-beta in mice predisposes to the development of mammary tumours. *Oncogene* 30.30:3381-3390.

Guo, H. and Bai, O. 2008. Relationship between the expression of ER, PR, Her -2 in breast cancer and its clinical pathological features. *Chinese Journal of Laboratory Diagnostics* 12:1390-1392.

Gusterson, B. A., Warburton, M. J., Mitchell, D., Ellison, M., Neville, A. M. and Rudland, P. S. 1982. Distribution of myoepithelial cells and basement membrane proteins in the normal breast and in benign and malignant breast diseases. *Cancer Research* 42:4763-4770.

Guyton, A. C. and Hall, J. E. 2000. Text book of Medical Physiology. 10th Ed. Saunders. Pp 56-59.

Halliwell, B. and Aruoma, O. I. 1991. DNA damage by oxygen-derived species. *FEBS Letters* 281:9-19.

Hamajima, N., Hirose, K., Tajima, K., Rohan, T. and Calle, E. E. 2002. Alcohol, tobacco and breast cancer-collaborative reanalysis of individual data from 53 epidemiological studies, including 58,515 women with breast cancer and 95,067 women without the disease. *British Journal of Cancer* 87: 1234-1245.

Hamilton, A. S. and Mack, T. M. 2003. Puberty and genetic susceptibility to breast cancer in a case-control study in twins. *New England Journal of Medicine* 348.23:2313-2322.

Hammond, M. E., Hayes, D. F., Dowsett, M., Allred, D. C., Hagerty, K. L., Badve, S., Fitzgibbons, P. L., Francis, G., Goldstein, N. S., Hayes, M., Hicks, D. G., Lester, S., Love, R., Mangu, P. B., McShane, L., Miller, K., Osborne, C. K., Paik, S., Perlmutter, J., Rhodes, A., Sasano, H., Schwartz, J. N., Sweep, F. C., Taube, S., Torlakovic, E. E., Valenstein, P., Viale, G., Visscher, D., Wheeler, T., Williams, R. B. 2010. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. *Journal of Clinical Oncology* 28:2784-2795.

Hampton, A. L. and Salamonsen, L. A. 1994. Eodometrial expression of messenger ribonucleic acid encoding matrix metalloproteinases and their tissue inhibitors coincides with menstruation. *The Journal of Endocrinology* 141: R1–R3.

Hanahan, D. and Weinberg, R. A. 2000. The hallmarks of cancer. *Cell* 100: 57-70.

Hankinson, S. E., Willet, W. C., Manson, J. E., Colditz, G. A., Hunter, D. J., Spiegelman, D., Barbieri, R. L. and Speizer, F. E. 1998. Plasma sex steroid levels and risk of breast cancer in postmenopausal women. *Journal of National Cancer Institute* 90: 1292-1299.

Hansen, R. K. and Bissell, M. J. 2000. Tissue architecture and breast cancer: the role of extracellular matrix and steroid hormones. *Endocrine-Related Cancer* 7: 95–113.

Harris, J.R., Lippman, M. E., Veronesi, U. and Willett, W. 1992. Breast Cancer (3). *New England Journal of Medicine* 327:473-480.

Harris, P. J., Roberton, A.M, Watson, M. E., Triggs, C. M. and Ferguson, L. R. 1993. The effects of soluble-fiber polysaccharides on the adsorption of a hydrophobic carcinogen to an insoluble dietary fiber. *Nutrition and Cancer* 19.1:43-54.

Haslam, S. Z., Osuch, J. R., Raafat, A. M. and Hofseth, L. J. 2002. Postmenopausal hormone replacement therapy : effects on normal mammary gland in humans and in a mouse postmenopausal model. *Journal of Mammary Gland Biology Neoplasia* 7:93-105.

Hartwig, A., Asmuss, M., Ehleben, I., Herzer, U., Kostelac, D., Pelzer, A., Schwerdtle, T and Burkle, A. 2002. Interference by toxic metal ions with DNA repair processes and cell cycle control: molecular mechanisms. *Environmental Health Perspectives* 5:797-799.

Heck, K. E. and Pamuk, E. R. 1997. Explaining the relation between education and postmenopausal breast cancer. *American Journal of Epidemiology* 145:366-372.

Hefti, M. M., Hu, R., Knoblauch, N.W., Collins, L. C., Haibe-Kains, B., Tamimi, R. M. and Beck, A. H. 2013. Estrogen receptor negative/progesterone receptor positive breast cancer is not a reproducible subtype. *Breast Cancer Research* 15:R68.

Hei, T. K. and Filipic, M. 2004. Role of oxidative damagein the genotoxicity of arsenic. *Free Radical. Biology and Medicine* 37:574–581.

Heldring, N., Pike, A., Andersson, S., Matthews, J., Cheng, G., Hartman, J., Tujague, M., Ström, A., Treuter, E., Warner, M. and Gustafsson, J-A. 2007. Estrogen Receptors: How Do They Signal and What Are Their Targets? *Physiological Reviews* 87.3: 905-931.

Helzlsouer, K. J., Alberg, A. J., Bush, T. L., Longcope, C., Gordon, G. B. and Comstock, G. W.

Hampton, A. L. and Salamonsen, L. A. 1994. Endometrial expression of messenger ribonucleic acid encoding matrix metalloproteinases and their tissue inhibitors coincides with menstruation. *The Journal of Endocrinology* 141: R1–R3.

Hanahan, D. and Weinberg, R. A. 2000. The hallmarks of cancer. *Cell* 100: 57-70.

Hankinson, S. E., Willet, W. C., Manson, J. E., Colditz, G. A., Hunter, D. J., Spiegelman, D., Barbieri, R. L. and Speizer, F. E. 1998. Plasma sex steroid levels and risk of breast cancer in postmenopausal women. *Journal of National Cancer Institute* 90: 1292-1299.

Hansen, R. K. and Bissell, M. J. 2000. Tissue architecture and breast cancer: the role of extracellular matrix and steroid hormones. *Endocrine-Related Cancer* 7: 95–113.

Harris, J.R., Lippman, M. E., Veronesi, U. and Willett, W. 1992. Breast Cancer (3). *New England Journal of Medicine* 327:473-480.

Harris, P. J., Roberton, A.M, Watson, M. E., Triggs, C. M. and Ferguson, L. R. 1993. The effects of soluble-fiber polysaccharides on the adsorption of a hydrophobic carcinogen to an insoluble dietary fiber. *Nutrition and Cancer* 19.1:43-54.

Haslam, S. Z., Osuch, J. R., Raafat, A. M. and Hofseth, L. J. 2002. Postmenopausal hormone replacement therapy : effects on normal mammary gland in humans and in a mouse postmenopausal model. *Journal of Mammary Gland Biology Neoplasia* 7:93-105.

Hartwig, A., Asmuss, M., Ehleben, I., Herzer, U., Kosteloc, D., Pelzer, A., Schwerdtle, T and Burkle, A. 2002. Interference by toxic metal ions with DNA repair processes and cell cycle control: molecular mechanisms. *Environmental Health Perspectives* 5:797-799.

Heck, K. E. and Pamuk, E. R. 1997. Explaining the relation between education and postmenopausal breast cancer. *American Journal of Epidemiology* 145:366-372.

Hefti, M. M., Hu, R., Knoblauch, N.W., Collins, L. C., Haibe-Kains, B., Tamimi, R. M. and Beck, A. H. 2013. Estrogen receptor negative/progesterone receptor positive breast cancer is not a reproducible subtype. *Breast Cancer Research* 15:R68.

Hei, T. K. and Filipic, M. 2004. Role of oxidative damage in the genotoxicity of arsenic. *Free Radical, Biology and Medicine* 37:574–581.

Heldring, N., Pike, A., Andersson, S., Matthews, J., Cheng, G., Hartman, J., Tujague, M., Ström, A., Treuter, E., Warner, M. and Gustafsson, J-A. 2007. Estrogen Receptors: How Do They Signal and What Are Their Targets? *Physiological Reviews* 87.3: 905.931.

Helzlsouer, K. J., Alberg, A. J., Bush, T. L., Longcope, C., Gordon, G. B. and Comstock, G. W.

1994. A prospective study of endogenous hormones and breast cancer. *Cancer Detection Preview* 18:79-85.

Hennessey, S., Huszti, E., Gunasekura, A., Salleh, A., Martin, L., Minkin, S., Chavez, S and Boyd, N.F. 2014. Bilateral symmetry of breast tissue composition by magnetic resonance in young women and adults. *Cancer Causes and Controls* 25.4:491-497.

Hernandez, L., Nucz-Villar, M. J., Martinez-Arribas, F., Pollan, M. and Schneider, J. 2005. Circulating hormone levels in breast cancer patients, correlation with serum tumour markers and the clinical and biological features of tumours. *Anticancer Research* 25:451-454.

Hiatt, R. A. and Bawol, R. D. 1984. Alcoholic beverage consumption and breast cancer incidence. *American Journal of Epidemiology* 120:676-683.

Hiatt, R. A., Klatsky, A. L. and Armstrong, M. A. 1988. Alcohol consumption and the risk of breast cancer in a prepaid health plan. *Cancer Research* 48: 2284–2287.

Hibbett, M. L., Stouffer, R. L, Wolf, D. P. and Zelinski-Wooten, M. B. 1996. Midcycle administration of a progesterone synthesis inhibitor prevents ovulation in primates. Proceedings of the National Academy of Sciences of the United States of America. 93:1897–1901.

Hinkula, M., Pukkala, E., Kyyronen, P. and Kauppila, A. 2001. Grand multiparity and risk of breast cancer: Population-based study of Finland. *Cancer Couse Control* 12: 491-500.

Hirose, K., Matsuo, K., Iwata, H. and Tajima, K. 2005. Dietary patterns and the risk of breast cancer in Japanese women. *Cancer Science* 98.9:1431-1438.

Ho, S.Y., Lam, T.H. and Janus, E.D. 2003. Hong Kong Cardiovascular Risk Factor Prevalence Study Steering Committee. Waist to stature ratio is more strongly associated with cardiovascular risk factors than other simple anthropometric indices. *Annals of Epidemiology* 13:683-691.

Ho. C. C. K., Rohaizak, M., Zulkifli, S. Z., Siti-Aishah, M. A., Nor-Aini. U. and Sharifah-Noor-Akmal, S. H. 2009. Serum sex hormone levels in pre- and postmenopausal breast cancer patients *Singapore Medical Journal* 50.5:513-518.

Horwitz, K.B., Jackson, T. A., Bain, D. L., Richer, J.K., Takimoto, G.S and Tung. L. 1996. Nuclear receptor co-activators and co-repressors. *Molecular Endocrinology* 10.10:1167-1177.

Horwitz, K. B., Koseki, Y. and McGuire, W. L. 1978. Estrogen control of progesterone receptor in human breast cancer: role of estradiol and antiestrogen. *Endocrinology* 103:1742-1751.

Horwitz, K. B. and McGuire W. L. 1975. Predicting response to endocrine therapy in human breast cancer: a hypothesis. *Science* 189:726-727.

Horwitz, K. B. and McGuire, W. 1978. Estrogen control of progesterone receptor in human breast cancer, correlation with nuclear processing of estrogen receptor. *Journal of Biological Chemistry* 253:2223-2228.

Horwitz, K. B. and McGuire, W. L. 1979. Estrogen control of progesterone receptor induction in human breast cancer: role of nuclear estrogen receptor. *Advanced Experimental Medicine and Biology* 117:95-110.

Hou, L., Zhang, X., Wang, D. and Baccarelli, A. 2012. Environmental chemical exposures and human epigenetics. *International Journal of Epidemiology* 41.1:79-105.

Howard, P.H., 1989. Handbook of Environmental Fate and Exposure Data, vol. 1. Lewis Publishers, Chelsea, MI.

Howe, G. R., Hirohata, T., Hislop, T. G., Iscovich, J. M., Yuan, J. M., Katsouyanni, K., Lubin, F., Marubini, E., Modan, B and Rohan, T. 1990. Dietary factors and risk of breast cancer: combined analysis of 12 case-control studies. *Journal of National Cancer Institute* 82:561-569.

Hoyert, D. L., Kochanek, K. D. and Murphy, S. L. 1997. Deaths: Final Data for 1997. Hyattsville, MD: National Center for Health Statistics.

Hsieh, S.D., Yoshinaga, H. and Muto, T. 2003. Waist-to-height ratio, a simple and practical index for assessing central fat distribution and metabolic risk in Japanese men and women. *International Journal of Obesity and Related Metabolic Disorders* 27:610-616.

Huang, B-M., Lai, H-Y. and Liu, M-Y 2002. Concentration dependency in lead-inhibited steroidogenesis in MA-10 mouse leydig cells. *Journal of Toxicology and Environmental Health, Part A* 65.7: 557-567

Huang, Y., Zhang, X., Li, W., Song, F., Dai, H., Wang, J., Gao, Y., Liu, X., Chen, C., Yan, Y., Wang, Y. and Chen, K. A. 2014. meta-analysis of the association between induced abortion and breast cancer risk among Chinese females. *Cancer Causes and Control* 25.2:227-236.

Hugo, E. R., Brandebourg, T. D., Woo, J. G., Loftus, J. and Alexander, J. W. 2008. Bisphenol-A

at environmentally relevant doses inhibits adiponectin release from human adipose tissue explants and adipocytes. *Environmental Health Perspectives* 116:1642-1647.

Huhtaniemi, I. 2010. Are gonadotrophins tumorigenic? A critical review of clinical and experimental data. *Molecular and Cellular Endocrinology* 329:56-61.

Huo, D., Ikpatt, F., Khramtsov, A., Dangou, J-M., Nanda, R., Dignam, J., Zhang, B., Grushko, T., Zhang, C., Oluwasola, O., Malaka, D., Malami, S., Odetunde, A., Adeoye, A.O., Iyare, F., Falusi, A., Perou, C.M. and Olopade, O. I. 2009. Population differences in breast cancer: Survey in indigenous African women reveals over-representation of triple negative breast cancer. *Journal of clinical Oncology* 27.27:4515-4521.

Ihekwaba, F. N. 1992. Breast cancer in Nigerian women. *British Journal of Surgery.* 79:771-779.

Ihemelandu, C. U., Lefall, L. D. Jr., Dewitty, R. L., Naab, T.J., Mezghebe, H.M., Makambi, K.H., Adams-Campbell, L. and Frederick, W.A 2007. Molecular breast cancer subtypes in premenopausal and postmenopausal African-American women: age-specific prevalence and survival. *Journal of Surgical Research* 143:109-118.

Ijaduola, T. G. and Smith, E. B. 1998. Pattern of breast cancer among white-American, African-American and non-imigrant west African women. *Journal of National Medical Association.* 90.9:547-551

Implanon (package insert) 2006. Roseland, NJ: Organon USA Inc.

International Agency for Research on Cancer (IARC). 1999. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Vol 72: Hormonal Contraception and Postmenopausal Hormonal Therapy. Lyon, France.

International Programme on Chemical Safety (IPCS)   2002.   Global assessment of the state-of-the-science of endocrine disruptors. Geneva, Switzerland, World Health Organization, International Programme on Chemical Safety.

Inter-Organization Programme for the Sound Management of Chemicals (IOMC). 2013. State of the Science of Endocrine Disrupting Chemicals 2012. Summary for Decision-Makers: An assessment of the state of the science of endocrine disruptors prepared by a group of experts for the United Nations Environment Programme and World Health Organization. Åke Bergman, Jerrold J. Heindel, Susan Jobling, Karen A. Kidd and R. Thomas Zoeller. Ed. Geneva: Switzerland.1-38.

Iwasaki, M., Otani, T., Inoue, M., Sasazuki, S. and Tsugane, S. 2007. The Japan Public Health

151

at environmentally relevant doses inhibits adiponectin release from human adipose tissue explants and adipocytes. *Environmental Health Perspectives* 116:1642-1647.

Huhtaniemi, I. 2010. Are gonadotrophins tumorigenic? A critical review of clinical and experimental data. *Molecular and Cellular Endocrinology* 329:56-61.

Huo, D., Ikpatt, F., Khramstov, A., Dangou, J-M., Nanda, R., Dignan, J., Zhang, B., Grushko, T., Zhang, C., Oluwasola, O., Malaka, D., Malami, S., Oderunde, A., Adeoye, A.O., Iyare, F., Falusi, A., Peiou, C.M. and Olopade, O. I. 2009. Population differences in breast cancer: Survey in indigenous African women reveals over-representation of triple negative breast cancer. *Journal of clinical Oncology* 27.27:4515-4521.

Ihekwaba, F. N. 1992. Breast cancer in Nigerian women. *British Journal of Surgery* 79:771-779.

Ihemelandu, C. U., Lefall, L. D. Jr., Dewitty, R. L., Naab, T.J., Mezghebe, H.M., Makambi, K.H., Adams-Campbell, L. and Frederick, W.A 2007. Molecular breast cancer subtypes in premenopausal and postmenopausal African-American women: age-specific prevalence and survival. *Journal of Surgical Research* 143;109-118.

Ijaduola, T. G. and Smith, E. B. 1998. Pattern of breast cancer among white-American, African-American and non-imigrant west African women. *Journal of National Medical Association.* 90.9:547-551

Implanon (package insert) 2006. Roseland, NJ: Organon USA Inc.

International Agency for Research on Cancer (IARC). 1999. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Vol 72: Hormonal Contraception and Postmenopausal Hormonal Therapy. Lyon, France.

International Programme on Chemical Safety (IPCS) 2002. Global assessment of the state-of-the-science of endocrine disruptors. Geneva, Switzerland, World Health Organization, International Programme on Chemical Safety.

Inter-Organization Programme for the Sound Management of Chemicals (IOMC). 2013. State of the Science of Endocrine Disrupting Chemicals 2012, Summary for Decision-Makers: An assessment of the state of the science of endocrine disruptors prepared by a group of experts for the United Nations Environment Programme and World Health Organization. Åke Bergman, Jerrold J. Heindel, Susan Jobling, Karen A. Kidd and R. Thomas Zoeller. Ed. Geneva; Switzerland. 1-38.

Iwasaki, M., Otani, T., Inoue, M., Sasazuki, S. and Tsugane, S. 2007. The Japan Public Health

151

Center-Based Prospective Study Group. Body size and risk for breast cancer in relation to estrogen and progesterone receptor status in Japan. *Annals of Epidemiology* 17.4:304-312.

Iyengar, M. 2005. Polychlorinated biphenyls-a review. 1-24.

Jacobs, T. W., Gown, A. M. and Yaziji, H. 1999. Specificity of Hercep test in determining HER-2/neu status of breast cancers using the United States food and drug administration-approved scoring system. *Journal of Clinical Oncology* 17:1983-1987.

Jarup. L. and Akesson, A. 2009. Current status of cadmium as an environmental health problem. *Toxicology and Applied Pharmacology* 238:201-208.

Jiang, X., Liu, H., Chen, X., Chen, P. H., Fischer, D., Sriraman, V., Yu, H. N., Arkinstall, S. and He, X. 2012. Structure of follicle-stimulating hormone in complex with the entire ectodomain of its receptor. *Proceedings of National Academy of Science USA* 109.31:12491–12496.

Jiang, C. H., Tsien, J. Z., Schultz, P. G. and Hu, Y. 2001. The effects of aging on gene expression in the hypothalamus and cortex of mice. *Proceedings of National Academy of Science USA* 98.4:1930-1934.

Jin, Y. H, Clark, A. B., Slebos, R. J., Resai, H.A., Taylor, J.A., Kunkel, T.A., Resnick, M.A and Gordenin, D.A 2003. Cadmium is a mutagen that acts by inhibiting mismatch repair. *Nature Genetics* 34:326-329.

Johnson, M. D., Kenney, N., Stoica, A., Hilakivi-Clarke, L., Singh, B, Chepko, G., Clarke, R., Sholler, P.F., Lirio, A.A., Foss, C., Reiter. R., Trock, B., Paik, S and Martin. M.B 2003. Cadmium mimics the in vivo effects of estrogen in the uterus and mammary gland. *National Medicine* 9:1081-1084.

Jones, S.B. 1999. Cancer in Developing Countries. *British Medical Journal* 319:505-508.

Joseph, P., Muchnok, T. K., Klishis, M. L., Roberts, J.R., Antonini, J.M., Whong, W.Z and Ong, T. 2001. Cadmium-induced cell transformation and tumorigenesis are associated with transcriptional activation of c-fos, c-jun, and c-myc proto-oncogenes: role of cellular calcium and reactive oxygen species. *Toxicological Science* 61, 295-303.

Joshi, J. V., Bhandarkar, S. D., Chadha, M., Balaiah, D. and Shah, R. 1993. Menstrual irregularities and lactation failure may precede thyroid dysfunction or goitre. *Journal of Postgraduate Medicine* 39:137–141.

152

Julia, B. A., Wolk, A. L., Bergkvist, L. M., Bottai, M. A. and Akesson, A. 2012. Dietary cadmium exposure and risk of postmenopausal breast cancer: a population-based prospective cohort study. Cancer Research 72.6:1459–1466.

Juracek, K. E. and Ziegler, A. C. 2006. The legacy of leaded gasoline in bottom sediment of small rural reservoirs. *Journal of Environmental Quality* 35:2092-2102.

Kaaks, R., Berrino, F., Key, T., Rinaldi, S., Dossus, L., Biessy, C., Secreto, G., Amiano, P., Bingham, S., Boeing, H., Bueno-de-Mesquita, H. B. and Chag-Claude, J. 2005. Serum sex steroids in premenopausal women and breast cancer risk within the European Prospective Investigation into Cancer and Nutrition (EPIC). *Journal of National Cancer Institute* 97:755-765.

Kabuto, M., Akiba, S., Stevens, R.G. and Neriishi, K. L. 2000. A prospective study of oestradiol and breast cancer in Japanese women. *Cancer Epidemiology Biomarkers Preview* 9:575-579.

Kaltreider, R. C., Pesce, C. A., Ihnat, M. A., Lariviere, J. P. and Hamilton, J. W. 1999. Differential effects of arsenic (III) and chromium (VI) on nuclear transcription factor binding. *Molecular Carcinogenesis* 25.3:219-229.

Kaneko, J.J. 1999. Clinical Biochemistry of animals 4th ed. Academic Press Inc. New York. P932.

Kang, J. H., Kondo, F. and Katayama, Y. 2006. Human exposure to bisphenol-A. *Toxicology* 226. 2/3:79–89.

Kang, J.H., Aosi, D. and Katayama, Y., 2007. Bisphenol A in the aquatic environment and its endocrine-disruptive effects on aquatic organisms. *Critical Review of Toxicology* 37:607-625.

Kannokar, N. and Jayaraman, G. 1988. Linear diffusion of lead in the intestinal wall: a theoretical study. *IMA Journal of Mathematics, Applied Medicine and Biology* 5.1:33-43.

Kashihara, H., Lee, J., Kawakubo, K., Tamura, M. and Akabayashi, A. 2009. Criteria of waist circumference according to computed tomography-measured visceral fat area and clustering of cardiovascular risk factors. *Circulation Journal* 73:1881-1886.

Kelsey, J. L. 1979. A review of the epidemiology of human breast cancer. *Epidemiology Review* 1:74-109.

Kene, T. S., Odigie, V. I., Yusuf, L. M., Yusuf, B. O. and Shehu, S.M. 2010 Pattern of

presentation and survival of breast cancer in a teaching hospital in North Western Nigeria. *Oman Medical Journal* 25:104-107.

Kerdivel, G., Habauzit, D. and Pakdel, F. 2013. Assesment and molecular actions of Endocrine-Disrupting Chemicals that interfere with Oestrogen Receptor Pathways. *International Journal of Endocrinology* 2013:1-14.

