MEDIAN TIME TO ACHIEVE UNDETECTABLE VIRAL LOAD AFTER INITIATION OF ANTIRETROVIRAL TREATMENT AMONG HIV-INFECTED ADULTS IN SOUTH-EAST NIGERIA

BY

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CERTIFICATION

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DEDICATION

This work is dedicated to God Almighty who made me and had sustained me all my life; and also to my parents, Mr Bernard and Mrs Christiana Obikeze, whose nurture for me and faith in God had played critical role in my growth and personal development.

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LIST OF ABBREVIATIONS AND ACRONYMS

AIDS   Acquired immune-deficiency syndrome

APIN   AIDS prevention initiative in Nigeria

ART   Anti-retroviral treatment

ARV   Anti-retroviral drug

BP   Blood Pressure

CD4   A measure of the number of helper T cells per cubic millimetre of blood, used to assess the prognosis of patients infected with HIV.

CI   Confidence interval

ELISA   Enzyme-linked immunosorbent assay

HAART   Highly active antiretroviral therapy

HIV   Human immunodeficiency virus

NRTI   Nucleoside reverse transcriptase inhibitor

NNRTI   Non-nucleoside reverse transcriptase inhibitor

PEPFAR   United States President's emergency plan for AIDS relief

Pep ID   PEPFAR identification number

RNA   Ribonucleic acid

TB   Tuberculosis

UNEC   University of Nigeria, Enugu Campus

UNTH   University of Nigeria Teaching Hospital, Enugu

UVL   Undetectable viral load

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ABSTRACT

Background

The burden of high HIV prevalence and poor access to treatment and care at the beginning of the last decade had necessitated APIN-Plus/Harvard PEPFAR intervention in Nigeria in 2004. So far, no published local study has documented the probability of survival in Nigeria for patients on HIV treatment by way of their time to achieve undetectable viral load after initiation of antiretroviral treatment, let alone determine those patient factors that may influence or predict the time to undetectable viral load while on antiretroviral treatment.

Objective

This study therefore assessed the median time to achieve undetectable viral load after initiation of antiretroviral therapy among HIV-infected adults in South-east Nigeria as well as those patient characteristics that influence or predict that outcome.

Methodology

It involved a retrospective review of records of 800 antiretroviral treatment-eligible but antiretroviral drug-naïve patients receiving treatment at the UNTH Enugu between June 2008 and May 2010. Categorical variables were presented using proportions, frequency tables and graphs while means, medians, standard deviations and range were used in presenting quantitative variables. Kaplan-Meier analysis was used to estimate the cohort survival probability in terms of time to undetectable viral load while Cox's regression analysis was used to model the predictors of the main study outcome. All tests of hypothesis were based on 5% significance

Results

The mean age of the 800 patients was 37.6 ±9.8 years with 72% aged between 25 – 44 years. About 45% of the cohort was married and 64.8% were females. Traders were in the majority with 34.3% while 51% attained secondary education or higher. Mean systolic blood pressure (BP) was 114.5mmHg ± 13.5 while mean diastolic BP was 73.4mmHg ±10.1 and 12.1% were hypertensive. Median CD4 count was 150.5 cells/ml with 71% of the cohort having CD4 count less than 200 cells/ml while the median RNA viral load was 153,007 copies/ml. The cohort's median time to achieve undetectable viral load was 12.5 weeks with 78.6% of them achieving