Kershaw, E. E. and Flier, J. S. 2004. Adipose tissue as an endocrine organ. *Journal of Clinical Endocrinology and Metabolism* 89:2548-2556.

Kessler, R. 2014. Lead-based decorative paints; where are they still sold and why? *Environmental Health Perspectives* 122.4 96-103.

Key, J., Hodgson, S., Omar, R. Z., Jensen, T. K. and Thompson, S. G. 2006. Meta-analysis of studies of alcohol and breast cancer with consideration of the methodological issues. *Cancer Causes Control* 17:759–770.

Key, T. J. and Pike, M. C. 1988. The role of oestrogens and progestogens in the epidemiology and prevention of breast cancer. *European Journal Cancer Clinical Oncology* 24:29-43.

Khokher, S., Qureshi, M. U., Mahmood, S. and Nagi, A. H. 2013. Association of immunohistochemically defined molecular subtypes with clinical response to pre-surgical chemotherapy in patients with advanced breast cancer. *Asian-Pacific Journal of Cancer Prevention* 14:3223-3228.

Khwaja, M. S., Nirodi, N. S. and Lawrie, J. H. 1980. Malignant tumours of the breast in Northern savannah of Nigeria. *The East African Medical Journal* 57:555-561.

Kim, M., Kim, S., Yun, S., Lee, M., Cho, B., Park, J., Son, S. And Kim, O. 2004. Comparison of seven indicator PCBs and three coplanar PCBs in beef, pork, and chicken fat. *Chemosphere* 54:1533–1538.

Kinney, A. Y., Millikan, R. C, Lin, Y. H, Moorman, P. G. and Newman, B. 2000. Alcohol consumption and breast cancer among black and white women in North Carolina (United States). *Cancer Causes Control* 11:345–357.

Kinney, C.A., Furlong, E.T., Zaugg, S.D., Burkhardt, M.R., Werner, S.L. and Cahill, J.D., Jorgensen, G.R., 2006. Survey of organic wastewater contaminants in biosolids destined for land application. *Environmental Science and Technology*, 40.7207-7215.

Kirkwood, T. B. 2002. Molecular gerontology. *Journal of Inherited Metabolic Disorders* 25.3:189-196.

Klaassen, C. D 1981. Pharmacokinetics in metal toxicity. *Fundamentals of Applied Toxicology* 1: 353 – 357.

Klassen C. D. 1996. Heavy metals and heavy metals antagonists. In: Hardman, J.G., Gillman A. G., Goodman, L. S., Rail, T. W, Murad, F. (eds). The Pharmacological Basis of Therapeutics. McGraw-Hill, New York, 1649-1672.

Kolawole, A. O. 2011. Feasible Cancer Control Strategies for Nigeria: Mini-Review American *Journal of Tropical Medicine & Public Health* 1.1:1-10.

Kopelman, P. G. 2000. Obesity as a medical problem. *Nature* 404:635-643.

Krassas, G. E. 2005. The male and female reproductive system in thyrotoxicosis. In: Braverman LE, Utiger RD, eds. Werner and Ingbar's the thyroid-a fundamental and clinical text. 9th ed. Philadelphia: Lippincott Williams & Wilkins; 621-628.

Krassas, G. E., Pontikides, N., Kaltsas, T., Papadopoulou, P. and Batrinos, M. 1994. Menstrual disturbances in thyrotoxicosis. *Clinical Endocrinology (Oxf)* 40:641-644.

Krassas, G. E., Pontikides, N., Kaltsas, T., Papadopoulou, P., Paunkovic, J., Paunkovic, N. and Duntas, L. H. 1999. Disturbances of menstruation in hypothyroidism. *Clinical Endocrinology (Oxf)* 50:655-659.

Krassas, G. E., Poppe, K. and Glinoer, D. 2010. Thyroid Function and Human Reproductive Health. *Endocrinology Reviews* 31:702-755.

Kroenke, C. H., Chen, W.Y., Rosner, B. and Holmes, M. D. 2005. Weight, weight gain, and survival after breast cancer diagnosis. *Journal of Clinical Oncology* 23,7:1370-1378.

Kuiper, G. G., Carlsson, B., Grandien, K., Enmark, E., Haggblad, J., Nilsson, S. and Gustafsson, J. A. 1997. Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta. *Endocrinology* 138:863–870.

Kuiper, G. G., Enmark, E., Pelto-Huikko, M., Nilsson, S. and Gustafsson, J. A. 1996. Cloning of a novel receptor expressed in rat prostate and ovary. *Proceedings of National Academy of Science* 93: 5925–5930.

Kwintkiewicz, J., Nishi, Y., Yanase, T. and Giudice, L. C. 2010. Peroxisome proliferator-activated receptor-gamma mediates bisphenol A inhibition of FSH-stimulated IGF-1, aromatase, and estradiol in human granulosa cells. *Environmental Health Perspectives* 118: 400-406.

La Vecchia, C., Altieri, A. and Tavani, A. 2001. Vegetables, fruit, antioxidants and cancer: a

review of Italian studies. *European Journal of Nutrition* 40.6:261-267.

La Vecchia, C., Ferraroni, M., Franceschi, S., Mezzetti, M., Decarli, A. and Negri, E. 1997. Fibers and breast cancer risk. *Nutrition and Cancer* 28:264-269.

Lahmann, P.H., Hoffmann, K., Allen, N., van Gils, C.H., Khaw, K.T., Tehard, B., Berrino, F., Tjonneland, A., Bigaard, J., Olsen, A., Overvad, K., Clavel-Chapelon, F., Nagel, G., Boeing, H., Trichopoulos, D., Economou, G., Bellos, G., Palli, D., Tumino, R., Panico, S., Sacerdote, C., Krogh, V., Peeters, P.H., Bueno-de-Mesquita, H.B., Lund, E., Ardanaz, F., Amiano, P., Pera, G., Quiros, J.R., Martinez, C., Tormo, M.J., Wirfalt, E., Berglund, G., Hallmans, G., Key, T.J., Reeves, G., Bingham, S., Norat, T., Biessy, C., Kaaks, R. and Riboli, E. 2004. Body size and breast cancer risk: findings from the European Prospective Investigation into Cancer and Nutrition (EPIC). *International Journal of Cancer* 111.5:762-771.

Lai, L. C. 2002. Role of steroid hormones and growth factors in breast cancer. *Clinical Chemistry and Laboratory Medicine* 10: 969-974.

Lajous, M., Boutron-Ruault, M. C., Fabre, A., Clavel-Chapelon, F. and Romieu, I. 2008. Carbohydrate intake, glycemic index, glycemic load, and risk of postmenopausal breast cancer in a prospective study of French women. *American Journal of Clinical Nutrition* 875:1384-1391.

Lakowicz, J. and Anderson, C. 1980. Permeability of lipid bilayers to methyl- mercury chloride: Quantification by fluorescence quenching of a carbazole-labeled phospholipid. *Chemico-Biological Interactions* 30:309-323.

Lambe, M., Hsieh, C. C, Chan, H. W., Ekbom, A., Trichopoulos, D. and Adami, H. O 1996. Parity, age at first and last birth, and risk of breast cancer: a population-based study in Sweden. *Breast Cancer Research and Treatment* 38:305-311.

Larsson, S. C., Bergkvist, L. and Wolk, A. 2009. Glycemic load, glycemic index and breast cancer risk in a prospective cohort of Swedish women, *International Journal of Cancer* 125.1:153-157.

Lawani, J., Ngu, V.A. and Osunkoya, B. O.1973. A clinico-pathological review o malignant disease of the breast in the University College Hospital. *Nigerian Medical Journal* 3:182-187.

Laycock, J. F. and Wise, P. H. 1996. Essential endocrinology. Oxford, UK: Oxford University

press.

Lean, M. E. J., Han, T. S. and Deurenberg, P. 1996. Predicting body compostion by densitometry from simple anthropometric measurements. *American Journal of Clinical Nutrition* 63:4-14.

Lee, M.Y., Jung, B.I., Chung, S.M., Bae, O.N., Lee, J.Y. and Park, J.D. 2003. Arsenic-induced dysfunction in relaxation of blood vessels. *Environmental Health Perspectives* 111:513-517.

Leers-Sucheta, S., Morohashi, K. and Mason, J. I. 1997. Synergistic activation of the human type II 3 beta-hydoxysteroid dehydrogenase-1 delta 5-delta-4 isomerase promoter by the transcription factor steroidogenic factor-1 adrenal 4 binding protein and phorbol ester. *Journal Biological Chemistry* 272:7960-7967.

Lemaire, M. and Baugnet,-Mahieu, L. 1986. Thyroid function in women with breast cancer. *European Journal of Cancer and Clinical Oncology* 22:301-307.

Lemieux, S., Prud'homme, D., Bouchardi C., Tremblav, A. and Depress, J. P. 1996. A single threshold value of waist girth identifies normal weight and overweight subjects with excess visceral adipose tissue. *American Journal of Clinical Nutrition* 64:685-693.

Leonard, S. S, Bower, J. J. and Shi, X. 2004. Metal-induced toxicity, carcinogenesis, mechanisms and cellular responses. *Molecular and Cell Biochemistry* 255.1-2.3-10.

Lethaby, A. E., Mason, B. H., Harvey, V. J. and Holdaway, I. M. 1996. Survival of women with node negative breast cancer in the Auckland region. *New Zealand Medical Journal* 109:330-333.

Li, C. I., Daling, J. R. and Malone, K. E. 2003. Incidence of invasive breast cancer by hormone receptor status from 1992 to 1998. *Journal of Clinical Oncology* 21.1:28-34.

Li, D-K., Miao, M., Zhou, Z., Wu, C., Shi, H., Liu, X., Wang, S and Yuan, W. 2013. Urine bisphenol-A level in relation to obesity and overweight in school age children. *PLoS ONE* 8.6: e65399.

Li, Y., Ling, M., Xu, Y., Wang, S., Li, Z., Zhou, J., Wang, X and Liu, Q. 2010.The repressive effect of NF-kappa B on p53 by mot-2 is involved in human keratinocyte transformation induced by low levels of arsenite. *Toxicological Sciences* 116;174-182

Liu, Q. Y., Niranjan, B., Gomes, P., Gomm, J. J, Davies, D., Coombes, R. C. and Buluwela, L.

1996. Inhibitory effects of activin on the growth and morphogenesis of primary and transformed mammary epithelial cells. *Cancer Research* 56:1155–1163.

Llanos, A. A., Makambi, K. H., Tucker, C. A., Shields, P. G. and Adams-Campbell, L. L. 2012. Alcohol, anthropometrics, and breast cancer risk in African American women. *Breast Journal* 18:394-395.

Loi, S., Milne, R. L., Friedlander, M. L., McCredie, M. R., Giles, G. G., Hopper, J. L. and Phillips, K. A. 2005. Obesity and outcomes in premenopausal and postmenopausal breast cancer. *Cancer Epidemiology and Biomarkers Preview* 14.7:1686-1691.

Longcope, C., Abend, S., Braverman, L. E. and Emerson, C. H. 1990. Androstenedione and estrone dynamics in hypothyroid women. *Journal of Clinical Endocrinology and Metabolism* 70:903–907.

Loutradis, D., Bletsa , R., Aravantinos, L., Kallianidis, K., Michalas, S. and Psychoyos, A. 1991. Preovulatory effects of the progesterone antagonist mifepristone (RU486) in mice. *Human Reproduction* 6:1238-1240.

Louvet, J., Harman, S. and Ross, G. 1975. Effects of human chorionic gonadotropin, human interstitial cell stimulating hormone and human follicle-stimulating hormone on ovarian weights in estrogen-primed hypophysectomized immature female rats. *Endocrinology* 96.5: 1179–1186.

Lovegrove J. A. 2002. Obesity, body fat distribution and breast cancer. *Nutrition Research Review* 15.2:389-412.

Low, S. C., Dixon, A. R., Bell, J., Ellis, I. O., Elston, C. W., Robertson, J.F. and Blamey R.W. 1992. Tumour estrogen receptor content allows selection of elderly patients with breast cancer for conservative tamoxifen treatment. *British Journal of Surgery* 79: 1314-1316.

Lubrano, L., Genovesi, G., Specchia, P., Constatini D, Stefania, M., Petrangeli, E., Lenzi, A. and Gnessi, L. 2013. Obesity and metabolic comorbidities; *Oxidative Medicine and Cellular Longetivity*. Article ID 640673. Pp 1-9. http://dx.doi.org/10.1155/2013/640673.

Lutzen, A., Liberti, S. E. and Rasmussen, L. J. 2004. Cadmium inhibits human DNA mismatch repair in vivo. *Biochemical and Biophysical Research Communication* 321.1:21-25.

Luu-the, V. 2001. Analysis and characteristic of multiple types of human 17 beta-hydroxysteroid dehydrogenase. *J. Steroid. Biochem. Molecular Biology* 76:143-151.

Lydon, J. P., DeMayo, F. J., Funk, C. R., Mani, S. K., Hughes, A. R., Montgomery Jr, C. A.,

Shyamala, G., Conneely, O. M. and O'Malley, B. W. 1995. Mice lacking progesterone receptor exhibit pleiotropic reproductive abnormalities. *Genes Development* 9:2266-2278.

Mahesh, V.B. 2011. Hirsutism, virilism, polycystic ovarian disease and the steroid gonadotropin-feedback system; a career retrospective. *AJP: Endocrinology and Metabolism* 302.1:4-18

Makanjuola, S. B. L., Ayodele, S.D., Javid, F. A., Obafunwa, J. O., Oludara, M. A. and Popoola, A. O. 2014. Breast cancer receptor status assessment and clinicopathological association in Nigerian women: A retrospective analysis. *Journal of Cancer Research and Therapy* 2:122-127.

Maki, D. D. and Grossman R.I. 2000. Patterns of disease spread in metastatic breast carcinoma: Influence of estrogen and progesterone receptor status. AJNR *American Journal of Neuroradiology* 21:1064-1066.

Mantovani, A. 2009. Cancer: inflaming metastasis. *Nature* 457: 36-37

Marino, M., Chiovato, L. and Pinchera, A. 2006. Graves' disease. In: DeGroot LJ, Jameson JL, eds. Endocrinology. 5th ed. Philadelphia: Elsevier Saunders. 1995–2028.

Mark, C. and Paul, G. 2001. Estrogen and the risk of breast cancer. *New England Journal of Medicine* 344.4:276-285.

MartÃnez-Campa, C., Alonso-GonzÃ¡lez, C., Mediavilla, M. D., Cos, S., GonzÃ¡lez, A., Ramos, S. and Sanchez-Barcelo, E.J. 2006. Melatonin inhibits both ER alpha activation and breast cancer cell proliferation induced by a metalloestrogen, cadmium. *Journal of Pineal Research* 40.4:291-296.

Martin, B., Rotten D., Jovlivet, A. and Guatray, J. P. 1981. Binding of steroids by proteins in follicular fluid of the human ovary. *Journal of Clinical Endocrinology and Metabolism* 35:443-447.

Martin, M. B., Reiter, R., Pham, T., Avellanet, Y. R., Camara, J., Lahm, M., Pentecost, E., Pratap, K., Gilmore, B.A., Divekar, S., Dagata, R.S., Bull J.L and Stoica, A. 2003. Estrogen-like activity of metals in MCF-7 breast cancer cells. *Endocrinology* 144.6:2425-2436.

Martin, M. B and Stoica, A. 2002. Insulin-like growth factor-1 and estrogen interactions in breast cancer. *Journal of Nutrition* 132: 3799S-3801S.

Martinez, A., Wang, K. and Hombuckle, K. C. 2010. Fate of PCB congeners in an industrial

harbor of lake Michigan. *Environmental Science and Technology* 44: 2803–2308.

Martinez, L., Castilla, J. A., Gil, T., Molina, J., Alarcon, J. L., Marcos, C. and Herruzo, A. 1995. Thyroid hormones in fibrocystic breast disease. *European Journal of Endocrinology* 6: 673-676.

Martinez, P., Roislien, J., Naidoo, N. and Clausen, T. 2011. Alcohol abstinence and drinking among African women: data from the World Health Surveys. *BMC Public Health* 11:160.

Mason, J.I., Keeney, D. S., Bird, I. M., Rainey, W.E., Morohashi, K., Leers-Sucheta, S., Meiner, M.H.1997. The regulation of 3 beta hydroxysteroid dehydrogenase expression. *Steroids* 62:164-168.

Matsui, K., Nishii, S. and Oka. M. 2005. P450 aromatase inhibition assay using a competitive ELISA. *Journal of Pharmacology and Biomedical Analysis* 38:307-312.

Mattisson, I., Wirfalt, E., Johansson, U., Gullberg, B., Olsson, H., Berglund, G. 2004. Intakes of plant foods, fibre and fat and risk of breast cancer--a prospective study in the MalmÄ Diet and Cancer cohort. *British Journal of Cancer* 90.1:122-127.

McCance, K. L. and Jones, R. E. 2003. Estrogen and insulin crosstalk: breast cancer risk implications. *Nurse Practioners* 28.5:12-23.

McCarver, D. G., Byun, R., Hines, R. N., Hichme, M. and Wegenck, W. 1998. A genetic polymorphism in the regulatory sequences of human CYP2E1: association with increased chlorzoxazone hydroxylation in the presence of obesity and ethanol intake. *Toxicology Applied Pharmacology* 152:276-281.

McCormack, V.A., DosSantos-Silva, I. 2006. Breast density and parenchymal patterns as markers of breast cancer risk: a meta analysis. *Cancer Epidemiology, Biomarkers Preview* 15:1159-1169.

McElroy, J. A., Shafer, M. M., Trentham-Dietz, A., Hampton, J. M. and Newcomb, P. A. 2006. Cadmium exposure and breast cancer risk. *Journal of National Cancer Institute* 98.12:869-873.

McKinney, J.D and Waller, C.L. 1994. Polychlorinated biphenyls as hormonally active structural analogues. *Environmental Health Perspectives* 102:290-297

McLaughlin, M. J., Palmer, L. T., Tiller, K. G., Beech, T. A. and Smart, A. K. 1997. Increased soil salinity causes elevated cadmium concentration in field-grown potato tubes. *Journal of Environmental Quality* 26:1644-1699.

Mcsorley, M. A., Alberg, A.J., Allen, D.S., Allen, N.E., Brinton, L.A. and Dorgan, J. F. 2009. Pre diagnostic circulating follicle stimulating hormone (FSH) concentrations and ovarian cancer risk. *International Journal of Cancer* 125.3:674-679.

McTiernan, A. 2005. Obesity and cancer: the risks, science, and potential management strategies. *Oncology (Williston Park)* 19.7:871-881.

Meerts, I., Letcher, R. J., Hoving, S., Marsh, G., Bergman, A., Lemmen, J. G., VanderBurg, B. and Brouwer, A. 2001. In vitro estrogenicity of polybrominated diphenyl ethers, hydroxylated PBDEs and polybrominated bisphenol A compounds. *Environmental Health Perspectives* 109:399.407.

Mertens, H. J., Heineman, M. J. and Evers, J. L. 2002. The expression of apoptosis-related proteins Bcl-2 and Ki67 in endometrium of ovulatory menstrual cycles. *Gynecologic and Obstetric Investigation* 53:224-230.

Micheli, A., Muti, P., Secreto, G., Krogh, V., Meneghini, E., Venturelli E., Sieri, S., Pala, V. and Berrino, F. 2004. Endogenous sex hormones and subsequent breast cancer in premenopausal women. *International Journal of Cancer* 112.2:312-318.

Michels, K. B. and Willett, W. C. 1996. Does induced or spontaneous abortion affect the risk of breast cancer? *Epidemiology* 7:521-528.

Milionis A and Milionis C. 2013. Correlation between body mass index and thyroid function in euthyroid individuals in Greece. *ISRN Biomarkers* Article ID: 651494, 7 Pages

Miller, W. L. 1988. Molecular biology of steroid hormone synthesis. *Endocrinology Review* 9.3:295-318.

Mindnich, R., Moller, G. and Adamski, J. 2004. The role of 17-beta hydroxysteroid dehydrogenase. *Molecular and Cellular Endocrinology* 218:7-20.

Missimer, S. A., Eliassen, A. H., Barbieri, R. L. and Hankinson, S.E. 2004. Endogenous estrogen, androgen and progesterone concentrations and breast cancer risk among premenopausal women. *Journal of National Cancer Institute* 96:1856.1865.

Mohamed, F. Z., Darwish, H., Betal, A. A. M. and Abd EL-razek, W. Y. 2015. Some tumour markers and hormonal receptors as Prognostic Parameters of Breast Cancer. *Indian Journal of Research* 4.2: 199.203.

Moorman, P. G. and Terry, P. D. 2004. Consumption of dairy product and the risk of breast cancer: a review of the literature. *American Journal of Clinical Nutrition* 80.5.14.

161

Mcsorley, M. A., Alberg, A.J., Allen, D.S., Allen, N.E., Brinton, L.A. and Dorgan, J. F. 2009. Pre-diagnostic circulating follicle stimulating hormone (FSH) concentrations and ovarian cancer risk. *International Journal of Cancer* 125.3:674-679.

McTieman, A. 2005. Obesity and cancer: the risks, science, and potential management strategies. *Oncology (Williston Park)* 19.7:871-881.

Meerts, I., Letcher, R. J., Hoving, S., Marsh, G., Bergman, A., Lemmen, J. G., VanderBurg, B. and Brouwer, A. 2001. In vitro estrogenicity of polybrominated diphenyl ethers, hydroxylated PBDEs and polybrominated bisphenol A compounds. *Environmental Health Perspectives* 109:399-407.

Mertens, H. J., Heineman, M. J. and Evers, J. L. 2002. The expression of apoptosis-related proteins Bcl-2 and Ki67 in endometrium of ovulatory menstrual cycles. *Gynecologic and Obstetric Investigation* 53:224-230.

Micheli, A., Muti, P., Secreto, G., Krogh, V., Meneghini, E., Venturelli E., Sieri, S., Pala, V. and Berrino, F. 2004. Endogenous sex hormones and subsequent breast cancer in premenopnusal women. *International Journal of Cancer* 112.2:312-318.

Michels, K. B. and Willett, W. C. 1996. Does induced or spontaneous abortion affect the risk of breast cancer? *Epidemiology* 7:521-528.

Milionis A and Milionis C. 2013. Correlation between body mass index and thyroid function in euthyroid individuals in Greece. *ISRN Biomarkers* Article ID: 651494, 7 pages

Miller, W. L. 1988. Molecular biology of steroid hormone synthesis. *Endocrinology Review* 9.3:295-318.

Mindnich, R., Moller, G. and Adamski, J. 2004. The role of 17-beta hydroxysteroid dehydrogenase. *Molecular and Cellular Endocrinology* 218:7-20.

Missimer, S. A., Eliassen, A. H., Barbieri, R. L. and Hankinson, S.E. 2004. Endogenous estrogen, androgen and progesterone concentrations and breast cancer risk among premenopausal women. *Journal of National Cancer Institute* 96:1856-1865.

Mohamed, F. Z., Darwish, H., Belal, A. A. M. and Abd EL-razek, W. Y. 2015. Some tumour markers and hormonal receptors as Prognostic Parameters of Breast Cancer. *Indian Journal of Research* 4.2: 199-203.

Moorman, P. G. and Terry, P. D. 2004. Consumption of dairy product and the risk of breast cancer: a review of the literature. *American Journal of Clinical Nutrition* 80:S-14.

Mourouzis I., Politi E and Pantos C. 2013. Thyroid hormone and tissue repair: new tricks for an old hormone. *Journal of Thyroid Research.* doi; 10.1155/2013/312104.312104.

Mourouzis I., Tzovaras A., Armonis B., Ardavanis A., Skondra M., Misitzis, J., Pectasides D. and Pantos C. 2015. Are thyroid hormones and tumour cell proliferation in human breast cancer positive for HER2 associated? *International Journal of Endocrinology* Doi: 10.1155/2015/765406

Msolly, A., Gharbi, O., Mahmoudi, K., Limem, S., Hochlef, M. and Ben-Ahmed, S. 2011. Association between body mass index and the risk of breast cancer in Tunisian women. *Annals of Saudi Medicine* 31.4:393-397.

Mueller, M., Anke, M., Hartmann, E.and Illing-Guenther, H.1996. Oral cadmium exposure of adults in Germany: Cadmium content of foodstuffs and beverages. *Food Additives and C ontaminant* 13:359-378.

Mulac-Jericevic, B. and Conneely O. M. 2004. Reproductive tissue selective actions of progesterone receptors. *Reproduction* 128:129.146.

Muscat, J.E., Britton, J.A., Djordjevic, M.V., Citron, M.L., Kemeny, M., Busch-Devereaux, E., Pittman, B. and Stellman, S.D. 2003. Adipose concentration of organochlorine compounds and breast cancer recurrence in Long Island, New York. *Cancer Epidemiology, Biomarkers and Prevention* 12:1474-1478.

Nadji, M, Gomez-Fernandez, C., Ganjei-Azar, P. and Morales, A. R. 2005. Immunohistochemistry of estrogen and progesterone receptors reconsidered: experience with 5,993 breast cancers. *American Journal of Clinical Pathology* 123:21-27.

Nagle, R. B., Bocker, W., Davis, J. R., Heid, H. W., Kaufmann, M., Lucas, D. O and Jarasch, E. D. 1986. Characterization of breast carcinomas by two monoclonal antibodies distinguishing myoepithelial from luminal epithelial cells. *Journal of Histochemistry and Cytochemistry* 34:869-881.

Nandi, S., Guzman, R. C. and Yang, J. 1995. Hormones and mammry carcinogenesis in mice, rats and humans: a unifying hypothesis. *Proceedings of National Academy of Science USA* 92: 3650-3657.

Nasca, P. C., Baptiste, M. S., Field, N. A., Metzger, B. B., Black, M., Kwon, C.S. and Jacobson,

H. 1990. An epidemiological case-control study of breast cancer and alcohol consumption. *International Journal of Epidemiology* 19:532–538.

National Institute of Health, 2008. NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Bisphenol-A. National Toxicology Program, US Department of Health and Human Services. Center for the Evaluation of Risks to Human Reproduction (CERHR-NTP), Washington D.C.

Neville, M. C., McFadden, T. B. and Forsyth, I. 2002. Hormonal regulation of mammary differentiation and milk secretion. *Journal of Mammary Gland Biological Neoplasia* 1:49-66.

Ngoma, T. 2006. Cancer control priorities in Africa during the HIV/AIDS era. UICC cancer congress.

Nieminen P. 2002. Effect of bisphenol A and phytosterols on the European polecat and the field vole. An Academic Dissertation. Department of Public Health, University of Helsinki. vii+33pp.

Nikolic, J. and Sokolovic, D. 2004. Lespefl an, a bioflavonoid, and amidinotransferase interaction in mercury chloride intoxication. *Renal Failure* 26.6:607-611.

Niswender, G. D., Juengel, J. L., Silva, J. P., Rollyson, M. K. and McIntush, E. W. 2000. Mechanisms Controlling the Function and Life Span of the Corpus Luteum. *Physiological Reviews* 80:1-29.

Nixon, A. J., Neuberg, D. and Hayes, D. F. 1994. Relationship of patient's age to pathologic features of the tumour and prognosis for patients with stage 1 or 2 breast cancer. *Journal of Clinical Oncology* 12:888-894.

Nnodu, O., Erinosho, L., Jamda, M., Olaniyi, O., Adelaiye, R., Lawson, L., Odedina, F., Shuaibu, F., Odumuh, T., Isu, N., Imam, H., Owolabi, O., Yaqub, N. and Zamani, A. 2010. Knowledge and Attitudes towards Cervical Cancer and Human Papillomavirus: A Nigerian Pilot Study. *African Journal of Reproductive Health* 14.1:95-108.

Nogueira, C. R. and Brentani, M. M. 1996. Triiodothyronine mimics the effects of estrogen in breast cancer cell lines. *Journal of Steroid Biochemistry and Molecular Biology* 59: 271-279.

Norback, D.H and Weltman, R.H. 1994. Polychlorinated biphenyls induction of hepatocellular carcinoma in the Sprague-Dawley rat. *Environmental Health Perspectives* 60:97-105

163

Nickim, A., Nufu, F. T., and Campbell, O. B. 2009. Breast cancer in young women in Ibadan, Nigeria. *African Health Sciences* 9.4: 242–246.

Nwachukwu, C., Grushko, T. A, Xu, J., Khramtsov, A. and Olopade, O. I. 2009. BRCA1 methylation contributes to the triple-negative breast cancer phenotype. *Cancer Research* 69:4050.

Oakley, G.G., Robertson, L.W. and Gupta, R.C. 1996. Analysis of polychlorinated biphenyls-DNA adducts by 32P-postlabelling. *Carcinogenesis (Lond)* 17:109-114

Oehlmann, J., Schulte-Oehlmann, U., Kloas, W., Jagnytsch, O., Lutz, I., Kusk, K.O., Wollenberger, L., Santos, E.M., Paull, G.C., Van Look, K.J. and Tyler, C.R., 2009. A critical analysis of the biological impacts of plasticizers on wildlife. *Philosophical Transactions of the Royal Society, B* 364: 2047-2062.

Ogundiran, T. O., Huo, D., Adenipekun, A., Campbell, O., Oyesegun, R., Akang, E., Adebamowo, C. and Olopade, O. I. 2010. Case-Control Study of Body Size and Breast Cancer Risk in Nigerian Women. *American Journal of Epidemiology* 1-9.

Ohanaka, C.E 2007. Breast cancer in young Nigerian women. *Nigerian Journal of Surgical Sciences* 17.2:86-90.

Okobia, M. N., Bunker, C. H., Okonofua, F. E. and Osimi, U. 2006. Knowledge, attitude and practice of Nigerian women towards breast cancer: A cross-sectional study. *World Journal of Surgical Oncology* 4: 1-9.

Okonofua, F. E., Lawal, A. and Bamgbose, J. K. 1990. Features of menopause and menopausal age in Nigerian women. *International Journal of Gynaecology and Obstetrics* 31:341-345.

Okorodudu, D. O., Jumean, M. F., Montori, V. H., Romero-Corral, A., Somers, V. K., Erwin P. J. and Lopez-Jimenez, F. 2010, Diagnostic performance of body mass index to identify obesity as defined by body adiposity: a systemic review and meta-analysis. *International Journal of Obesity* 34:791-799.

Olayioye, M. A. 2001. Update on HER 2 as a target for cancer therapy: Intracellular signaling pathways of ErbB2/HER 2 and family members. *Breast Cancer Research* 3.6: 385-389

Olivotto, I. A., Truong, P. T., Speers, C. H., Bernstein, V., Allan, S. J., Kelly, S. J. and Lesperance, M. L. 2004. Time to stop progesterone receptor testing in breast cancer management. *Journal of Clinical Oncology* 22:1769-1770.

Olopade, F. 2004. Why take it if you don't Have Anything? Breast Cancer Risk Perceptions and Prevention Choices at a Public Hospital. *Canada Pubmed Online Journal of the National Library of Medicine and the National Institute of Health* 21.7:779-785

Olsson, I. M., Bensryd, I., Lundh, T., Ottosson, H., Skerfving, S. and Oskarsson, A. 2002. Cadmium in blood and urine: Impact of sex, age, dietary intake, iron status and for mer smoking Association of renal effects. *Environmental Health Perspectives* 110:1185-1190.

Olsson, I.M., Eriksson, J., Obom, I., Skerfving S. and Oskarsson, A.2005. Cadmium in food production systems: a health risk for sensitive population groups. *Ambio* 34:344-351.

Oluwatosin, O. A. and Oladepo, O. 2006. The level of the knowledge of breast cancer and it's early detection measures among rural women in Akinyele local area. Ibadan. Nigeria. *BMC Cancer* 6:271.

Oluwole, S.F., Ali, A. O., Adu, A., Blanc, B. P., Barlow, B., Oropeza, R. and Freeman H. P. 2003. Impact of a cancer screening program on breast cancer stage at diagnosis in a medically underserved urban community. *Journal of American College of Surgeons* 196: 180-188.

Omar, S., Khaled, H., Gaafar, R., Zekry, A. R., Eissa, S. and El-khatib, O. 2003. Breast cancer in Egypt: a review of disease presentation and detection strategies. *Eastern Mediterrinean Health Journal* 9.3: 448-463.

Oppenheimer, J. H., Koerner, D., Schwartz, H. L. and Surks, M. I. 1972. Specific nuclear triiodothyronine binding sites in rat liver and kidney. *Journal of Clinical Endocrinology and Metabolism* 35:330-333.

Ottesen, M., Feldt-Rasmussen, U., Andersen, J., Hippe, E. and Schouboe, A. 1995. Thyroid function and autoimmunity in pernicious anemia before and during cyanocobalamin treatment. *Journal of Endocrinology Investigation* 18:91-97.

Pace, P., Taylor, J., Suntbaralingam, S., Coombes, R. C. and Ali, S. 1997. Human estrogen receptor beta binds DNA in a manner similar to and dimerizes with estrogen receptor alpha. *Journal of Biological Chemistry* 272:25832-25838.

Pall, M., Mikuni, M., Mitsube, K. and Brannstrom, M. 2000. Time-dependent ovulation

165

inhibition of a selective progesterone-receptor antagonist (Org 31710) and effects on ovulatory mediators in the in vitro perfused rat ovary. *Biology of Reproduction* 63:1642-1647.

Pallud S., Ramauge M. and Gavaret J-M. 1999. Regulation of type 3 iodothyronine deiodinase expression in cultured rat astrocyte: role of Erk cascade. *Endocrinology.* 140.6:2917-2923

Parker, K. L and Schimmer, B. P. 1995. Transcriptional regulation of the genes encoding the cytrochrome P-450 steroid hydroxylases. *Vitamin Hormones.* 51:339-370

Parkin, D. M. and Fernandez, L. M., 2006. Use of statistics to assess the global burden of breast cancer. *Breast Journal* 12.1:70-80.

Parkin, D. M., Ferlay, J., Hamdi-Cherif, M., Sitas, F., Thomas, J.O., Wabinga, H. and Whelan, S.L. 2003. Cancer in Africa Epidemiology and Prevention. IARC (WHO) Scientific Publications no. 153, IARC Press, Lyon, France.

Parl, F. F., Schmidt, B. P., Dupont, W. D. and Wagner, R. K. 1984. Prognostic significance of estrogen receptor status in breast cancer in relation to tumor stage, axillary node metastasis, and histopathologic grading. *Cancer* 54:2237-2242.

Patel, T., Gupta, A. and Shah, M. 2013. Pathological predictive factors for tumor response in locally advanced breast carcinomas treated with anthracyclin-based neoadjuvant chemotherapy. *Journal of Cancer Research and Therapy* 9:245-249.

Payne, S. J., Bowen, R. L., Jones, J. L. and Wells, C. A. 2008. Predictive markers in breast cancer – the present. *Histopathology* 52:82-90.

Pechoux, C., Gudjonsson, T., Ronnov-Jessen, L., Bissell, M. J. and Petersen, O. W. 1999. Human mammary luminal epithelial cells contain progenitors to myoepithelial cells. *Developmental Biology* 20: 688-699.

Pedersen, O., Richelsen, B., Bak, J., Arnfred, J., Weeke, J. and Schmitz, O. 1988. Characterization of the insulin resistance of glucose utilization in adipocytes from patients with hyper- and hypothyroidism. *Acta Endocrinologia (Copenh)* 119.228-234.

Peer, N., Lombard, C., Steyn, K. and Levitt, N. 2014. Rising alcohol consumption and a high prevalence of problem drinking in black men and women in Cape Town: the CRIBSA study. *Journal of Epidemiology and Community Health* 68:446-452

Pelkonen, O and Nerbert, D.W, 1982.Metabolism of polycyclic aromatic hydrocarbons: etiologic role in carcinogenesis. *Pharmacology Revision* 34:189-222

Peretz, J., Vrooman, L., Ricke, W.A., Hunt, P. A., Ehrlich, S., Hauser, R., Padmanabhan, V., Taylor, H.S., Swan, S.H., VandeVoort, C.A. and Flaws, J.A. 2014. Bisphenol-A and reproductive Health: Update of experimental and human evidence, 2007-2013. *Environmental Health Perspectives* 122.8:775-785

Perez, A. L. and Anderson, K. A. 2009. Estimates cadmium accumulation in wheat and potato from phosphate fertilizer applications. *Science Total Environment* 407:5096-5103.

Petersen, O. W. and van Deurs, B. 1988. Growth factor control of myoepithelial cell differentiation in cultures of human mammary gland. *Differentiation* 39:197–215.

Pettersson, K., Grandien, K., Kuiper, G. G. and Gustafsson, J. A. 1997. Mouse estrogen receptor beta forms estrogen response element-binding heterodimers with estrogen receptor alpha. *Molecular Endocrinology* 11.1486–1496.

Pfeifer, S. M. and Strauss, J. F. III. 1996. Progestins In: Reproductive Endocrinology, surgery and technology, pp 495.503. (Eds) E. Y Adashi, J. A Rock and Z Rosenwaks. Philadelphia: Lippincott-Raven.

Philips, A., Chalbos, D. and Rochefort, H. 1993. Estradiol increases and antiestrogens antagonize the growth factor-induced activator protein-1 activity in MCF7 breast cancer cells without affecting c-fos and c-jun synthesis. *Journal of Biological Chemistry* 268:14103–14108.

Pierce, J. P., Natarajan, L., Caan, B. J., Parker, B. A., Greenberg, E. R., Flatt, S.W., Rock, C.L., Kealey, S., Al-Delaimy, W.K., Bardwell, W.A., Carlson, R.W., Emond, J.A., Faciber, S., Gold, E.B., Hajek, R.A., Hollenback, K., Jones, L.A., Karanja, N., Madlensky, L., Marshall, J., Newman, V.A., Ritenbaugh, C., Thomson. C.A., Wasserman, L. and Stefanick, M. L. 2007. Influence of a diet very high in vegetables, fruit, and fiber and low in fat on prognosis following treatment for breast cancer: the Women's Healthy Eating and Living (WHEL) randomized trial. *Journal of American Medical Association* 298.3:289-298.

Pierce, J. P., Stefanick, M. L., Flatt, S. W., Natarajan, L., Sternfeld, B., Madlensky, L. Al-Delaimy W.K., Thomson, C.A., Kealey, S., Hajek, R., Parker, B. A., Newman, V.A. Caan B. and Rock, C.L. 2007. Greater survival after breast cancer in physically active

women with high vegetable-fruit intake regardless of obesity. *Journal of Clinical Oncology* 25.17:2345-2351.

Pike, M. C., Krailo, M. D., Henderson, B. E., Casagrande, J. T. and Hoel, D. G.1983. Hormonal risk factors, breast tissue age and the age-incidence of breast cancer. *Nature* 303:767-770.

Pinheiro, S. P., Holmes, M. D., Pollack, M. N., Barbieri, R. L. and Hankinson, S. E. 2005. Racial differences in premenopausal endogenous hormones. *Cancer Epidemiology Biomarkers Previews* 14:2147-2153.

Pinto, A. E., Andre, S., Laranjeira, C. and Soares, J. 2005. Correlations of cell cycle regulators (p53, p21, pRb and mdm2) and c-erbB-2 with biological markers of proliferation and overall survival in breast cancer. *Pathology* 37:45-50.

Pogrinby, I.P and Rusyn, I. 2013. Environmental toxicants, epigenetics and cancer. *Advanced Experimental Medical Biology* 754: 215-232

Pontikides, N., Kaltsas, T. H. and Krassas G.E. 1990. The hypothalamic-pituitary-gonadal axis in hyperthyroid female patients before and after treatment. *Journal of Endocrinology Investigation* 13.2 Suppl: 203.

Popoola., A.O., Ibrahim, N. A., Omodele, F. O., Oludara, M.A., Adebowale, S.A. and Igwilo, A.I. 2012. Pattern of Spread of Breast Cancer among Patients attending Cancer Unit of Lagos State University Teaching Hospital. *Asian Journal of Medical Sciences* 4.3:89-94.

Pot, J. and Simmins, D. 1994. Sex and ethnic group differences in fat distribution in young United Kingdom South Asians and Europids. *Journal of Clinical Epidemiology* 47:837-841.

Powell, B. L., Piersma, D., Kevenaar, M. E., Vanstaveren, L. L., Themmen, A. P. N., Iacopetta, B. J. and Berns, E. M. J. J. 2003. Luteinizing Hormone Signaling and Breast Cancer: Polymorphisms and Age of Onset. *The Journal of Clinical Endocrinology & Metabolism* 88.4:1653–1657.

Prat, A., Cheang, M. C., Martin, M., Parker, J. S., Carrasco, E., Caballero, R., Tyldesley, S., Gelmon, K., Bernard, P. S., Nielsen, T. O. and Perou, C. M. 2013. Prognostic significance of progesterone receptor-positive tumor cells within immunohistochemically declined luminal A breast cancer. *Journal of Clinical Oncology* 31:203-209.

Privalsky, M. 2002. Regulation of oestrogen response by co-repressor. *British Medical Journal* 12:79-85.

Prya P. N., Pillai, A. and Gupta, S. 2004. Effect of simultaneous exposure to lead and cadmium on gonadotropin binding and steroidogenesis on granulose cells: an in vitro study. *Indian Journal and Experimental Biology* 42:143-148.

Program for Appropriate Technology in Health (PATH) 1997. 15.1.

Pujol, P., Daures, J. P., Brouillet, J. P., Chang S., Rouanet P., Bringer J., Greiner J. and Maudelonde T. 2001. A prospective prognostic study of the hormonal milieu at the time of surgery in premenopausal breast carcinoma. *Cancer* 91: 1854-1861.

Putnam, J. and Gerrior, S. 1999. Trends in the U.S. food supply, 1970-1997. In Frazião E (ed): "America's Eating Habits: Changes and Consequences." Washington, DC: Economic Research Service, U.S. Department of Agriculture.

Qiao, E. Q., Ji, M., Wu, J., Li, J., Xu, X, Ma, R., Zhang, X., He, Y., Zha, Q., Song, X., Zhu, L. and Tang, J-H. 2013. Joint detection of multiple immunohistochemical indices and clinical significance in breast cancer. *Molecular and Clinical Oncology* 1:703-710.

Qian, F., Ogundiran, T., Hou N., Ndom, P., Gakwaya, A. and Jombwe, J. 2014. Alcohol consumption and breast cancer risk among women in three sub-Saharan African countries. *PLOS ONE* 9.9:e106908.

Radu, A., Pichon, C., Camparo, P., Antoine, M., Allory, Y., Couvelard, A., Fromont, G., Hai, M. T. and Ghinea, N. 2010. Expression of follicle-stimulating hormone receptor in tumor blood vessels. *New England Journal of Medicine* 363.17:1621–1630.

Ragab, A. R., Farouk, O., Afify, M. M., Attia, A. M., Samanoudy, A. E. And Taalab, Y. M. 2014. The Role of Oxidative Stress in Carcinogenesis Induced By Metals in Breast Cancer Egyptian Females Sample at Dakahlia Govemorate. *Journal of Environmental Analysis and Toxicology* 4:207.

Rakha, E. A., El-Sayed, M. E., Green, A. R., Paish, E. C., Powe, D. G., Gee, J., Nicholson, R. I., Lee, A. H., Robertson, J. F. and Ellis, I. O. 2007. Biologic and clinical characteristics of breast cancer with single hormone receptor positive phenotype. *Journal of Clinical Oncology* 25:4772-4778.

Ramsey, S. D., Henry, N. L. and Gralow, J 2015. Tumor marker usage and medical care costs among older early-stage breast cancer survivors. *Journal of Clinical Oncology* 33:149-155.

Rapiti, E., Fiorella, G., Verkooijen, H. M., Vlastos, G., Schafer, P., Sappino, A-P., Kurtz, J.,

Neyroud-Casper I. and Bouchardy, C. 2005. Survival of young and older breast cancer patients in Geneva from 1990-2001. *European Journal of Cancer* 41:1446-1452.

Rasmuson, B., Feldt-Rasmussen, U., Hegedus, L., Perrild, H., Bech, K. and Hoier-Madsen, M. 1987. Thyroid function in patients with breast cancer. *European Journal of Cancer and Clinical Oncology* 23:553-556.

Ravdin, P. M., Green, S., Dorr, T. M., McGuire, W. L., Fabian, C., Pugh, R. P., Carter, R. D., Rivkin S. E., Borst, J. R. and Belt, R. J. 1992. Prognostic significance of progesterone receptor levels in estrogen receptor positive patients with metastatic breast cancer treated with tamoxifen; results of a prospective Southwest oncology group study. *Journal of Clinical Oncology* 10:1284-1291.

Recarcanu, F., Simionnescu, C., Georgescu, C. V. and Pirici, E. 2011. Ductal invasive mammary carcinoma-clinicopathological prognostic factors related to immunohistochemical expression of hormonal receptors and Her2/neu oncoprotein. *Romanian Journal of Morphology and Embrology* 52:1059-1064.

Redmond, G. P. 2004. Thyroid dysfunction and women's reproductive health. *Thyroid* 14.Suppl 1:S5–S15.

Reeves, P. G. and Chaney, R. L. 2008. Bioavailability as an issue in risk assessment and management of food cadmium: A review. *Science of the Total Environment* 398:13-19.

Reincke, M., Beuschlein, F., Menig, H., Hofmockel, G., Arlt, W., Lehmann, R. and Karl, M. 1998. Localization and expression of adrenocorticotropic hormone receptor mRNA in normal and neoplastic human adrenal cortex. *Journal of Endocrinology* 156:415-423.

Reiner, A., Neumeister, B., Spona, J., Reiner, G., Schemper, M. and Jakesz, R. 1990. Immunocytochemical localisation of estrogen and progesterone receptor and prognosis in human primary breast cancer. *Cancer Research* 50:7057-7061.

Remennick, L. I. 1990. Induced abortion as cancer risk factor: a review of epidemiological evidence. *Journal of Epidemiology and Community Health* 44:259-264.

Renehan, A. G., Tyson, M., Egger, M., Heller, R. F. and Zwahlen, M. 2008. Body mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies. *Lancet* 371:569-578.

Reuben, S. H. 2010. Reducing environmental cancer

170

risk, what we can do now. 2008-2009 Annual Report, President's Cancer Panel. U.S. Department of Health and Human Services, National Institutes of Health, National Cancer Institute.

Revillion, F., Bonneterre, J. and Peyrat, J. P. 1998. ERBB2 oncogene in human breast cancer and its clinical significance. *European Journal of Cancer* 34:791-808.

Rhodes, A. and Jasani, B. 2009. The oestrogen receptor-negative/progesterone receptor-positive breast tumour: a biological entity or a technical artefact? *Journal of Clinical Pathology* 62:95-96.

Riboli, E. and Norat, T. 2003. Epidemiologic evidence of the protective effect of fruit and vegetables on cancer risk. *American Journal of Clinical Nutrition* 78.3 Suppl: 559S-569S.

Robbins, J. 1992. Thyroxine transport and the free hormone hypothesis. *Endocrinology* 131:546-547.

Rock, C. L., Flatt, S. W., Natarajan, L., Thomson, C. A., Bardwell, W. A., Newman, V. A., Hollenback, K. A., Jones, L., Caan, B.J., Pierce, J. P. 2005. Plasma carotenoids and recurrence-free survival in women with a history of breast cancer. *Journal of Clinical Oncology* 23:6631-6638.

Rock, C. L., Flatt, S. W., Thomson, C. A., Stefanick, M. L., Newman, V. A., Jones, L. A., Natarajan, L., Ritenbaugh, C., Hollenback, K. A., Pierce, J. P and Chang, R. J. 2004. Effects of a high-fiber, low-fat diet intervention on serum concentrations of reproductive steroid hormones in women with a history of breast cancer. *Journal of Clinical Oncology* 22.12:2379-2387.

Rohan, T. E., Howe, G. R., Friedenreich, C. M., Jain, M. and Miller, A. B. 1993. Dietary fiber, vitamins A, C, and E, and risk of breast cancer: a cohort study. *Cancer Causes Control* 4:29-37.

Romieu, I., Lazcano-Ponce, E., Sanchez-Zamorano, L. M., Willett, W. and Hernandez-Avila, M. 2004. Carbohydrates and the risk of breast cancer among Mexican women. *Cancer Epidemiology Biomarkers Previews* 13.8:1283-1289.

Ronis, M. J., Badger, T. M., Shema, S. J., Robertson, P. K. and Shaikh, F. 1996. Reproductive toxicity and growth effects in rats exposed to lead at different periods during development. *Toxicology and Applied Pharmacology* 136:361-371.

171

Ronnov-Jessen, L., Petersen, O. W. and Bissell, M. J. 1996. Cellular changes involved in conversion of normal to malignant breast: importance of the stromal reaction. *Physiological Reviews* 76:69–125.

Rosenberg, C. R., Pasternack, B. S., Shore, R. E., Koenig. K. L.and Toniolo, P. G., 1994. Premenopausal estradiol levels and the risk of breast cancer: a new method of controlling for day of the menstrual cycle. *American Journal of Epidemiology* 140:518-525.

Rossner, P. Jr, Gammon, M. D., Terry, M. B., Agrawal, M., Zhang, F. F., Teitelbaum, S. L., Eng, S.M., Gaudet, M.M., Neugut, A.I and Santella, R.M. 2006. Relationship between urinary 15-F2t-Isoprostane and 8-Oxodeoxyguanosine levels and breast cancer risk. *Cancer Epidemiology Biomarkers Previews* 15.4:639-644.

Rothenberg, S. J., Karchmer, S., Schnaas, L., Perroni, E., Zea, F. and Fernandez, A. J. 1994. Changes in serial blood lead levels during pregnancy. *Environmental Health Perspectives* 102.10:876-880.

Rotstein, A. 2011. Sex Hormone Synthesis. *Endocrinology Reviews* 32.1:81-151

Roy, P., Salminen, H., Koskimies, P., Simola, J., Smeds, A., Saukko, P. and Huhtaniemi, I.T., 2004. Screening of some anti-androgenic endocrine disruptors using a recombinant cell-based in vitro bioassay. *Journal of Steroid Biochemistry and Molecular Biology* 88:157-166.

Rudland, P. S. 1993. Epithelial stem cells and their possible role in the development of the normal and diseased human breast. *Histology and Histopathology* 8:385–404.

Rudland, P.S., Fernig, D. G. and Smith, J. A. 1995. Growth factors and their receptors in neoplastic mammary gland. *Biomedical Pharmacotherapy* 49:389-399.

Ruiz-Ramos, R., Lopez-Carrillo, L., Rios-Perez, A. D., De Vizcaya-Ruiz, A. and Cebrian, M. E. 2009. Sodium arsenite induces ROS generation, DNA oxidative damage, HO-1 and c-myc Proteins, NF-kB activation and cell proliferation in human breast cancer MCF-7 cells. *Mutation Research* 674.1-2:109-115.

Russo, I. H. and Russo, J. 1998. Role of hormones in mammary cancer initiation and progression. *Journal of Mammary Gland Biology and Neoplasia* 349–361.

Russo, J., Hu, Y. F, Silva, I. D. C. G. and Russo, I. H. 2001. Cancer Risk Related to Mammary Gland Structure and Development. *Microscopic Research Technology* 52:204–223.

Rutkowski. J.M., Davis, K. E. and Scherer, P.E 2009. Mechanisms of obesity and related pathologies: The macro and microcirculation of adipose tissue. *FEBS Journal* 276.20:5738-5746.

Ryu, S. Y., Kim, C. B., Nam, C. M., Park, J. K., Kim, K. S., Park, J., Yoo, S.Y. and Cho. K. S. 2001. Is body mass index the prognostic factor in breast cancer? a meta-analysis. *Journal of Korean Medical Science* 16.5:610-614.

Sacks, N. P. and Baum, M. 1993. Primary management of carcinoma of the breast. *Lancet* 342:1402-1408.

Saji, S., Jensen, E. V., Nilsson, S., Rylander, T., Warner, M. and Gustafsson, J. A. 2000. Estrogen receptors alpha and beta in the rodent mammary gland. *Proceedings of National Academy of Science* 97: 337–342.

Salamonsen, L. A., Butt, A. R., Hammond, F. R., Garcia, S. and Zhang, J. 1997. Production of endometrial matrix metalloproteinases but not their tissue inhibitors is modulated by progesterone withdrawal in an in vitro model for menstruation. *The Journal of Clinical Endocrinology and Metabolism* 82: 1409–1415.

Santen, R. J., Yue W.and Bocchinfuso, W. 2004. Estradiol-induced carcinogenesis via formation of genotoxic metabolites. In: Ingle JN, Dowsett M, eds. Advances in Endocrine Therapy of Breast Cancer: Proceedings of the 2003. Gleneagles Conference. New York. NY: Summit Communications. 163-177.

Sapozhnikova, Y., Bawardi, O. and Schlenk, D. 2004. Pesticides and PCBs in sediments and fish from the Salton Sea, California, USA. *Chemosphere* 55:797–809.

Saraiva, P. P., Figueiredo, N. B., Padovani, C. R., Brentani, M. M. and Nogueir, C. R. 2005. Profile of thyroid hormones in breast cancer patients. *Brazilian Journal of Medical and Biological Research* 38:761-765.

Sarlis, N. J., Gourgiolis, L., Pucino, F. and Tolis G. J. 2002. Lack of association between Hashimoto thyroiditis and breast cancer: a quantitative research hypothesis. *Hormones* 1:35-41.

Satarug. S., Garrett, S.H., Sens, M.A, Sens, D.A. 2010. Cadmium, environmental exposure and health outcomes. *Environmental Health Perspectives* 118:182-190.

Saten, R. J, Leszczynski, D and Tilson-Mallet, M. 1986. Enzymatic control of oestrogen

production in human breast cancer: relative significance of aromatase versus sulfatase pathways. *Annals of New York Academy of Science* 464:126-137.

Sawin, C. T., Hershman, J. M., Chopra, I. J. 1977. The comparative effect of T4 and T3 on the TSH response to TRH in young adult men. *Journal of Clinical Endocrinology and Metabolism* 44:273-278.

Scawn, R., Shousha, S. 2002. Morphologic spectrum of Estrogen receptor Negative Breast Carcinoma. *Archive of Pathology and Laboratory Medicine* 126:325-330.

Schoppmann, S.F., Horvat, R. and Birner, P. 2002. Lymphatic vessels and lymphangiogenesis in female cancer: Mechanisms, clinical impact and possible implications for anti-lymphangiogenic therapies (Review). *Oncology Reports* 9: 455-460.

Scippo, M. L., Argiris, C., Van De Weerdt, C., Muller, M., Willemsen, P., Martial, J. and Maghuin-Rogister, G. 2004. Recombinant human estrogen, androgen and progesterone receptors for detection of potential endocrine disruptors. *Annals Bioanalytical Chemistry* 378:664-669.

Shak, S. 1999. Overview of the trastuzumab (Herceptin) anti-HER2 monoclonal antibody clinical program in HER2 overexpressing metastatic breast cancer. Herceptin Multinational Investigator Study Group. *Seminars in Oncology* 12:71-77.

Shannon, J., Ray, R., Wu, C., Nelson, Z., Gao, D. L., Li, W., Hu, W., Lampe, J., Horner, N., Satia, J., Patterson, R., Fitzgibbons, D., Porter, P. and Thomas, D. 2005. Food and botanical groupings and risk of breast cancer: a case-control study in shanghai, china. *Cancer Epidemiology Biomarkers Previews* 14.1:81-90.

Shao, Z., Sheikh, M. S., Rishi, A. H., Dawson, M. I., Li, X., Wilber, J. F., Feng, P. and Fontana, J. A 1995. Thyroid hormone enhancement of estradiol stimulation of breast carcinoma proliferation. *Experimental Cell Research* 218: 1-8.

Shareef, A., Angove, M.J., Wells, J.D. and Johnson, B.B., 2006. Aqueous solubilities of estrone, 17b-estradiol, 17a-ethynylestradiol, and bisphenol A. *Journal of Chemical and Engineering Data* 51: 879-881.

Shih, C. M, Ko, W. C, Wu, J. S., Wei, Y.H., Wang, L.F., Chang, E.E., Lo, T.Y., Cheng, H.H and Chen, C. T. 2004. Mediating of caspase-independent apoptosis by cadmium through the mitochondria-ROS pathway in MRC-5 fibroblasts. *Journal of Cell Biochemistry* 91: 384-397.

174

Shu, X. O., Jin, F., Dai, Q., Shi, J. R., Potter, J. D., Brinton, L.A., Hebert, J.R., Ruan, Z., Gao, Y-T. and Zheng, W. 2001. Association of body size and fat distribution with risk of breast cancer among Chinese women. *International Journal of Cancer* 94.3:449-455.

Siddiqui, M. K., Jyoti- Singh, S., Mehrotra, P. K., Singh, K. and Sarangi, R. 2006. Comparison of some trace elements concentration in blood, tumour free breast and tumour tissues of women with benign and malignant breast lesions, an Indian study. *Environmental Interactions* 32.5:630-637.

Sidhu, S., Gullett, B., Striebich, R., Klosterman, J., Contreras, J. and DeVito, M., 2005. Endocrine disrupting chemical emissions from combustion sources: diesel particulate emissions and domestic waste open burn emissions. *Atmosphere and Environment* 39: 801-811.

Sieri, S., Pala, V., Brighenti, F., Pellegrini, N., Muti, P., Micheli, A., Evangelista, A., Grioni, S., Contiero, P., Berrino, F. and Krogh, V. 2007. Dietary glycemic index, glycemic load, and the risk of breast cancer in an Italian prospective cohort study. *American Journal of Clinical Nutrition* 86.4:1160-1166.

Siewt, C. L., Gengler, B., Vegas, E., Puckett, R. and Louie, M. C. 2010. Cadmium promotes breast cancer cell proliferation by potentiating the interaction between ER alpha and c-jun. *Molecular Endocrinology* 24:981-992.

Süteri, P. K., Murai, J. T., Hammod, G. L., Nisker, J. A., Raymond, W. J. and Kuhn, R. W. 1982. The serum transport of steroid hormones. *Recent Progress in Hormone Research* 38:457-510.

Simard, J., Ricketts, M.L., Gingras, S., Saucy P., Feltus, F.A. and Meiner, M.H.2005. Molecular biology of the 3-beta-hydroxysteroid dehydrogenase/delat 5-Delta 4 isomerase gene family. *Endocrine Reviews* 26:525-582.

Sivrikaya, A., Menevşe, E., Altıntepe, L, and Tiftik, A. M. 2013. The Relations between Levels of Cadmium and Thyroid Parameters in Hemodialysis Patients. *Journal of Clinical and Analytical Medicine* 4,1: 1-4.

Sjögren, S., Inganäs, M., Lindgren, A., Holmberg, L. and Bergh, J. 1998. Prognostic and Predictive value of c-erbB-2 overexpression in primary breast cancer, alone and in combination with other prognostic markers. *Journal of Clinical Oncology* 16:462-469.

Slavin, J. 2003. Why whole grains are protective: biological mechanisms. *Proceedings of*

175

Nutrition Society 62.1:129-134.

Slavin, J. L. 2000. Mechanisms for the impact of whole grain foods on cancer risk. *Journal of American College Nutrition* 19.3 Suppl: 300S-307S.

Smith, R. E. and Good, B. C. 2003. Chemoprevention of breast cancer and the trials of the National Surgical Adjuvant Breast and Bowel Project and others. *Endocrine Related Cancer* 10:347-357.

Smyth, P. P. A., Smith, D., McDermott, E., Murray, M., Geraghty, J. and O'Higgins, N. A. 1996. A relationship between thyroid enlargement and breast cancer. *Journal Clinical Endocrinology and Metabolism* 81:937-941.

Smyth, P. P. A., Shering, S., Kilbane, M. T., Murray, M., McDermott, E. W. M., Smith, D. F.and O'Higgins, N. J. 1998. Serum thyroid peroxidase autoantibodies, thyroid volume and outcome in breast cancer. *Journal of Clinical Endocrinology and Metabolism* 83:2711-2716.

Smyth, P. P. 1997. The thyroid and breast cancer: a significant association? *Annals of Medicine* 29: 189-191.

Snijder, M. B., Dam, R. M. V., Visser, M. and Seidell, J. C. 2006. What aspects of body fat are particularly hazardous and how do we measure them? *International Journal of Epidemiology* 35:83-92.

Somm, E., Schwitzgebel, V. M., Toulotte, A., Cederroth, C. R., Combescure, C., Nef, S., Aubert. M. L. and Huppi, P. S. 2009. Perinatal exposure to bisphenol-A alters early adipogenesis in the rat. *Environmental Health Perspectives* 117:1549-1555.

Song, H., Zhang, T., Yang, P., Li, M., Yang, Y., Wang, Y., Du, J., Pan, K and Zhang, K. 2015. Low doses of BPA stimulates the proliferation of Breast cancer cells via ERK 1/2/ERR gamma signals. *Toxicology in vitro* 30(1 Pt B): 521-528

Sprangler, L. D. 1996. Xenoestrogens and breast cancer, nowhere to run to. *Women wise magazine* 1-8.

Stahlhut, R. W., Welshons, W. V. and Swan, S.H., 2009. Bisphenol-A data in NHANES suggest longer than expected half-life, substantial nonfood exposure, or both. *Environmental Health Perspectives* 117:784-789.

Staples, C.A., Dorn, P.B., Klecka, G.M., O'Block, S.T. and Harris, L.R., 1998. A review of the environmental fate, effects, and exposures of bisphenol-A. *Chemosphere* 36:2149-2173.

Stark, A., Kleer, C., Martin, I., Awuah, B., Nsiah-Asare, A, Takiyi V., Bramen M., Quayson SE., Zarbo R., Wicha M. and Newman. 2010. African Ancestry and Higher Prevalence of Triple-Negative Breast Cancer: Findings From an International Study. *Cancer* 116: 4926-4932.

Steenland, K.,Selevan, S. and Landrigan, P. 1992. The mortality of lead smelter workers. An update. *American Journal of Public Health* 82:1641-1644.

Stierer, M., Rosen, H., Weber, R., Hanns, H., Jurgen, S. and Heinz, T. 1993. Immunohistochemical and biochemical measurement of estrogen and progesterone receptors in primary breast cancer correlation of histopathology and prognostic factors. *Annals of Surgery* 218.1: 13-21.

Stoica, A., Katzenellenbogen, B. S. and Martin, M. B. 2000b. Activation of estrogen receptor-alpha by the heavy metal cadmium. *Molecular Endocrinology* 14:545-553.

Stoica, A., Pentecost, E. and Martin. M. B. 2000a. Effects of arsenite on estrogen receptor-alpha expression and activity in MCF-7 breast cancer cells. *Endocrinology* 141.10:3595-3602.

Stoll, B. A. 1996. Can supplementary dietary fibre suppress breast cancer growth? *British Journal of Cancer* 73.5:557-559.

Stradtman. E. W. 1993. Thyroid Dysfunction and Ovulatory Disorders. In: Carr BR, Blackwell RE (ed.), Textbook of Reproductive Medicine. Norwalk, Connecticut: Appleton & Lange.

Sturgeon, S. R., Potischman, N., Malone, K. E, Dorgan, J. F., Daling, J., Schairer, C. and Brinton, L. A. 2004. Serum levels of sex hormones and breast cancer risk in premenopausal women: a case-control study (USA). *Cancer Causes Control* 15:45-53.

Sule, E. A. 2011. Age distribution and histological types of Breast Cancer in two major Hospitals in the Niger Delta. *Continental Journal of Biomedical Sciences* 5.1: 37-42.

Sundaram, V., Hanna, A. N., Koneru, L., Newman, H. A. and Falko, J. M. 1997. Both hypothyroidism and hyperthyroidism enhance low density lipoprotein oxidation. *Journal of Clinical Endocrinology and Metabolism* 82:3421-3424.

Sun, X., Fontaine, J. M., Barti, I., Behnam, B., Welsh, M. J. and Benndorf, R. 2007. Induction of Hsp22 (HspB8) by estrogen and the metalloestrogen cadmium in estrogen receptor-positive breast cancer cells. *Cellular Stress Chaperones* 12.4: 307-319.

Surks. M. I., Ortiz, E., Daniels, G. H., Sawin, C. T., Col, N. F., Cobin RH, Franklyn, J. A.,

Hershman, J. M., Burman, K. D., Denke, M. A., Gorman, C., Cooper, R. S. and Weissman, N. J. 2004. Subclinical thyroid disease: Scientific review and guidelines for diagnosis and management. *Journal of American Medical Association* 291.2:228-238.

Surks, M. I., Schadlow, A. R., Stock, J. M. and Oppenheimer, J. H. 1973. Determination of iodothyronine absorption and conversion of L-thyroxine (T4) to L-triiodothyronine (T3) using turnover rate techniques. *Journal of Clinical Investigation* 52:805-811.

Suvarchala, S. B. and Nageswararao, R. 2011. Carcinoma Breast-Histopathological and Hormone Receptors Correlation. *Journal of Bioscience and Technology* 2.4:340-348.

Suzuki, R., Orsini, N., Mignone, L., Saji, S. and Wolk, A. 2008. Alcohol intake and risk of breast cancer defined by estrogen and progesterone receptor status—a meta-analysis of epidemiological studies. *International Journal of Cancer* 122: 1832-1841.

Suzuki, T., Sasano, H., Kimura, N., Tamura, M., Fukaya, T., Yajima, A. and Nagura, H. 1994. Immunohistochemical distribution of progesterone, androgen and oestrogen receptors in the human ovary during the menstrual cycle: relationship to expression of steroidogenic enzymes. *Human Reproduction* 9: 1589-1595.

Sweeney, C., Blair, C. K., Anderson, K. E., Lazovich, D. and Folsom, A. R. 2004. Risk factors for breast cancer in elderly women. *American Journal of Epidemiology* 160.9:868-875.

Taiwo, K.A and Akanbi, O.C. 1997. The effects of soaking and cooking time on the cooking properties of two cowpea varieties. *Journal of Food Engineering.* 33:337-346

Takagi, S., Hummel, B. C. and Walfish, P. G. 1990. Thionamides and arsenite inhibit specific T3 binding to the hepatic nuclear receptor. *Biochemistry and Cell Biology* 68.3:616-621.

Takatani, O., Okumoto, T., Kosano, H., Nishida, M., Hiraide, H. and Tamakuma, S. 1989. Relationship between the levels of serumthyroid hormones or estrogen status and the risk of breast cancer genesis in Japanese woman. *Cancer Research* 49: 3109-3112

Tanaka, T., Tamai, H., Kuma, K., Matsuzuka, F. and Hidaka, H. 1981. Gonadotropin response to luteinizing hormone releasing hormone in hyperthyroid patients with menstrual disturbances. *Metabolism* 30:323-326.

Taylor, E. F., Burley, V. J., Greenwood, D. C. and Cade, J. E. 2007. Meat consumption and risk of breast cancer in the UK Women's Cohort Study. *British Journal of Cancer* 96.7:1139-1146.

Teebor, O. W., Boorstein, R. J. and Cadet, J. 1988. The repairability of oxidative free radical

mediated damage in DNA: a review. *International Journal of Radiation Biology* 54:131-150.

Tekin, D., Kayaalti, Z., Aliyev, V. and Soylemezoglu, T. 2012. The effects of metallothionein 2A polymorphism on placental cadmium accumulation: Is metallothionein a modifying factor in transfer of micronutrients to the fetus? *Journal of Applied Toxicology* 32: 270-275.

Tellez-Plaza, M., Navas-Acien, A., Crainconu, C.M. and Giualler, E.2008. Cadmium exposure and hypertension in the 1991-2004 National Health and Nutrition Examination Survey (NHANES). *Environmental Health Perspectives* 116:51-56.

Templeton, D. M. and Liu, Y. 2010. Multiple roles of cadmium in cell death and survival. *Chemical and Biological Interaction* 188: 267-275.

Terry, P., Jain, M., Miller, A. B., Howe, G. R. and Rohan, T. E. 2002. No association among total dietary fiber, fiber fractions, and risk of breast cancer. *Cancer Epidemiology Biomarkers Preview* 11.11:1507-1508.

The National Academy of Clinical Biochemistry (NACB) 1996. Standards of Laboratory Practice. Laboratory Support for the Diagnosis and Monitoring of Thyroid Disease. American Association of Clinical Chemistry. 1-64.

Thomas, H. V., Key, T. J., Allen, D. S., Moore, J. W., Dowsell, M., Fentiman, I. S. and Wang, D. Y. 1997. A prospective study of endogenous serum hormone concentrations and breast cancer risk in postmenpausal women on the island of Guemsey. *British Journal of Cancer* 76:401-405.

Thomas, P. and Dong, J. 2006. Binding and activation of the seven-transmembrane estrogen receptor GPR30 by environmental estrogens: a potential novel mechanism of endocrine disruption. *Journal of Steroid Biochemistry and Molecular Biology* 102.1-5:175-179.

Thomasset, N., Lochter, A., Sympson. C. J., Lund, L. R., Williams, D. R., Behrendtsen, O., Werb, Z. and Bissell, M. J. 1998. Expression of autoactivated stromelysin-1 in mammary glands of transgenic mice leads to a reactive stroma during early development. *American Journal of Pathology* 153: 457–467.

Thompson, 2006. Breast Cancer Metastosis: Markers and Models: Clinical Features of Breast Cancer Metastasis. *Journal Citation Reports.* Retrived from www. nature. com/ review/ cancer.2005.

Thor, A. D., Berry, D. A., Budman, D. R., Muss, H. B., Kute, T. And Henderson, I. C. 1998. C-erb B2 expression, p53 and efficacy of adjuvant therapy in lymphnode positive breast cancer. *Journal of National Cancer Institute* 98:1346-1360.

Thun, M. J., DeLancey, J. O., Center, M. M., Jemal, A. and Ward, E. M. 2010. The global burden of cancer: priorities for prevention. *Carcinogenesis* 31.1: 100–110.

Thun, M. J., Peto, R., Lopez, A. D., Monaco, J. H. and Henley, S. J. 1997. Alcohol consumption and mortality among middle-aged and elderly U.S. adults. *New England Journal of Medicine* 337: 1705–1714.

Tosovic, A., Bondesson, A. G., Bonseson, L., Ericsson, U. B., Malm, J. and Manjer, J. 2010. Prospectively measured triiodothyronine levels are positively associated with breast cancer risk in postmenopausal women. *Breast Cancer Research* 12:R33.

Tosovic, A., Becker, C., Bondeson, A-G., Ericsson, U-B., Malm, J. and Manjer, J. 2012. Prospectively measured thyroid hormone and thyroid peroxidase antibodies in relation to breast cancer risk. *International Journal of Cancer* 131.9: 2126-2133.

Travis, R. C. and Key, T. J. 2003. Oestrogen exposure and breast cancer risk. *Breast Cancer Research* 5.5: 239-247.

Trentham-Dietz, A., Newcomb, P. A., Storer, B. E., Longnecker, M.P., Baron, J., Greenberg, E.R. and Willett, W.C. 1997. Body size and risk of breast cancer. *American Journal of Epidemiology* 145.11: 1011–1019.

Trussell, J. 1998. Contraceptive efficacy. In: Hatcher RA, Trussell J, Stewart F, et al. eds. Contraceptive Technology. 17th ed. New York, NY: Ardent Media. 779-844.

Tsai, W. T., 2006. Human health risk on environmental exposure to bisphenol-A: a review. *Journal of Environmental Science and Health* 24:225-255.

Tsai, Y. C., Lu, Y., Nichols, P. W., Zlotnikov, G., Jones, P. A. and Smith, H. S. 1996. Contiguous patches of normal human mammary epithelium derived from a single stem cell: Implications for breast carcinogenesis. *Cancer Research* 56: 402-404.

Tsang, B. K., Amstrong, D. T. and White-field, J. F. 1980. Steroid biosynthesis by isolated human ovarian follicular cells in vitro. *Journal of Clinical Endocrinology and Metabolism* 51: 1407.1411.

Tseng, C. H., Huang, Y. K., Huang, Y. L., Chung, C. J., Yang, M. H. and Chen, C. J. 2005.

Arsenic exposure, urinary arsenic speciation, and peripheral vascular disease in blackfoot disease-hyperendemic villages in Taiwan. *Toxicology and Applied Pharmacology* 206.3:299-308.

Tseng, C.H 2005. Waist-to-height ratio is independently and better associated with urinary albumin excretion rate than waist circumference or waist-to-hip ratio in Chinese adult type 2 diabetic women but not men. *Diabetes Care* 28: 2249-2251.

Tulinius, H., Sigvaldason, H and Olafsdottir, G. 1990. Left and right sided breast cancer. *Pathology Research and Practice* 186.1:92-94.

Tunizicker-Dunn, M. and Maizels, E. T. 2006. FSH signaling pathways in immature granulose cells that regulate target gene expression; branching out from protein kinase A. *Cell Signalling* 1351-1359.

Turken, O., Narin, Y., Demirbas, S., Onde, M. E., Sayan, O., Kandemir, E. G., Yaylac, I. M and Ozturk, A. 2003. Breast cancer in association with thyroid disorders. *Breast Cancer Research* 5: R110-R113.

Tworoger, S.S., Eliassen, A.H. and Kelesidis, T. 2007. Plasma adiponectin concentration and risk of incident breast cancer. *Journal of Clinical Endocrinology and Metabolism* 92:1510-1516.

Umayahara, Y., Kawamori, R., Watada, H., Imano, E., Iwama, N., Morishima, T., Yamasaki, Y., Kajimoto, Y. and Kamada, T. 1994. Estrogen regulation of the insulin-like growth factor 1 gene transcription involves an AP-1 enhancer. *Journal of Biological Chemistry* 269. 16433–16442.

Unden, A. B., Sandstedt, B., Bruce, K., Hedblad, M. and Stahle-Backdahl, M. 1996. Stromelysin-3 mRNA associated with myofibroblasts is overexpressed in aggressive basal cell carcinoma and in dermatofibroma but not in dermatofibrosarcoma. *Journal of Investigative Dermatology* 107:147–153.

United States Department of Agriculture (USDA) 2000. Nutrition and Your Health: Dietary Guidelines for Americans. 5th ed. Washington, DC: U.S. Department of Agriculture, U.S. Department of Health and Human Services.

Urbatzka, R., van Cauwenberge, A., Maggioni, S., Vigano, L., Mandich, A., Benfenati, E., Lutz,

Arsenic exposure, urinary arsenic speciation, and peripheral vascular disease in blackfoot disease-hyperendemic villages in Taiwan. *Toxicology and Applied Pharmacology* 206.3:299-308.

Tseng, C.H 2005. Waist-to-height ratio is independently and better associated with urinary albumin excretion rate than waist circumference or waist-to-hip ratio in Chinese adult type 2 diabetic women but not men. *Diabetes Care* 28: 2249-2251.

Tulinius, H., Sigvaldason, H and Olafsdottir, G. 1990. Left and right sided breast cancer. *Pathology Research and Practice* 186.1:92-94.

Tunizicker-Dunn, M. and Maizels, E. T. 2006. FSH signaling pathways in immature granulose cells that regulate target gene expression; branching out from protein kinase A. *Cell Signalling* 1351-1359.

Turken, O., Narin, Y., Demirbas, S., Onde, M. E., Sayan, O., Kandemir, E. G., Yaylac, I. M and Ozturk, A. 2003. Breast cancer in association with thyroid disorders. *Breast Cancer Research* 5: R110-R113.

Tworoger, S.S., Eliassen, A.H. and Kelesidis, T. 2007. Plasma adiponectin concentration and risk of incident breast cancer. *Journal of Clinical Endocrinology and Metabolism* 92:1510-1516.

Umayahara, Y., Kawamori, R., Watada, H., Imano, E., Iwama, N., Morishima, T., Yamasaki, Y., Kajimoto, Y. and Komada, T. 1994. Estrogen regulation of the insulin-like growth factor I gene transcription involves an AP-1 enhancer. *Journal of Biological Chemistry* 269. 16433–16442.

Unden, A. B., Sandstedt, B., Bruce, K., Hedblad, M. and Stahle-Backdahl, M. 1996. Stromelysin-3 mRNA associated with myofibroblasts is overexpressed in aggressive basal cell carcinoma and in dermatofibroma but not in dermatofibrosarcoma. *Journal of Investigative Dermatology* 107:147–153.

United States Department of Agriculture (USDA) 2000. Nutrition and Your Health: Dietary Guidelines for Americans. 5th ed. Washington, DC: U.S. Department of Agriculture. U.S. Department of Health and Human Services.

Urbatzka, R., van Couwenberge, A., Maggioni, S., Vigano, L., Mandich, A., Benfenati, E., Lutz,

and Kloas, W., 2007. Androgenic and antiandrogenicactivities in water and sediment samples from the river Lambro, Italy, detected by yeast androgen screen and chemical analyses. *Chemosphere* 67:1080-1087.

Ursin, G., Longnecker, M. P., Haile, R. W. and Greenland, S. 1995. A meta-analysis of body mass index and risk of premenopausal breast cancer. *Epidemiology* 6:137-141.

US Environmental Protection Agency (USEPA), 2010. Bisphenol A Action Plan. Washington D.C., USA.

Vahter, M., Berglund, M., Nermell, B.and Akesson, A.1996. Bioavailability of cadmium from shelfishand mixed diet in women. *Toxicology and Applied Pharmacology* 136:332-341.

Vaissiere, T., Sawan, C. and Herceg, Z. 2008. Epigenetic interplay between histone modifications and DNA methylation in gene silencing. *Mutation Research* 659:40.48.

Valavinides, A. T., Vlahogianne, M. and Dassenakis, S. 2006. Molecular biomarkers of oxidative stress in aquatic organism in relation to toxic environmental pollutants. *Ecotoxicology Environment* 64:178-189.

Valenti, G., Ceda, G. P., Denti, L., Tarditi, E. and Speroni, G. 1984. Gonadotropin secretion in hyperthyroidism and hypothyroidism. *Ricerca Clin Lab* 14:53–63.

Valko, M., Leibfritz, D., Moncol, J., Cronin, M.T.D., Mazur, M.and Telser, J. 2007. Free radicals and antioxidants in normal physiological functions and human disease. *International Journal of Biochemistry and Cell Biology* 39: 44-84.

Valko, M., Rhodes, C.J., Moncol, J., Izakovic, M. and Mazur, M. 2006. Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chemico-Biological Interactions* 160: 1-40.

van den Brandt, P. A., Spiegelman, D., Yaun, S. S., Adami, H.O., Beeson, L., Folsom, A.R., Fraser, G., Goldbohm, R.A., Graham, S., Kushi, L., Marshall, J.R., Miller, A.B., Rohan, T., Smith-Warner, S.A., Speizer, F.E., Willett, W.C., Wolk, A. And Hunter, D.J. 2000. Pooled analysis of prospective cohort studies on height, weight, and breast cancer risk. *American Journal of Epidemiology* 152.6:514–527.

van Kruijsdijk, R. C. M., van der Wall, E. and Visseren, F. L. J. 2009. The role of dysfunctional adipose tissue. *Cancer Epidemiology Biomarkers and Prevention* 18: 2569-2578.

Van-Emon, J. M., Chuang, J. C., Bronshtein, A. C.and Altstein M, 2013. Determination of polychlorinated biphenyls in soil and sediment by selective pressurized liquid extraction with immunochemical detection. *Science of the Total Environment* 463–464.

182

Varoni, M.V., Palomba, D., Gianorso, S. and Anania, V. 2003. Cadmium as an environmental factor of hypertension in animals: new perspectives on mechanisms. *Veterinary Research Communication* 27.Suppl 1:807-810.

Venna, M. and Srivastava, S. 2002. Epigenetics in cancer: implications for early detection and prevention. *Lancet Oncology* 3: 755-763

Vessey, M. P., McPherson, K., Yeates, D. and Doll, R. 1982. Oral contraceptive use and abortion before first term pregnancy in relation to breast cancer risk. *British Journal of Cancer* 45:327-331.

Vidal, J. L. M., Frias, M. M., Frenich, A. G., Olea-Serrano, F. and Olea, N. 2002. Determination of endocrine-disrupting pesticides and polychlorinated biphenyls in human serum by GC-ECD and GC-MS-MS and evaluation of contributions to the uncertainty of the results. *Anal of Bioanalytical Chemistry* 372: 766-775.

Vo An. T. and Millis, RM. 2012. Epigenetics and breast cancers. *Obstetrics and Gynaecology International* Article ID;602720, 10 pages

Vohl, M. C., Sladek, R., Robitaille, J., Gurd, S., Marceau, P., Richard, D. and Hudson, T. J. 2004. A survey of genes differentially expressed in subcutaneous and visceral adipose tissue in men. *Obesity Research* 12:1217-1222.

Von Basedow, C. A. 1840. Exophthalmus durch Hypertrophie des Zellgewebes in der Augenhochle. *Wochenschrift fuer die Gesamte Heilkunde* 6:197-204; 220-228.

Wajchenberg, B. L. 2000. Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome. *Endocrine Reviews* 21:697-738.

Wakai, K., Tamakoshi, K., Date, C., Fukui, M., Suzuki, S., Lin, Y.,Niwa, Y., Nishio, K., Yatsuya, H., Kondo, T., Tokudome, S., Yamamoto, A., Toyoshima, H., Tamakoshi. 2005. Dietary intakes of fat and fatty acids and risk of breast cancer: a prospective study in Japan. *Cancer Science* 96,9:590-599.

Wang Bin., Mi Mantian., Wang Jian., Wei Na., Zhang Qianyong., Zhu Jundong., Yang Shu., Guo Botao., Xu Jing and Yang Xinhua 2009. Does the increase of endogenous steroid hormone levels also affect breast cancer risk in Chinese women? A case-control study in Chongqing, China. *International Journal of Cancer* 124: 1892-1899,

Wang, Y., Fang, J., Leonard, S. S. and Rao, K. M. 2004. Cadmium inhibits the electron transfer

chain and induces reactive oxygen species. *Free Radical Biology and Medicine* 36:1434–1443.

Wayne, S. J., Neuhouser, M. L., Ulrich, C. M., Koprowski, C., Baumgartner, K. B., Baumgartner, R. N., McTiernan, A., Bernstein, L. and Ballard-Barbash. 2007. Dietary fiber is associated with serum sex hormones and insulin-related peptides in postmenopausal breast cancer survivors. *Breast Cancer Research and Treatment* Dec 5.

Wellings, S. R., Jensen, H. M. and Marcum, R. G. 1975. An atlas of sub- gross pathology of the human breast with special reference to possible precancerous lesions. *Journal of the National Cancer Institute* 55: 231-273.

Welshons, W. V., Nagel, S. C. and Vom Saal, F. S. 2006. Large effects from small exposures. III. Endocrine mechanisms mediating effects of bisphenol A at levels of human exposure. *Endocrinology* 147: S56-69.

White, R. and Parker, M. G. 1998. Molecular mechanisms of steroid hormone action. *Endocrine-Related Cancer* 5:1-14.

Whiteman, M. K., Hillis, S. D., Curtis, K. M., McDonald, J. A., Wingo, P. A. and Marchbanks, P. A. 2005. Body mass and mortality after breast cancer diagnosis. *Cancer Epidemiology Biomarkers Previews* 14.8:2009-2014.

WHO. 2002. National Cancer Control Programmes: policies and managerial guidelines. 2nd edition.

WHO. 2005. Global action against cancer now! Retrieved on June 1, 2008 from http://www.who.int/cancer/media/GlobalActionCancerEnglfull.pdf.

WHO. 2006a. Cancer. Fact sheet No 297. www.who.int/mediacentre/factsheets/fs297/en/print/html.

WHO. 2006b. Epidemiological Factsheet on HIV/AIDS and sexually Transmitted Infections. Nigeria. Available at http://www.who.int/globalatlas/predefined Reports/EFS PDFs/EFS2006 NG.pdf.

WHO. 2008. The impact of cancer - Nigeria. http://www.who.int/infobase/report.aspx.

Wilson, N. K., Chuang, J. C., Lyu, C.W., Menton, R. and Morgan, M. 2003. Aggregate exposures of nine preschool children to persistent organic pollutants at day care and at home *Journal of Experimental Analysis and Environmental Epidemiology* 13: 187–202.

Wingo, P. A., Newsome, K., Marks, J. S., Calle, E. E. and Parker, S. L et al. 1997. The risk of

184

breast cancer following spontaneous or induced abortion. *Cancer Causes Control* 8:93–108.

Wolf, C., Rouyer, N., Lutz, Y., Adida, C., Loriot, M., Bellocq, J. P., Chambon, P. and Basset, P. 1993. Stromelysin 3 belongs to a subgroup of proteinases expressed in breast carcinoma fibroblastic cells and possibly implicated in tumor progression. *Proceedings of National Academy of Science* 90:1843–1847.

Wolff, M.S., Toniolo, P.G., Lee, E.W., Rivera, M. and Dublin, N.1993. Blood levels of organochlorine residues and risk of breast cancer. *Journal of National Cancer Institute (Bethesda)* 85:648-652

Wong, O. and Harris, F. 2000. Cancer mortality study of employees at lead battery plants and lead smelters, 1947-1995. *American Journal of Industrial Medicine* 38:255-270.

World Cancer Research Fund (WCRF) 2007. Food, nutrition, physical activity, and the prevention of cancer: a global perspective. Washington. DC: American Institute for Cancer Research.

Writing Group for the Women's Health Initiative Investigators (WGWHII) 2002. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results from the Women's Health Initiative randomized controlled trial. *Journal of American Medical Association* 288.3:321-333.

Wu, L. L., Chiou, C. C, Chang, P. Y. and Wu, J.T. 2004. Urinary 8-OHdG: a marker of oxidative stress to DNA and a risk factor for cancer, atherosclerosis and diabetics. *Clin Chim Acta* 339:1–9.

Wysowski, D. K., Comstock, G. W., Helsing, K. J. and Lau, H. L 1987. Sex hormone levels in serum in relation to the development of breast cancer. *American Journal Epidemiology* 125:791-799

Yaffe, K. 2003. Hormone therapy and the brain déjà vu all over again? *Journal of American Medical Association* 289.271 7-2719.

Yager, J. D. 2000. Endogenous estrogens as carcinogens through metabolic activation. *Journal of National Cancer Institute Monograph* 27:67-73.

Yager, J. D. and Davidson, N. E. 2006. Mechanisms of disease: estrogen carcinogenesis in breast cancer. *New England Journal of Medicine* 354:270-282.

Yamauchi, H., Stearns, V. and Hayes, D. F. 2001. When is a tumour marker ready for prime

time? A case study of c-erbB-2 as a predictive factor in breast cancer. *Journal of Clinical Oncology* 19:2334-2356.

Yang, X. R., Sherman, M. E., Rimm, D. L., Lissowska. J., Brinton, L.A., Peplonska, B., Hewitt, S.M., Anderson, W.F., Szeszenia-Dabrowska, N., Bardin-Mikolajczak, A., Zatonski, W., Caman, R., Mandich, D., Rymkiewicz, G., Ligaj, M., Lukaszck, S., Kordek, R and Garcia-Closas, M. 2007. Differences in risk factors for breast cancer molecular subtypes in a population-based study. *Cancer Epidemiology Biomarkers Previews* 16: 439-443.

Yeh, J. and Adashi, E. Y. 1999. The ovarian lifecycle In: Yen SSC., Jaffe RB., Barbieri RL eds. Reproductive endocrinology: Physiology, pathophysiology and clinical management. 4th ed. Philadelphia: WB Saunders Co.153-190.

Yen, S. S. C. 1999. The human menstrual cycle: neuroendocrine regulation In: Yen SSC., Jaffe RB., Barbieri RL eds. Reproductive endocrinology: Physiology, pathophysiology and clinical management. 4th ed. Philadelphia: WB Saunders Co.191-217.

Ying, S., Myers, K., Bottomley, S., Holleday, T. and Bryant, H. E. 2009. BRCA2-dependent homologous recombination is required for repair of Arsenite-induced replication lesions in mammalian cells. *Nucleic Acid Research* 37.15: 5103-5113.

Yu, H., Shu, X. O., Shi, R. H., Dai, Q., Jin, F., Gao, Y. T., Li, B. D. L. and Zheng, W. 2003. Plasma sex steroid hormones and breast cancer risk in Chinese women. *International Journal of Cancer* 105:92-97.

Yu, X., Filardo, E. J. and Shaikh, Z. A. 2010. The membrane estrogen receptor GPR30 mediates cadmium-induced proliferation of breast cancer cells. *Toxicology and Applied Pharmacology* 245.1: 83-90.

Zähringer, S., Tomova, A., von Werder, K., Brabant, G., Kumanov, P. and Schopohl, J. 2000. The influence of hyperthyroidism on the hypothalamic-pituitary-gonadal axis. *Experimental Clinical Endocrinology and Diabetes* 108:282-289.

Zhang, J. and Salamonsen, L. A. 2002. In vivo evidence for active matrix metalloproteinases in human endometrium supports their role in tissue breakdown at menstruation *The Journal of Clinical Endocrinology and Metabolism* 87: 2346-2351.

Zhang, M. and Holman, C. D. 2011. Low-to-moderate alcohol intake and breast cancer risk in Chinese women. *British Journal of Cancer* 105: 1089-1095.

Zhang, S., Hunter, D. J., Forman, M. R., Rosner, B. A., Speizer, F. E., Colditz, G. A., Manson,

J.E., Hankinson, S.E. and Willett, W. C. 1999. Dietary carotenoids and vitamins A, C, and E and risk of breast cancer. *Journal of National Cancer Institute* 91.6:547-556.

Zhao, C.Q., Young, M.R., Diwan, B.A., Coogan, T.P and Waalkes, M.P. 1997. Association of arsenic-induced malignant transformation with DNA hypomethylation and aberrant gene expression. *Proceedings of National Academy of Science USA* 94; 10907-10912.

Zheng, W., Gustafson D. R., Sinha, R., Cerhan, J. R., Moore, D., Hong, C. P., Anderson, K.E., Kushi, L.H., Seller, T.A and Folsom, A. R. 1998. Well done meat intake and the risk of breast cancer. *Journal of National Cancer Institute* 90.22:1724-1729.

Zhang, X.L., Liu, U., Weng, S.F.and Wang, H.S. 2016. Bisphenol-A increases the migration and activation of triple negative breast cancer cells via ERR gamma. *Basic Clinical Pharmacology and Toxicology* Doi:10.111/bcpt.12591

Zhou, J., Chen, Y., Huang, Y., Long, J., Wan, F. and Zhan, S. 2013. Serum follicle-stimulating hormone level is associated with human epidermal growth factor receptor type 2 and Ki67 expression in post-menopausal females with breast cancer. *Oncology Letters* 6.4:1128–1132.

Zhou, X., Sun, H. Ellen, T.P., Chen, H. and Costa, M. 2008. Arsenite alters global histone H3 methylation. *Carcinogenesis* 29:1831-1836

Zhu, K., Davidson, N. E., Hunter, S., Yang, X. and Payne-Wilks, K. 2003. Methyl-group dietary intake and risk of breast cancer among African-American women: a case-control study by methylation status of the estrogen receptor alpha genes. *Cancer Causes Control* 14:827–836.

Zhu, S., Heshka, S., Wang Z Shen, W., Allison, D. B., Ross, R. and Heymsfield, S. 2004. Combination of BMI and waist circumference for identifying cardiovascular risk factors in whites. *Obesity Research* 12:633.645.

Zoeller, R.T., Bansal, R. and Parris, C., 2005. Bisphenol-A, an environmental contaminant that acts as a thyroid hormone receptor antagonist in vitro, increases serum thyroxine, and alters RC3/neurogranin expression in the developing rat brain. *Endocrinology* 146:607-612.

Zreik, T. G., Mazloom, A., Chen, Y Vannucci M., Pinnix CC., Fulton S., Hadziahmetovic M.,

Asmar N., Munkarah AR., Ayoub CM., Shinadeh F., Berjawi G., Hannoun A., Zalloua P., Wogan C. and Dabaja B. 2010. Fertility drugs and the risk of breast cancer: a meta-analysis and review. *Breast Cancer Research and Treatment* 124:13-16

APPENDCES

APPENDIX 1(a)

INSTITUTE FOR ADVANCED MEDICAL RESEARCH AND TRAINING (IMRAT)
COLLEGE OF MEDICINE, UNIVERSITY OF IBADAN, IBADAN, NIGERIA.
E-Mail - imratcomui@yahoo.com

UI/UCH EC Registration Number: NHREC/05/01/2008a
NOTICE OF FULL APPROVAL AFTER FULL COMMITTEE REVIEW

Re: *Radioactive Phosphors and their interaction with Hormones and their Receptors in Normal and Cancerous Breast.*

UI/UCH Ethics Committee assigned number. UI/EC/18/0193

Name of Principal Investigator . **Utolape O. Ajayi**
Address of Principal Investigator: Department of Chemical Pathology,
University College Hospital, Ibadan

Date of receipt of valid application: 30/11/2010

[faded paragraph text illegible due to document quality]

Related Areas •Genetics & Bioethics •Malaria •Environmental Sciences •Epidemiology Research & Service •Behavioural & Social Sciences •Pharmaceutical Sciences •Cancer Research & Services •HIV/AIDS

APPENDIX 1(b)

INSTITUTE FOR ADVANCED MEDICAL RESEARCH AND TRAINING (IAMRAT)
COLLEGE OF MEDICINE, UNIVERSITY OF IBADAN. IBADAN, NIGERIA.
Director: Prof. A. Ogunniyi, ~~~~~~~~~~~~~~~~~~~~~~~~
Tel 08023038583. 08038094173
E-mail: aogunniyi@comui.edu.ng

UI/UCHEC Registration Number: NHREC/05/01/2008a

Notice of Approval for Amendment

Re: Endocrine Disruptors and their Interaction with Hormones and their Receptors in Normal and Cancerous Breasts

UI/UCH Ethics Committee assigned number: UI/EC/10.0193

Name of Principal Investigator: Olabisi O. Ajosi
Address of Principal Investigator: Department of Chemical Pathology, University College Hospital, Ibadan

Date of receipt of application for approval of amendment: 13/01/2015

Approval for Amendment

Status:

This is to inform you that the UI/UCH Ethics Committee has reviewed your application for approval of amendment to your research protocol. The ~~~~~~~~~~ indicates that expression of oestrogen and progesterone receptors, CerB2 will be determined by Immunohistochemistry and its reaction will be carried out in the Genetics and Bioethics Laboratory, Institute for Advanced Medical Research and Training Whole Building, College of Medicine, University of Ibadan.

The Committee notes the amendment and having found it satisfactory, hereby approves the amended protocol.

All informed consent forms used in this study must carry the UI/UCH EC assigned number and ~~~~~~~~~~ of UI/UCH EC approval of the study. It is expected that you submit your annual report as well as an ~~~~~~~~~ request for the project renewal in the UI/UCH EC early in order to obtain renewal of your approval and avoid disruption of your research.

The National Code for Health Research Ethics requires you to comply with all institutional guidelines, rules and regulations and with the tenets of the Code including ensuring that all adverse events are reported promptly to the UI/UCH EC. No changes are permitted in the research without prior approval by the UI/UCH EC except in circumstances outlined in the Code. The UI/UCH EC reserves the right to ~~~~~~~~~~~~~~~~ to your research site without previous notification.

Dr. W.O.~~~~~~
Vice-Chairman, UI/UCH Ethics Committee
E-mail: uhuchirc@yahoo.com

190

APPENDIX 2

INFORMED CONSENT FORM

IRB Research Approval.................................

This approval will elapse on..............................

TITLE OF RESEARCH

ENDOCRINE DISRUPTORS AND THEIR INTERACTION WITH HORMONES AND THEIR RECEPTORS IN NORMAL AND CANCEROUS BREASTS

Name and Affiliation of Researcher

This study is being conducted by AJAYI Olulope of the Department of Chemical Pathology, University of Ibadan, Ibadan..

This questionnaire is being administered to you to help in assessing the possible interaction of certain endocrine disruptors (lead, cadmium, arsenic, bisphenol-A, Polychlorinated bisphenyls) with the female reproductive hormones and their receptors.

All information provided by you will be kept very confidential. You will not be exposed to any risk or harm except the discomfort of needle prick during the collection of blood sample. Ten milliliters of blood will be collected from you using a new disposable pyrogen-free needle for some laboratory investigations. The result will be kept confidential.

This exercise might cause you minor discomfort, however, this will only last for a short space of time. You have the right to refuse participation in the research and also withdraw at any time you so desire.

If there are abnormal results, I shall contact you for necessary action. Your efforts in filling this questionnaire will be highly appreciated.

STATEMENT OF PERSON OBTAINING INFORMED CONSENT

I have fully explained this research to ....and have given sufficient information, including the risks and benefits to make an informed decision.

191

DATE...................................SIGNATURE...................................

NAME...

Statement of person giving consent

I have read the description of the research or have had it translated into the language I understand. I have also talked it over with the researcher to my satisfaction. I understand that my participation is voluntary. I know enough of the purpose, methods, risks and benefits of the research to judge that I want to take part in it. I have received a copy of this consent form and additional information

DATE.................................SIGNATURE...................................

NAME...

This research has been approved by the Health Research Joint Ethics Committee of the University of Ibadan/University College Hospital. The chairman of this committee can be contacted at:

Biode Building

2nd floor, room T10,

IAMRAT,

College of Medicine, University of Ibadan

E-mail: uiuchire@yahoo.com

If you have any question about participation in this research, you can contact my supervisor Dr Mabel A. Charles-Davies, Department of Chemical Pathology, College of Medicine, University of Ibadan (08023045256). Thanks.

APPENDIX 3

DEPARTMENT OF CHEMICAL PATHOLOGY

COLLEGE OF MEDICINE

UNIVERSITY OF IBADAN

QUESTIONNAIRE

Good day madam. This questionnaire is being administered to you to determine the interactions of certain chemicals in the environment (e.g. lead, cadmium e. t. c) with the female reproductive hormones in breast cancer. This will help in proper management of breast cancer. Your kind cooperation in providing correct information to the questions below will be highly appreciated. All information provided shall be kept very confidential.

Date.. Hospital Number.............................

SECTION A. DEMOGRAPHIC CHARACTERISTICS

1. Gender: Female( )
2. Age:.................
3. Place of residence.................
4. Ethnic group.................
5. State of Origin:.................
6. Marital Status: Married ( ), single( ), widow ( ), divorced/separated ( )
7. Highest educational attainment: None ( ), Pry School ( ), Secondary School ( ), ND/NCE ( ), HND/B.Sc. ( ), PG ( ).
8. Occupation:.................

SECTION B. DIET HISTORY

9. Dairy product (butter, cheese, milk) intake? Daily ( ), weekly ( ), occasionally ( ), never ( )

10. Vegetable intake? Daily ( ), weekly ( ), occasionally ( ), never ( )

193

11. Fruit intake? Daily ( ), weekly ( ), occasionally ( ), never ( )

12. Consumption of red meat? Daily ( ), weekly ( ), occasionally ( ), never ( )

13. Cereal products intake? Daily ( ), weekly ( ), occasionally ( ), never ( )

14(a). Alcohol intake? Yes ( ), No ( )

14(b) If response is yes, how? 1 bottle/day ( ), 1 bottle/week ( ), 1 bottle occasionally ( ).

14 (c) If consumption is more than 1 bottle, pls, specify...

15(a) Cigarette smoking? Yes ( ), No ( )

15(b) If response is yes pls, indicate the number of sticks and how?......................................

SECTION C. ANTHROPOMETRIC MEASUREMENTS

16. Blood pressure..

17. Weight...

18. Height...

19. BMI..

20. Waist circumference....................................

21. Hip circumference......................................

SECTION D. OBSTETRICS/ GYNAECOLOGICAL AND BREAST CANCER HISTORY

22. Is your menstrual cycle regular or irregular?......................

23. Your menstruation lasts for how many days?...............

24. Duration of menstrual cycle............................

25. Present day of menstrual cycle..... (Note. Day 1 refers to 1ˢᵗ day of the last menstruation.

26. Age at menarche..

194

27. Age at first full pregnancy...

28. Age at menopause (if applicable)..................................

29. Use of contraceptive Yes ( ), No ( ). If response is yes, kindly state the type(s)

30. Any family history of breast cancer? Yes ( ), No ( ), if response is yes pls, state the

relationship e.g. mother, sister.................................

31. Any history of breast cancer? Yes ( ), No ( ). If response is yes, how was it managed?

....

32. Cancer stage (breast cancer subjects)...........................

33. Are you currently on hormone replacement therapy? Yes ( ), No ( )

APPENDIX 4

CALCULATION OF SAMPLE SIZE (COMPARISON OF TWO MEANS)

$$N = \frac{(Z_\alpha + Z_{2\beta})^2 (\sigma_1^2 + \sigma_0^2)}{(\mu_1 - \mu_0)^2}$$

NOTE: N=Sample size

$\mu_1 - \mu_0$ = Difference between the means to be detected assumed to be 10

$\sigma_1^2 + \sigma_0^2$ = Standard deviations

Z_α = Standard normal deviate corresponding to the null hypothesis i.e. 1.96

$Z_{2\beta}$ = Standard normal deviate corresponding to the alternate hypothesis i.e. 1.28

α = level of significance

β = type II error

Oestradiol (pg/ml)

	Mean	Standard Deviation
Test	113.72	16.40
Control	156.00	19.80

$$N = \frac{(1.28 + 1.96)^2 (16.40^2 + 19.80^2)}{(10)^2}$$

N=69.89 (approximately 70).

N.B: The mean and standard deviation values used in the calculation of the sample size of this study were obtained from *(Egbe, 2007). He determined mean±SD of oestradiol in breast cancer patients and control.

APPENDIX 5

Reference Intervals of Hormonal Assay

Oestradiol (E$_2$)

Follicular Phase: 90-1100 (pmol/L)

Luteal Phase: 90-1200 (pmol/L)

Postmenopausal: ≤170 (pmol/L)

Progesterone

Follicular: ≤2.8 (nmol/L)

Luteal: 15-80 (nmol/L)

Postmenopausal: ≤ 1.59 (nmol/L)

Luteinizing Hormone (LH)

Follicular: 1.0-13.0 (IU/L)

Luteal: 0.5-15.0 (IU/L)

Postmenopausal: 14.0-62.0 (IU/L)

Follicle-Stimulating Hormone (FSH)

Follicular: 3.0-11.0 (IU/L)

Luteal: 1.5-10.8 (IU/L)

Postmenopausal: 36.0-168.0 (IU/L)

Free Triiodothyronine (FT$_3$)

3.2-6.0 (pmol/L)

Free Thyroxine (FT$_4$)

10.6-21.0 (pmol/L)

Thyroid. Stimulating Hormone (TSH)

0.38-4.31 (mIU/L)

Reference: TOSOH enzyme immunoassay protocol leaflet

197

APPENDIX 6

Coexpression of ER/PR in Women with Breast Cancer

ER/PR Co-expression	Frequency (n)	Percentage (%)
ER+/PR+	4	5.1
ER+/PR-	6	7.6
ER-/PR-	65	82.3
ER-/PR+	4	5.1

198

APPENDIX 7

REAGENT PREPARATION FOR THE DETERMINATION OF THE ANALYTES

Reagent Preparation for Progesterone Determination

(a) Substrate Solution

All reagents were brought to 18-25 ^oC before preparing the working reagent. The contents of the AIA-PACK Substrate reconstituent II (100mL) were added to the lyophilized AIA-PACK Substrate reagent II and mixed thoroughly to dissolve the solid material.

(b) Wash Solution

The entire contents of the AIA-PACK concentrate (100mL) were added to approximately 2.0L of Chemical and Allied Product (CAP) Class reagent grade water and mixed well. The final volume was adjusted to 2.5L.

(c) Diluent

The entire content of the AIA-PACK Diluent Concentrate (100mL) was added to approximately 4.0L of CAP Class I reagent grade water. This was thoroughly mixed and adjusted to 5.0L.

CAP=Chemical and allied product

Calibration Procedure

The calibrators met the criteria of Institute for Reference Materials and Measurement and European Reference Materials (IRMM ERM). Calibration stability was monitored by quality control performance. The calibrator lot and concentration numbers were correctly entered into the software. Progesterone calibrators were run and were within a 10% range.

Quality Control: Manufacturer's control i.e. levels 1, 2 and 3 were run with the assay

Sensitivity: The minimal detectable concentration of progesterone by the method used is 0.318nmol/L (0.1 ng/ml).

Specificity: The specificity of progesterone is 100%.

Reagent Preparation for E₂ Determination

(a) Substrate Solution

All reagents were brought to 18-25 $^{\circ}$C before preparing the working reagent. The contents of the AIA-PACK Substrate reconstituent II (100mL) were added to the lyophilized AIA-PACK Substrate reagent II and mixed thoroughly to dissolve the solid material.

(b) Wash Solution

The entire contents of the AIA-PACK concentrate (100mL) were added to approximately 2.0L of CAP Class reagent grade water and mixed well. The final volume was adjusted to 2.5L.

(c) Diluent

The entire content of the AIA-PACK Diluent Concentrate (100mL) was added to approximately 4.0L of CAP Class I reagent grade water. This was thoroughly mixed and adjusted to 5.0L

Calibration Procedure

The calibrators met the criteria of Institute for Reference Materials and Measurement and European Reference Materials (IRMM ERM). Calibration stability was monitored by quality control performance. The calibrator lot and concentration numbers were correctly entered into the software. Oestradiol calibrators were run and were within a 10% range.

Quality Control: Manufacturer's control i.e. levels 1, 2 and 3 were run with the assay.

Sensitivity: The minimal detectable concentration of E₂ by the method used was 52.85pmol/L (14.4pg/ml).

Specificity: The specificity of E₂ is 100%.

Reagent Preparation for FSH Determination

(a) Substrate Solution

All reagents were brought to 18-25 °C before preparing the working reagent. The contents of the AIA-PACK Substrate reconstituent II (100mL) were added to the lyophilized AIA-PACK Substrate reagent II and mixed thoroughly to dissolve the solid material.

(b) Wash Solution

The entire contents of the AIA-PACK concentrate (100mL) were added to approximately 2.0L of CAP Class reagent grade water and mixed well. The final volume was adjusted to 2.5L.

(c) Diluent

The entire content of the AIA-PACK Diluent Concentrate (100mL) was added to approximately 4.0L of CAP Class I reagent grade water. This was thoroughly mixed and adjusted to 5.0L

Calibration Procedure

The calibrators met the criteria of Institute for Reference Materials and Measurement and European Reference Materials (IRMM ERM). Calibration stability was monitored by quality control performance. The calibrator lot and concentration numbers were correctly entered into the software. FSH calibrators were run and were within a 10% range.

Quality Control: Manufacturer's control i.e. levels 1, 2 and 3 were run with the assay.

Sensitivity: The minimal detectable concentration of follicle-stimulating hormone by the method used was 1.0 IU/L (1.0mIU/mL).

Specificity: The specificity of FSH is 100%

Reagent Preparation for LH Determination

(a) Substrate Solution

All reagents were brought to 18-25 °C before preparing the working reagent. The contents of the AIA-PACK Substrate reconstituent II (100mL) were added to the lyophilized AIA-PACK Substrate reagent II and mixed thoroughly to dissolve the solid material.

(b) Wash Solution

The entire contents of the AIA-PACK concentrate (100mL) were added to approximately 2.0L of CAP Class reagent grade water and mixed well. The final volume was adjusted to 2.5L.

(c) Diluent

The entire content of the AIA-PACK Diluent Concentrate (100mL) was added to approximately 4.0L of CAP Class I reagent grade water. This was thoroughly mixed and adjusted to 5.0L

Calibration Procedure

The calibrators met the criteria of Institute for Reference Materials and Measurement and European Reference Materials (IRMM ERM). Calibration stability was monitored by quality control performance. The calibrator lot and concentration numbers were correctly entered into the software. LH calibrators were run and were within a 10% range.

Quality Control: Manufacturer's control i.e. levels 1, 2 and 3 were run with the assay.

Sensitivity: The minimal detectable concentration of LH by the method used was 0.2 IU/L (0.2mIU/mL).

Specificity: The specificity of LH is 100%.

Reagent Preparation for FT3 Determination

(a) Substrate Solution

All reagents were brought to 18-25 °C before preparing the working reagent. The contents of the AIA-PACK Substrate reconstituent II (100mL) were added to the lyophilized AIA-PACK Substrate reagent II and mixed thoroughly to dissolve the solid material.

(b) Wash Solution

The entire contents of the AIA-PACK concentrate (100mL) were added to approximately 2.0L of CAP Class reagent grade water and mixed well. The final volume was adjusted to 2.5L.

(c) Diluent

The entire content of the AIA-PACK Diluent Concentrate (100mL) was added to approximately 4.0L of CAP Class I reagent grade water. This was thoroughly mixed and adjusted to 5.0L

Calibration Procedure

The calibrators met the criteria of Institute for Reference Materials and Measurement and European Reference Materials (IRMM ERM). Calibration stability was monitored by quality control performance. The calibrator lot and concentration numbers were correctly entered into the software. FT_4 calibrators were run and were within a 10% range.

Quality Control: Manufacturer's control i.e. levels 1, 2 and 3 were run with the assay.

Sensitivity: The minimal detectable concentration of FT_4 by the method used was 1.29 pmol/L (0.1ng/dL).

Specificity: The specificity of FT_4 is 100%.

Reagent Preparation for FT_3 Determination

(a) Substrate Solution

All reagents were brought to 18-25 °C before preparing the working reagent. The contents of the AIA-PACK Substrate reconstituent II (100mL) were added to the lyophilized AIA-PACK Substrate reagent II and mixed thoroughly to dissolve the solid material.

(b) Wash Solution

The entire contents of the AIA-PACK concentrate (100mL) were added to approximately 2.0L of CAP Class reagent grade water and mixed well. The final volume was adjusted to 2.5L.

(c) Diluent

The entire content of the AIA-PACK Diluent Concentrate (100mL) was added to approximately 4.0L of CAP Class 1 reagent grade water. This was thoroughly mixed and adjusted to 5.0L

Calibration Procedure

The calibrators met the criteria of Institute for Reference Materials and Measurement and European Reference Materials (IRMM ERM). Calibration stability was monitored by quality control performance. The calibrator lot and concentration numbers were correctly entered into the software. FT_3 calibrators were run and were within a 10% range.

Quality Control: Manufacturer's control i.e. levels 1, 2 and 3 were run with the assay.

Sensitivity: The minimal detectable concentration of FT_3 by the method used was 0.77pmol/L (0.5pg/mL).

Specificity: The specificity of FT_3 is 100%

Reagent Preparation for TSH Determination

(a) Substrate Solution

All reagents were brought to 18-25 °C before preparing the working reagent. The contents of the AIA-PACK Substrate reconstituent II (100mL) were added to the lyophilized AIA-PACK Substrate reagent II and mixed thoroughly to dissolve the solid material.

(b) Wash Solution

The entire contents of the AIA-PACK concentrate (100mL) were added to approximately 2.0L of CAP Class reagent grade water and mixed well. The final volume was adjusted to 2.5L.

(c) Diluent

The entire content of the AIA-PACK Diluent Concentrate (100mL) was added to approximately 4.0L of CAP Class I reagent grade water. This was thoroughly mixed and adjusted to 5.0L.

Calibration Procedure

The calibrators met the criteria of Institute for Reference Materials and Measurement and European Reference Materials (IRMM ERM). Calibration stability was monitored by quality control performance. The calibrator lot and concentration numbers were correctly entered into the software. TSH calibrators were run and were within a 10% range.

Quality Control: Manufacturer's control i.e. levels 1, 2 and 3 were run with the assay.

Sensitivity: The minimal detectable concentration of TSH by the method used was 0.01 mIU/L (0.01μIU /mL).

Specificity: The specificity of TSH is 100%

Reagent Preparation for Lead Determination

1. Five ml of triton X-100 was made up to 100 ml with deionised water to give a 5% solution (v/v). The solution of the mixture was enhanced in warm water by placing on hot plate.

2. Stock lead standard

(a) Lead standard solution was prepared by diluting 1.60g of lead nitrate ($PbNO_3$) in 100mL of de-ionized water

3. Working Standards

(a) 1.0, 2.5 and 5.0μg/L were prepared by dilution from the stock standard with acidified deionised water

(b) The working standards were used to prepare a calibration curve which was used to compare the digested samples.

Reagent Preparation for Cadmium Determination

205

1. Five ml of triton X-100 was made up to 100 ml with deionised water to give a 5% solution (v/v). The solution of the mixture was enhanced in warm water by placing on hot plate

2. Stock cadmium standard

Cadmium standard solution was prepared by dissolving 2.10 g of cadmium nitrate in 250 mL of de-ionized water and was made up to 1 litre.

3. Working Standards

(a) 1.0, 2.5 and 5.0µg/L were prepared by dilution from the stock standard with acidified deionised water.

(b) The working standards were used to prepare a calibration curve which was used to compare the digested samples.

Reagent Preparation for Arsenic Determination

1. Five ml of triton X-100 was made up to 100 ml with deionised water to give a 5% solution (v/v). The solution of the mixture was enhanced in warm water by placing on hot plate

2. Stock arsenic standard

Arsenic standard solution was prepared by dissolving 1.0g of arsenic powder in 50 mL concentrated nitric acid and was diluted to 1 litre with de-ionized water.

3. Working Standards

(a) 1.0, 2.5 and 5.0µg/L were prepared by dilution from the stock standard with acidified deionised water.

(b) The working standards were used to prepare a calibration curve which was used to compare the digested samples.
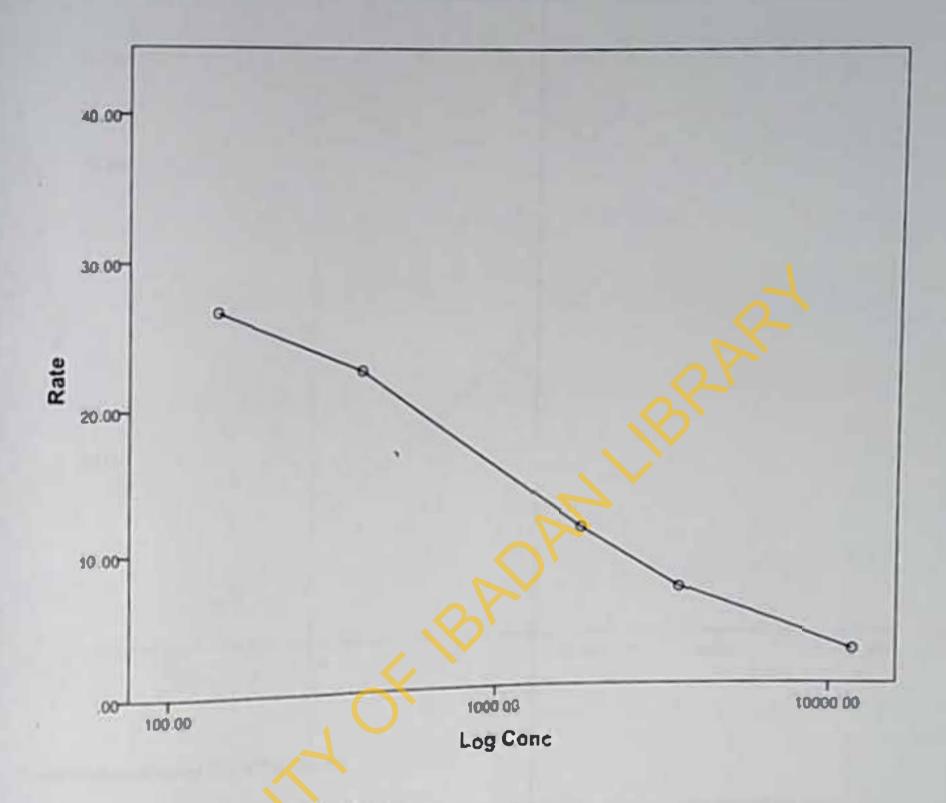
Quality Control of the Toxic Metals

To ensure quality control of these analytes, de-ionized water samples in three different bottles were included with the serum samples and blinded to the laboratory technician. This was done to ascertain the reliability of the results.

Quality Control of the Determination of Hormone Receptors by Immunohistochemistry
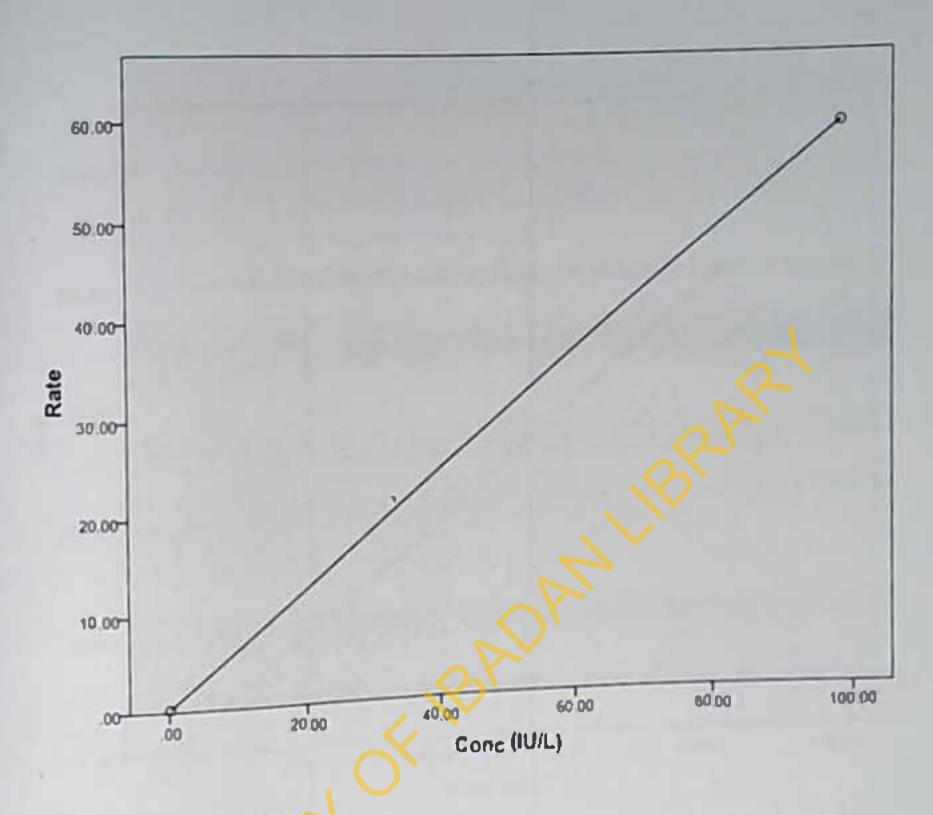
N.B-All tissue sections were performed at the same time and submitted to standard methods. Known positive and negative cases were used as external controls. ER, and PR were considered positive when >10% of the nuclei were stained in 10 high power field (HPF) (Ferrero-Pous et al., 2001; Pinto et al., 2005). The HER 2 was considered negative when with score 0 and +1, and positive with score +2 and +3. To be considered as +2, +3 the cellular membrane was completely stained in more than 10% of the tumour cells. Cells without staining, or with weak staining in part of the cell membrane and in less than 10% of the tumour cells were considered negative (Jacobs et al., 1999).

APPENDIX 8

Calibration Curves of the Hormones

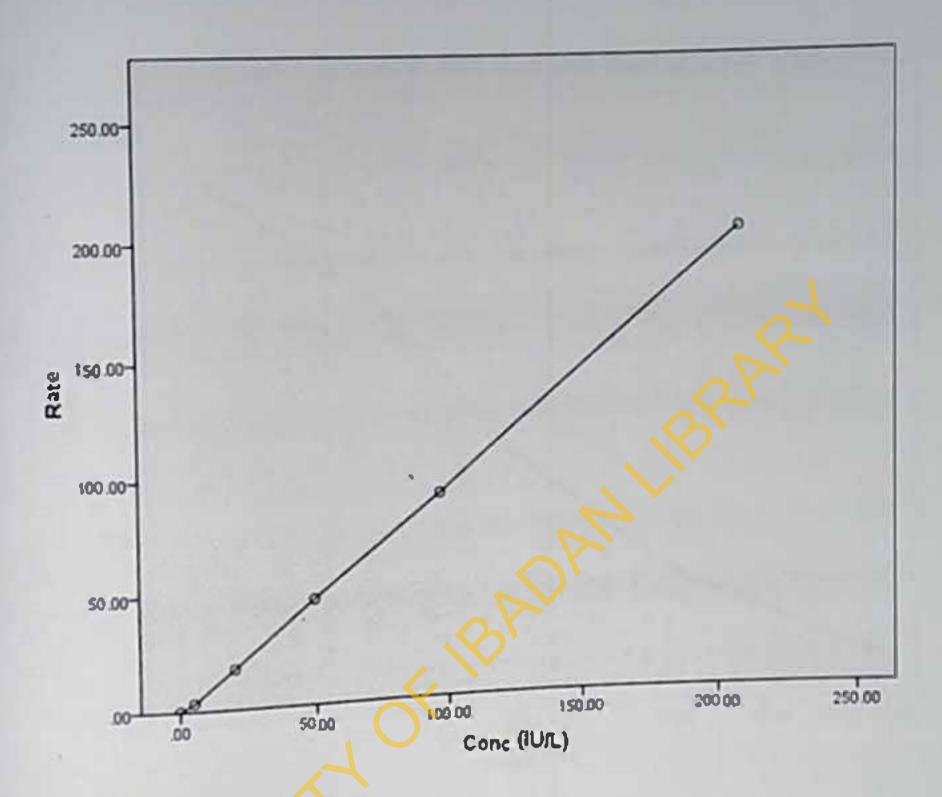Calibration Curve for Progesterone Assay
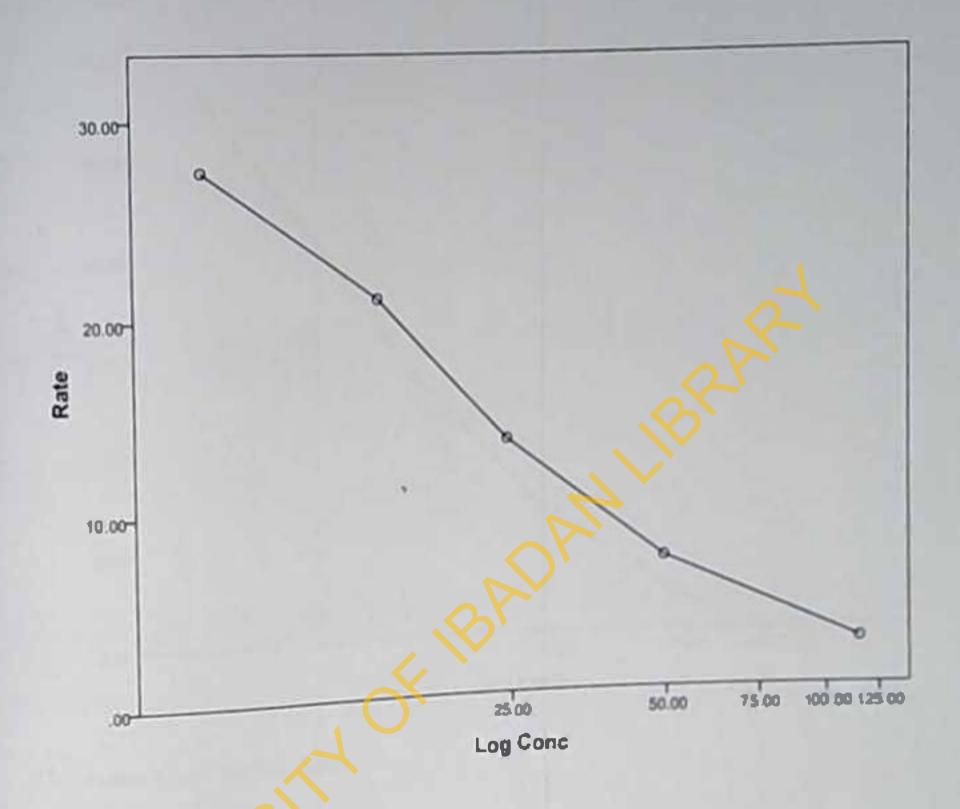
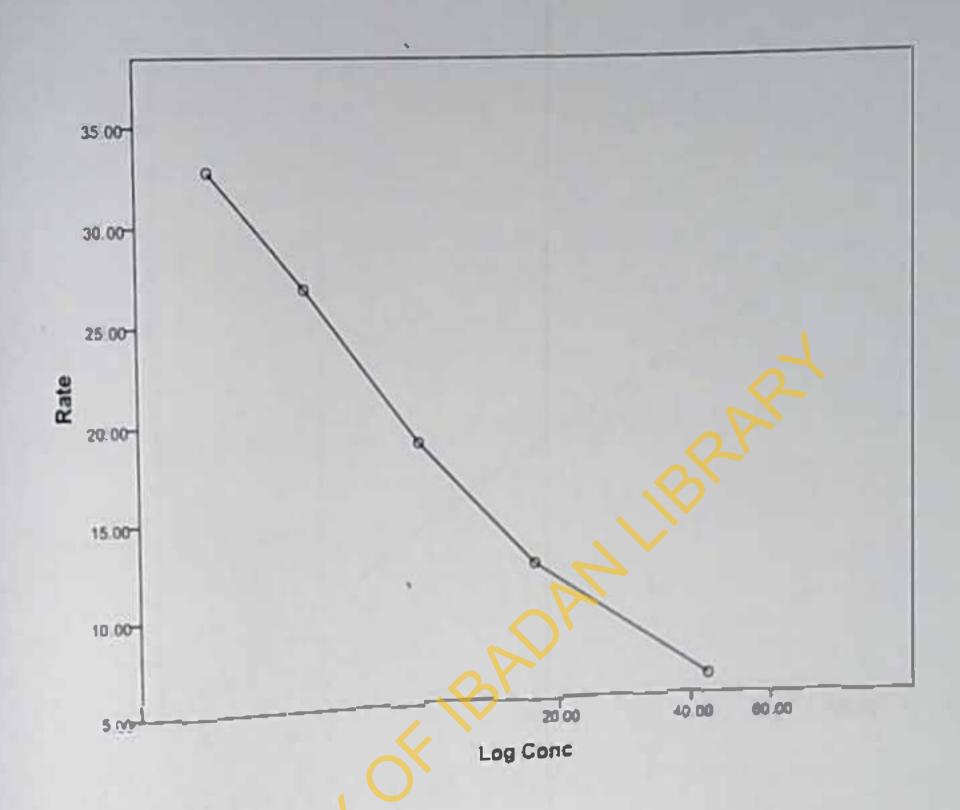Calibration Curve for Oestradiol Assay

Calibration Curve for FSH Assay

N.B: Two points were plotted on the calibration curve for FSH assay as determined by the manufacturer (Check Appendix 10)
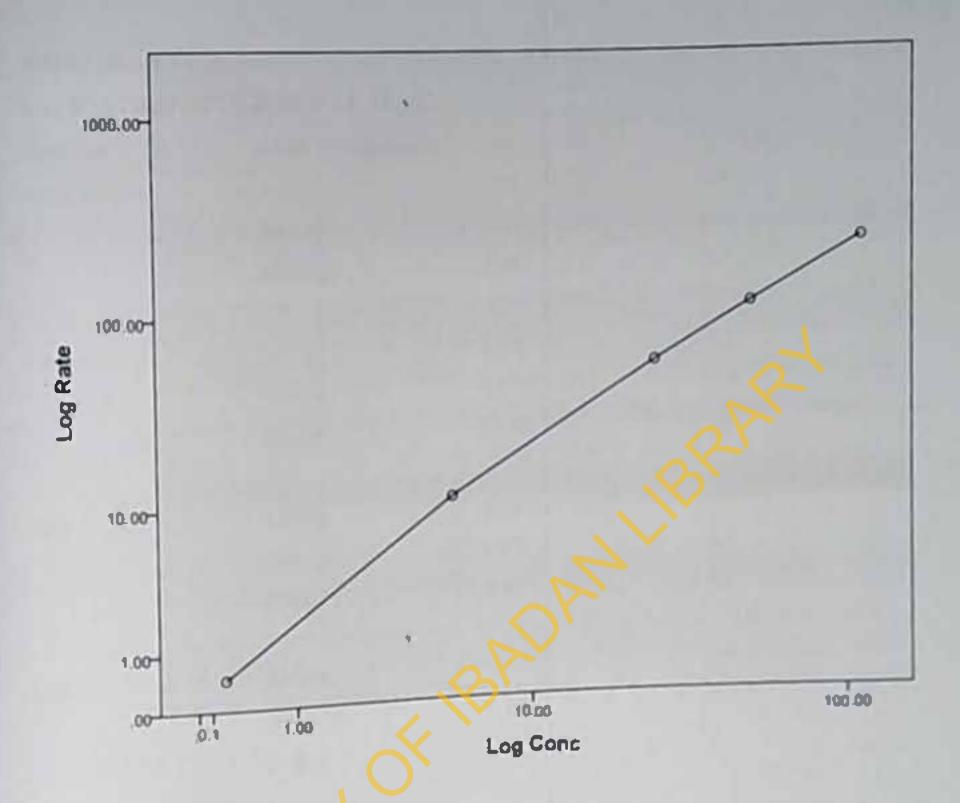
Calibration Curve for LH Assay

Calibration Curve for FT₄ Assay

Calibration Curve for FT₃ Assay

Calibration Curve for TSH Assay

APPENDIX 9

VALIDATION OF HORMONAL ASSAY

Hormone	Mean concentration	SD	CV (%)
Progesterone	A=2.07	0.23	11.3
	B=9.69	0.67	6.9
	C=25.02	1.56	6.2
Oestradiol	A=152.1	6.30	4.1
	B=623.3	15.73	2.5
	C=1993.2	57.66	2.9
FSH	A=4.94	0.28	5.6
	B=16.37	0.77	4.7
	C=60.15	2.57	4.3
LH	A=4.94	0.13	2.7
	B=25.27	0.54	2.1
	C=98.14	2.03	2.1
FT_4	A=7.727	0.41	5.3
	B=25.8	0.85	3.3
	C=59.5	1.59	2.7
FT_3	A=2.75	0.11	3.9
	B=5.10	0.16	3.2
	C=14.2	0.29	2.1
TSH	A=2.19	0.11	5.0
	B=8.26	0.39	4.8
	C=80.63	3.51	4.4

SD=Standard deviation. CV=Coefficient of variation. A=QC 1, B=QC 2, C=QC 3

215

APPENDIX 9

VALIDATION OF HORMONAL ASSAY

Hormone	Mean concentration	SD	CV (%)
Progesterone	A=2.07	0.23	11.3
	B=9.69	0.67	6.9
	C=25.02	1.56	6.2
Oestradiol	A=152.1	6.30	4.1
	B=623.3	15.73	2.5
	C=1993.2	57.66	2.9
FSH	A=4.94	0.28	5.6
	B=16.37	0.77	4.7
	C=60.15	2.57	4.3
LH	A=4.94	0.13	2.7
	B=25.27	0.54	2.1
	C=98.14	2.03	2.1
FT_4	A=7.727	0.41	5.3
	B=25.8	0.85	3.3
	C=59.5	1.59	2.7
FT_3	A=2.75	0.11	3.9
	B=5.10	0.16	3.2
	C=14.2	0.29	2.1
TSH	A=2.19	0.11	5.0
	B=8.26	0.39	4.8
	C=80.63	3.58	4.4

SD= Standard deviation. CV= Coefficient of variation. A=QC 1, B=QC 2, C=QC 3

215

Research Article

Serum Polychlorinated Biphenyls and Bisphenol-A Levels in Nigerian Women with Breast Cancer

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Abstract

Endocrine disruptors (EDs)-polychlorinated biphenyls (PCB) and bisphenol-A (BPA) have been associated with increased risk of breast cancer (B.Ca). In Nigeria, there is increase in breast cancer incidence. This has been attributed to increased use and exposure to EDs. Although, several studies have been done on breast cancer, there is paucity of information on the serum levels of these EDs in Nigerian women. This study therefore aims at determining the serum levels of PCB and BPA in breast cancer patients. Eighty women aged 18-70 years were recruited with informed consent after institutional ethical approval. They were 40 participants with breast cancer pre-treatment (cases) matched with 40 apparently healthy women (controls). They were recruited from a tertiary hospital in Ibadan, Nigeria. Demographic indices: age, age at menarche (AM), age at menopause (AMP) and anthropometric indices: height, weight, body mass index (BMI), waist circumference (WC), hip circumference (HC), waist-hip ratio (WHR) and waist-height ratio (WHT) were obtained from the pre-test questionnaire administered and standard methods respectively. 10 ml of venous blood was collected from each participant and sera obtained were analyzed for PCB and BPA. These were determined by High performance liquid chromatography (HPLC). Data were analyzed using Student t-test and Pearson correlation coefficient, p was significant at <0.05. Results showed increases in the height, weight, WC, HC, WHR, WHT, PCB and BPA serum levels in cases compared with controls. Positive correlations were observed among WC and WHR, WHT, HC; HC and WHT; WHR and WHT in both cases and controls. However, positive correlations among height, weight, WC and WHR; BMI and weight were observed in cases only. Weight correlated negatively with age at menarche (AM) only in controls. The differences and correlations were statistically significant (p<0.05). Mechanisms involving PCB, BPA and adiposity may be involved in breast cancer aetiology. Reduction of environmental pollution and dietary modulation may be helpful.

Keywords: Breast cancer, bisphenol-A, polychlorinated biphenyls, adiposity, age at menopause

INTRODUCTION

Breast cancer is one of the most common types of cancer and affects millions of women around the world with a noticeable fatality rate (Msolly *et al.*, 2011). In Nigeria, the incidence of breast cancer has been reported to be on the increase (Adesunkanmi *et al.*, 2006). This increase in incidence rate has been attributed to changes in demography, socio-economic status and epidemiological risk factors. Mortality rates are on the decline in the developed countries (America, Australia and Western Europe) due to early diagnosis, screening and improved cancer treatment programs. Conversely, mortality rates are on the increase in the developing countries as well as in Eastern and Central Europe (Adesunkanmi *et al.*, 2006). Body mass index (BMI) has been reported as a measure of overall adiposity, while waist circumference (WC) and waist-hip-ratio (WHR) are reliable proxy measures of abdominal fat (Chakraborty and Bose, 2009). Therefore, they could be used to identify overweight and obesity. However, the waist circumference alone is a somewhat better indicator of visceral adiposity than the waist to hip ratio (Donohoe *et al.*, 2011). These measures of adiposity have been widely

recommended for epidemiological surveys because of their independent association with major non-communicable metabolic diseases including breast cancer (Chakraborty and Bose, 2009). The relationships between adiposity and menopausal status coupled with prognosis of breast cancer have been the subject of considerable research for the past 40 years or more (Renehan *et al.*, 2008).

The adipose tissue is a heterogenous mixture of adipocytes, stromal, pre-adipocytes, immune cells and endothelium (Halberg *et al.*, 2008). It functions as a complex endocrine organ secreting a host of factors collectively referred to as adipokines (Halberg *et al.*, 2008). Moreover, the adipose tissue vascularisation functions in a delicate balance in tissue homeostasis, the perturbation initiated by tumour growth dysregulate all involved cell types (Haighton *et al.*, 2002). This is seen in breast cancer in which transformed ductal epithelial cell break through the basal lamina and invade the mammary stromal compartment which is highly enriched in adipose tissue after filling the lumen of mammary duct (Haighton *et al.*, 2002).

Human exposure to some environmental chemicals described as endocrine disruptors has been shown to alter endocrine functions resulting in adverse physiologic functions. These endocrine disruptors include polychlorinated biphenyls (PCDs) and bisphenol-A (BPA). The main release mode of exposure is oral (Monk *et al.*

2007). Endocrine disruptors mimic the action of oestrogens binding to the receptor, move into the nucleus and disrupt cell growth and division leading to uncontrolled cell proliferation. It has been reported that some of these toxicants increase the risk of breast cancer and its progression (Brophy et al., 2012).

Bisphenol-A is a momomer of plastic materials that are widely used in daily life. BPA is used for the production of epoxy resins, phenol resins, polycarbonates, polyacrylates, polyesters and lacquer coatings on food cans. Hence, there is widespread human exposure (Brody et al, 2007; Betancourt et al., 2012).

Human exposure to bisphenol-A of up to 50ug/dl/day is considered safe by United States Environmental Protection Agency (USEPA). Currently, the safety of BPA products is controversial. Some studies have demonstrated the ability of BPA to weakly stimulate oestrogen receptors (Fernandez and Russo, 2010). BPA binds with higher affinity to oestrogen receptor beta (ERβ) which is expressed in almost every tissue in the body (Brisken, 2008). High levels of these endocrine disruptors have been suggested in the serum breast cancer patients (Calafat et al., 2013).

Polychlorinated biphenyls are members of a chemical family that were widely used in the past in industry as lubricants, coatings and insulation materials for dielectric equipment like transformers and capacitors (Iyengar, 2005). PCBs were also used in common consumer items such as hydraulic fluid, fluorescent lights, televisions and other appliances. The release of PCBs to the environment has been reported to be through poorly maintained hazardous waste dumps and city landfills, illegal or improper dumping of hydraulic fluids/coolants, leaks from electrical transformers and other equipment, burning of medical, industrial or city waste from older consumer goods like televisions (Gray et al. 2009). However, there is paucity of information on the serum level of PCBs in non-occupationally exposed women in Nigeria. This study estimated the serum levels of PCBs and BPA in Nigerian breast cancer patients.

MATERIALS AND METHODS

Study design and duration: This case-control study was conducted after ethical approval (UI/EC/10/0193) was obtained from the joint ethical committee of University of Ibadan/University College Hospital, Ibadan, Nigeria.

Subjects: A total of 80 women aged 18-70 years were recruited for this study after informed consent. These were 40 breast cancer patients who have not commenced treatment (cases) and 40 apparently healthy women (controls) were age-matched with the cases. Subjects with other types of cancer, diabetes mellitus, hypertension, pregnancy, lactating mothers and subjects on hormonal therapy were excluded from the study. Moreover, breast cancer patients who had commenced treatment were excluded. The cases were recruited by surgical oncologist at the Surgical Oncology Division, Department of Surgery, University College Hospital (UCH), Ibadan, Nigeria.

Controls: These were apparently healthy individuals without breast cancer. They were randomly recruited within Ibadan city. Fasting blood glucose was determined to exclude type 2 diabetes mellitus.

Demographic and Anthropometric Indices

Demographic indices: Age, age at menarche and age at menopause were obtained through questionnaires administered to the subjects.

Anthropometric indices: weight, height, body mass index, waist circumference, hip circumference, waist-hip ratio, waist-height ratio were measured.

Weight: This was taken with a bathroom weighing scale placed on a flat surface. The subjects while wearing light clothing and without any shoes on were made to stand on the scale with the indicator at zero. The reading was recorded to the nearest 0.5kg.

Height: This was measured against a flat, vertical surface with the subjects standing bare footed in an upright position without any head gear on. Without raising the heels from the ground nor the feet kept together. Measurements were taken with a sliding headpiece brought to the vertex of the subject's head. The reading at this level was taken to the nearest 0.1m.

Body Mass Index (BMI): This was calculated from the body weight and height of the subjects using the formula stated below.

$$BMI\ (Kg/m^2) = weight\ (kg)/height\ (m^2).$$

Waist and hip circumferences: Waist circumference (in cm) was measured using a measuring tape placed at the navel level while hip circumference (in cm) was measured at the widest circumference of the hip over light clothing using a non-stretchable measuring tape without any pressure on the body surface. Both indices were recorded to the nearest 0.1cm.

Waist-hip ratio: This was calculated from the ratio of the waist circumference to the hip circumference i.e. waist/height.

Waist-height ratio: This was calculated from the ratio of the waist circumference to the height.

Sample Collection: Ten (10) ml of venous blood was obtained from antecubital fossa vein of each subject. The blood sample was allowed to clot, retracted and centrifuged at 3500 rpm for 5 minutes after which serum was separated and stored at -20°C until analysis was done.

Biochemical Investigations: The biochemical indices estimated were serum polychlorinated biphenyls (PCBs) and bisphenol A (BPA). Serum PCBs and BPA were estimated by high performance liquid chromatography (ALLIANCE, e2695; Waters, USA).

Analytical Method for BPA: Serum samples were fortified with 12.5 nanograms of isotopically labeled phthalate metabolites, 50 nanograms of labelled bisphenol-A, 250 nanograms of 4-methylumbelliferone glucuronide, 100 microlitres of ammonium acetate buffer (PH 6.5) and 10 microlitres of β-glucuronidase (Escherichia coli K12, Roche Diagnostics). The samples were mixed and incubated at 37°C overnight to allow for the deglucuronidation.

Following enzymatic hydrolysis, a 20μl, aliquot of the sample was added to 70μL of HPLC- grade water and 10ng of labeled 4-methylumbelliferone to determine deglucuronidation efficiency. The remaining sample was loaded on to Zymark rapid trace solution for automated solid phase extraction (SPE). The 60milligram/3mL Oasis-HLB cartridges were conditioned with HPLC-grade methanol (2ml) and 0.1 M formic acid (2mL). The samples were diluted with 5 mL of 0.1 M formic acid and loaded on the SPE cartridge at a rate of 1.0mL/min. The cartridge was washed with water (1mL) and 10% methanol in water (2mL) at a flow rate of 1 mL/min. The samples were eluted with 1.0mL of acetonitrile at a flow rate of 0.5 mL/min. The eluate was evaporated to dryness under a stream of dry nitrogen and the residue was resuspended in 85% methanol in water (200 microliters) and transferred to glass autosampler vials. Quality control of the analysis was maintained by analyzing a method blank (calf serum) and two spiked calf serum samples (20ng/mL). The detection limit (0.2ng/mL) was based upon a lower calibration standard (0.5ng/ml) which gave an instrument signal to noise response of 3:1.

Analytical Method for PCBs: Serum samples were mixed with methanol and a mixture of internal standards were added to correct for recovery and ensure quality control. The samples were then extracted three times with n-hexane-diethyl-ether (1:1 v/v). After evaporation of the solvents the fat content was determined gravimetrically. The fat was redissolved in n-hexane and treated with concentrated sulphuric acid. The PCBs were separated from the bulk of the chlorinated compounds by elution through a silica gel column (4.5g of 3% water-deactivated silica-gel). The first fraction, containing the PCBs was eluted with 30ml of n-hexane. The columns were of different polarity to ease identification of analytes which was based on retention times relative to internal standards. Quantification was performed using multilevel calibration curves obtained by injection of standard solutions of at least three different concentrations. The limit of determination (LOD) was determined as three standard deviations (SD) above the value of the blank and varied between 2 and 7 pg/g serum (not lipid adjusted). Samples with concentrations of LODs three SD above the blank have a 99% probability of being non-zero. To increase this probability, the quantification limits (LOQ) were set at higher levels than the LODs. In this case the lowest standard concentration was used; 10pg/g serum. The reproducibility of the method was demonstrated by 21 replicate determinations using an in-house control serum sample included in the analytical batches during the course of the study.

Statistical Analysis

Statistical Package for Social Sciences (SPSS) software version 17.0 computer software was used for the analysis of data. Two-tailed independent t-test of significance at 95% confidence limit with p<0.05 were considered significant for the variables. Pearson correlation coefficient was used to find relationships between quantitative variables.

RESULTS

Table 1 compares mean±SE of age, age at menarche(AM), age at menopause(AMP), weight, height, body mass index (BMI), waist circumference (WC), hip circumference (HC), waist-hip ratio (WHR), waist-height ratio (WHR), serum polychlorinated biphenyls and bisphenol-A in breast cancer subjects(cases) and controls. Comparison showed significant difference (p<0.05) in mean±SE of weight, height, waist circumference, hip circumference, waist-hip ratio, waist-height ratio, polychlorinated biphenyls and bisphenol-A in breast cancer subjects and controls. There were no significant differences in age, age at menarche, age at menopause and body mass index.

Table1.
Comparison of demographic, anthropometric, and biochemical indices breast cancer subjects and controls using Student's t. test.

PARAMETERS	CASES		CONTROL			
	N	Mean±SE	N	Mean±SE	t	P
Demographic Indices						
Age (years)	40	47.90±1.03	40	51.10±2.32	-1.081	0.283
Age at Menarche (years)	39	15.23±0.31	40	14.93±0.35	1.508	0.136
Age at Menopause (years)	12	46.75±1.93	18	49.11±0.66	-1.599	0.122
. Anthropometric Indices						
Weight(Kg)	39	69.62±2.41	40	58.89±1.61	3.715	0.000*
Height(m)	39	1.63±0.01	40	1.56±0.01	5.439	0.000*
BMI (kg/m²)	39	23.09±0.84	40	23.32±0.64	1.742	0.083
Waist Circumference (cm)	39	86.54±1.55	40	76.68±1.45	4.645	0.000*
Hip Circumference (cm)	39	99.00±1.44	40	94.82±1.24	2.620	0.011*
Waist-Hip	39	0.87±0.01	40	0.82±0.01	4.073	0.000*
Waist-Height	38	52.52±0.90	40	48.98±0.89	2.793	0.007*
Biochemical Parameters						
PCB (ng/ml)	40	0.81±0.47	40	0.11±0.01	6.805	0.000*
BPA (ng/ml)	40	0.79±0.48	40	0.79±0.01	10.115	0.000*

Values are mean±SE; *=significant, SE= standard error, n= number of subjects, p=probability, PCB=polychlorinated Biphenyls, BPA=bisphenol-A, BMI=body mass index

Table 2.

Correlation of demographic with anthropometric indices in females with breast cancer and controls.

Indices	Breast cancer n=40 (r.p-values)	Controls n=40 (r.p-values)
Height vs Weight	0.349, 0.029*	0.139, 0.391
Height vs WC	0.360, 0.026*	0.222, 0.169
Height vs WHR	0.354, 0.029*	0.204, 0.207
Weight vs BMI	0.925, 0.000*	0.197, 0.224
Weight vs AM	0.021, 0.899	-0.328, 0.039*
WC vs WHR	0.595, 0.000*	0.697, 0.000*
WC vs WHT	0.930, 0.000*	0.937, 0.000*
WC vs HC	0.770, 0.000*	0.685, 0.000*
HC vs WHT	0.779, 0.000*	0.660, 0.000*
WHR vs WHT	0.485, 0.002*	0.618, 0.000*
BMI vs WC	0.205, 0.216	0.566, 0.000*
BMI vs HC	0.295, 0.072	0.562, 0.000*
BMI vs WHT	0.251, 0.128	0.651, 0.000*
AMP vs BMI	-0.629, 0.028	-0.083, 0.743

*=significant; p=probability, r=pearson correlation coefficient, BMI=body mass index, WC=waist circumference, HC= hip circumference, WHR=waist-hip ratio, WHT=waist-height ratio, AM=age at menarche, AMP= age at menopause

Table 2 shows the correlation of demographic with anthropometric indices in females with breast cancer and controls. Positive correlations among height, weight, waist circumference (WC) and waist-hip ratio (WHR), weight and body mass index (BMI) were observed in cases only. Positive correlations were observed among waist circumference (WC) correlated positively with waist-hip ratio (WHR), waist-height ratio (WHT) and hip circumference (HC). Hip circumference correlated positively with waist-height ratio (WHT). Waist-hip ratio (WHR) correlated positively with waist-height ratio (WHT) in both cases and controls. Non-waist-height ratio (WHT) in both cases and controls. Non-significant negative correlation was observed between age at menopause (AMP) and body mass index (BMI) in both cases and control. Positive correlations were observed among body mass index (BMI) with hip circumference (HC) and waist-height ratio (WHT). Weight however correlated negatively with age at menarche (AM). These correlations were statistically significant (p<0.05).

DISCUSSION

Recent studies have shown that the incidence of breast cancer in Nigeria is on the increase in recent years particularly in younger women because of its obscure aetiology (Adesunkanmi et al. 2006, Anieto and Charles-Davies, 2008). The most established risk factors are related to life-time hormone exposure, early menarche, late menopause and recently, exposure to endocrine disrupting substances (Brophy et al. 2012; Fernandez and Russo, 2010; Korenkamp, 2008).

This present study showed that demographic indices (age, age at menarche, age at menopause) are similar in both breast cancer patients (cases) and non-breast cancer patients (controls). This could be because the subjects were age matched.

Statistically significant increases were observed in weight, bsi, bt, waist circumference (WC), hip circumference (HC), waist-hip ratio (WHR), weight-height ratio (WHT) in cases when compared with controls (p<0.05). Non-significant increase was observed in body mass index (BMI) in cases when compared with the controls. This signifies that adiposity could be a contributory factor in the aetiology of breast cancer. While WC has been shown as an accurate predictor of visceral adiposity either alone or in combination with BMI, visceral fat remains more associated with increased risk of cancer development (Steffen et al. 2009). The systemic effects exerted by visceral adiposity are focus of much research (Donohoe et al. 2011). WHR measurements may be more relevant in determining cardiovascular risk than WC alone, whether this is the case for cancer risk is not yet known (Donohoe et al. 2011). BMI is associated with an increased incidence of many types of cancer including breast cancer (Renehan et al. 2008; Donohoe et al. 2011). There is still paucity of information on the involvement of HC and WHT in the aetiology of breast cancer (Donohoe et al. 2011). Positive correlations were observed among WC and WHR, WHT, HC; HC and WHT; WHR and WHT in both cases and controls. However, positive correlations among height, weight, WC and WHR; BMI and weight were observed in cases only. Weight correlated negatively with age at menarche (AM) only in controls. The differences and correlations were statistically significant (p<0.05). This is equally a pointer to the fact that adiposity plays a major role in cancer aetiology.

Humans are exposed daily to a variety of compounds. It is thus likely that even if none of these chemicals reach an effective level, the combination or mixture of chemicals may become dangerous. These chemicals enter the food chain and accumulate in animals up to humans (Lubrano et al. 2013). Bisphenol-A (BPA), a breakdown product of coatings in food and beverage containers, may act as oestrogen receptor agonist. In this present study, there was a significant increase in the serum BPA level in cases when compared with the controls. This is in consonance with the outcome of a similar study (Fernandez and Russo, 2010).

In this present study, significant increase was observed in the serum PCB levels in cases when compared with the controls. There is currently paucity of information on females who are non-occupationally exposed, particularly in Nigeria. Some Polychlorinated biphenyls (PCBs) activate Aryl hydrocarbon receptor (AhR) (Lubrano et al. 2013). Although the mechanism through which AhR regulates energy metabolism are not clearly established, crosstalk with oestrogen receptor may be involved (Lubrano et al. 2013). Recently, much evidence has emerged indicating that environmental toxicants including BPA and PCBs can affect mitochondrial function and cause pro-oxidative reaction leading to pathological conditions like cancer (Valavlakes et al. 2006; Altern et al. 2010; Farabel et al. 2011). This further supports the fact that oxidative stress could be one of

the mechanisms of action of these toxicants in the aetiology of breast cancer.

In conclusion, mechanisms involving PCBs and BPA as well as adiposity may be involved in breast cancer aetiology reduction of environmental pollution and dietary modulation may be helpful.

Recommendation

Policies aimed at discouraging the practice of packaging of food products or water with materials containing BPA should be put in place by the relevant authority. Adequate maintenance of hazardous waste dumps and city landfills as well as control of illegal or improper dumping of hydraulic fluids/coolants, leaks from electrical transformers and other equipment, burning of medical, industrial or city waste from older appliances goods like televisions may reduce environmental pollution due to PCBs. These strategies together with consumption healthy diet and lifestyle may prevent, protect and reduce the high mortality associated with breast cancer in Nigeria.

REFERENCES

Adesunkanmi AR., Lawal OO, Adelusola KA, Durosimi MA (2006). The severity, outcome and challenges of breast cancer in Nigeria. *Breast*.15(3): 399-409

Albers G, Echteld MA, de Vetite, Onwuteaka-Philipsen BD, van der Linden MH, Deliens L (2010). Evaluation of quality of life measures for use in palliative care: A systematic review. *Palliative Med.* 24:17-37

Arinola OG and Charles Davies MA(2008). Micronutrient levels in the plasma of Nigerian females with Breast cancer. *African Journal of Biotech.*7:11.1620-23

Betancourt Angela M, Wang Jun, Sarah Jenkins Sarah, Mobley Jim, Russo Jose, and Lamartiniere Coral A(2012). Altered Carcinogenesis and Proteome in Mammary Glands of Rats after Prepubertal Exposures to the Hormonally Active Chemicals Bisphenol A and Genistein. *J. Nutr.*142: 7 1382S-1388S

Brisken C (2008). Endocrine disruptors and breast cancer.*Chimia.* 62(5).406-9

Brody JG, Moysich KB, Humblet O, Attfield KR, Beehler GP.S

Rudel RA(2007).Environmental pollutants and breast cancer: epidemiologic studies.*Cancer*.109(12 Suppl):2667-2711

Brophy James T,Keith Margaret M, Watterson Andrew, Park Robert,Gilbertson Michael, Maticka-Tyndale Eleanor, Beck Mathias, Abu-Zahra Hakam,Schneider Kenneth, Reinhartz Abraham, DeMatteo Robert and Luginaah Isaac (2012).Breast cancer risk in relation to occupations with exposure to carcinogens and endocrine disruptors: a Canadian case-control study. *Environmental Health.* 11:87. doi:10.1186/1476-069X-11-87

Calafat Antonia M, KochHolger M, Swan Shanna H, Hauser Russ,Goldman Lynn R, Lanphear Bruce P, Longnecker Matthew P, Rudel Ruthann A, Teitelbaum Susan L, WhyattRobin MandWolff Mary S(2013).Misuse of blood serum to assess exposure to bisphenol A and

phthalates.*Breast Cancer Research*.15:403. doi:10.1186/bcr3491

Chakraborty R and Bose K (2009). Central adiposity, body mass index and percent body fat among Bengalee Hindu Male slum dwellers of dumdum, West-Bengal, India. *The open Obesity Journal*.1:32-37

Donohoe CL, Doyle SL, Reynolds JV (2011). Visceral adiposity, insulin resistance and cancer risk.Diabetology and Metabolic Syndrome. 3(12) doi: 10.1186/1758.5996-3-12

Farahat FM, Ellison CA, Donner MR, McCurragle BP, Crane AL, Fenske RA (2011). Biomarkers of chlorpyrifos exposure and effect in Egyptian cotton field workers. *Environ. Health Persp.*119:801-806

Fernandez SV, Russo J (2010).Estrogen and xenoestrogens in breast cancer. *Toxicol Pathol.* 38(1):110-122

Gray J, Evans N, Taylor B, Rizzo J, Walker M (2009): State of the evidence: the connection between breast cancer and the environment. *Int J Occup Environ Health* 15:43-78

Haighton LA, Hlywka JJ, Doull J, KroesR(2002). An evaluation of the possible carcinogenicity of BPA to humans. *Regulatory Toxicol and Pharmacol.*35:23 8-54

Halberg N, Wernstedt-Asterholm L, Scherer PE (2008) The adipocyte as an endocrine cell.*Endocrinol Metab Clin North Am.*37:753-68

Iyengar M (2005). Polychlorinated biphenyls-a review.Pp 1-24

King SW, Rane WS, Kire SJ, Gavrison JL, Taunton J, Hegde RS(2006).Substrate-specific translocation attenuation during ER stress defines a pre-emptive quality control pathway. *Cell* 1:27(5) 999-1013

Kortenkamp A (2006). Environmental contaminants and breast cancer. The growing concerns about endocrine disrupting chemicals. A briefing paper for WWF-UK.Pp 1-8

Lubrano L.,Genovesi G. Specchia, P., Cassandri D, Stefania, M, Petrogelli E, Lenzi A, Gnessi L(2013). Obesity and metabolic comorbidities: Oxidative Medicine and Cellular Longetivity.Article ID 640673 pp 1-9. http://dx.doi.org/10.1155/2013/640673

Msoll A, Ghorbi O., Mahmoudi K, Limem S, Hochlaf M, Ben Ahmed S (2011) Association between body mass index and the risk of breast cancer in Tunisian women. *Annals of Saudi Medicine*.31(4):393-397.

Reichin AG, Tyson M, Egger M, Heller RF, Zwahlen M (2008). Body mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies. *Lancet* 371:569-578

Steffen A., Schulze MB, Pischon T, Dierrich T, Molina E, Chirlaque MD, Barricarte A, Amiano P, Quiros JR, TjonnelaR(2009). Anthropometry and esophageal cancer risk in the European Prospective Investigation into cancer and nutrition. *Cancer Epidemiol Biomarkers Prev.*18: 2079-2089

Valavanidis AT, Vlahogianni M, Dassenakis, Scoullos (2006).Molecular biomarkers of oxidative stress in aquatic organisms in relation to toxic environmental pollutants. *Ecotoxicol Environ.* 64.178-189.

7. TOSOH AIA-1200 series or AIA-600 Immunoassay Analyzers can NOT be used to perform the ST AIA-PACK FSH assay.

STORAGE AND STABILITY
All unopened materials are stable until the expiration date on the label when stored at the specified temperature.

Materials	Cat. No.
2-8°C	
ST AIA-PACK FSH	0025265
AIA-PACK FSH CALIBRATOR SET	0020565
AIA-PACK FSH SAMPLE DILUTING SOLUTION	0020955
AIA-PACK SUBSTRATE SET II	0020965
AIA-PACK WASH CONCENTRATE	0020956
AIA-PACK DILUENT CONCENTRATE	0018591
1-30°C	
AIA-PACK DETECTOR STANDARDIZATION TEST CUP	0020970
AIA-PACK SAMPLE TREATMENT CUP	0020971

ST AIA-PACK FSH cups may be stored at 2-8°C up to 1 day. Cups and Sample Diluting Solution should be used within 1 day and 7 days of opening respectively, provided that vials are kept tightly sealed and stored between 2-8°C. ...

SPECIMEN COLLECTION AND HANDLING
- Serum or heparinized plasma is appropriate for the assay. EDTA and citrated plasma SHOULD NOT BE USED.
- ...
- ...
- ...
- The sample required for analysis is 50 µL.

PROCEDURE
For the AIA Nex IA / AIA-21, AIA-600 II, AIA-1800, AIA-2000 and AIA-360, please refer to each Operator's Manual for detailed instructions.

1. Reagent Preparation
A) Substrate Solution
Bring all reagents to 18-25°C before preparing the working reagent. Add the entire contents of the AIA-PACK SUBSTRATE RECONSTITUENT II (100 mL) to the lyophilized AIA-PACK SUBSTRATE REAGENT II, mix thoroughly to dissolve the solid material.

B) Wash Solution
Add the entire contents of the AIA-PACK WASH CONCENTRATE (100 mL) to approximately 2.0 L of CAP Class I or NCCLS Type I Reagent Grade water, mix well, and adjust the final volume to 2.5 L.

C) Diluent
Add the entire contents of the AIA-PACK DILUENT CONCENTRATE (100 mL) to approximately 4.0 L of CAP Class I or NCCLS Type I Reagent Grade water, mix well, and adjust the final volume to 5.0 L.
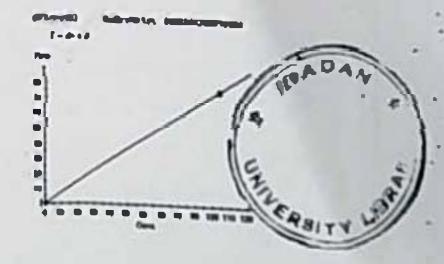
2. Calibrator Procedure
A) Calibration Curve
...

ST AIA-PACK FSH

For the quantitative measurement of follicle stimulating hormone (FSH) in serum or heparinized plasma.

NAME AND INTENDED USE
ST AIA-PACK FSH is designed for IN VITRO DIAGNOSTIC USE ONLY for the quantitative measurement of follicle-stimulating hormone (FSH) in human serum or heparinized plasma on TOSOH AIA System Analyzers.

SUMMARY AND EXPLANATION OF TEST
Follicle-stimulating hormone (FSH) is a glycoprotein which, like LH, hCG and TSH, consists of alpha and beta chains. The alpha chain is virtually identical in all four hormones, whereas the beta chains are different and determine both the specific biological activity and immunological characteristics of each hormone (1,2). The FSH molecule contains approximately 16% carbohydrate and has a molecular weight of approximately 34,000 to 30,000 daltons (3). FSH stimulates ovarian follicle growth and estrogen production in women, and testicular spermatogenesis in men (4-7).
Hypothalamic control of both FSH and LH secretion by the anterior pituitary appears to be by a common releasing hormone, gonadotropin-releasing hormone (GnRH) with negative feed back at the hypothalamic level by estrogen in the female and testosterone in the male.
Determination of FSH concentrations is essential in assessment and monitoring of patients with suspected infertility. Other physiological disorders associated with abnormal FSH secretion have been reported (3, 8-11).

PRINCIPLE OF THE ASSAY
The ST AIA-PACK FSH is a two-site immunoenzymometric assay which is performed entirely in the AIA-PACK test cups. FSH present in the test sample is bound with monoclonal antibody immobilized on a magnetic solid phase and enzyme-labeled monoclonal antibody in the AIA-PACK test cups. The magnetic beads are washed to remove unbound enzyme-labeled monoclonal antibody and are then incubated with a fluorogenic substrate 4-methylumbelliferyl phosphate (4MUP). The amount of enzyme-labeled monoclonal antibody that binds to the beads is directly proportional to the FSH concentration in the test sample. A standard curve is constructed and unknown sample concentrations are calculated using this curve.

MATERIAL PROVIDED (ST AIA-PACK FSH, Cat. No. 0025265)
Plastic test cups containing lyophilized twelve magnetic beads coated with anti-FSH mouse monoclonal antibody and 100 µL of anti-FSH mouse monoclonal antibody (to human FSH) conjugated to bovine alkaline phosphatase with sodium azide as a preservative.

MATERIALS REQUIRED BUT NOT PROVIDED
The following materials are not provided but are required to perform follicle-stimulating hormone analysis using the ST AIA-PACK FSH (Cat. No. 0025265) on the TOSOH AIA System Analyzers. They are available separately from TOSOH.

Materials	Cat. No.
AIA Nex IA or AIA-21	0018533
AIA Nex IA or AIA-21 LA	0018540
AIA-1800 ST	0019834
AIA-1800 LA	0019837
AIA-2000 ST	0022101
AIA-2000 LA	0023101
AIA-600 II	0019014
AIA-600 II BCR	0019328
AIA-360	0019943
	0020965
AIA-PACK SUBSTRATE SET II	
AIA-PACK SUBSTRATE REAGENT II	
AIA-PACK SUBSTRATE RECONSTITUENT II	0020365
AIA-PACK FSH CALIBRATOR SET	
ZERO CALIBRATOR 0 mIU/mL	
POSITIVE CALIBRATOR 100 mIU/mL (approx.)	0020565
AIA-PACK FSH SAMPLE DILUTING SOLUTION	0020955
AIA-PACK WASH CONCENTRATE	0020956
AIA-PACK DILUENT CONCENTRATE	0018591
SAMPLE CUPS	0020970
AIA-PACK DETECTOR STANDARDIZATION TEST CUP	0020971
AIA-PACK SAMPLE TREATMENT CUP	
Additional Requirements for AIA Nex IA / AIA-21 only:	
PIPETTE TIPS	0018552
PRELOADED PIPETTE TIPS	0018583
Additional Requirements for AIA-600 II, AIA-1800 and AIA-2000	
PIPETTE TIPS	0019215
TIP RACK	0019216
PRELOADED PIPETTE TIPS	0022103

Only materials obtained from TOSOH should be used. Materials obtained elsewhere should not be substituted since assay performance is characterized based solely on TOSOH materials.

WARNINGS AND PRECAUTIONS
1. The ST AIA-PACK FSH is intended for in vitro diagnostic use only.
2. Test cups from different lots or different assays should not be mixed within a tray.
3. The ST AIA-PACK FSH contains sodium azide, which may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of such reagents, flush with large volumes of water to prevent azide build-up.
4. Human serum is not used in the preparation of this product; however, since human serum will be used for samples and other quality control products in the lab may be obtained from human serum, please use standard laboratory safety procedures in handling all ...
5. Do not use beyond the expiration date.
6. The ST AIA-PACK FSH has been designed so that the high dose "hook effect" is not a problem for the vast majority of samples. Samples with FSH concentrations between 200 and 20,000 mIU/mL will read 200 mIU/mL. The "hook effect" phenomenon only occurs at concentrations > 20,000 mIU/mL.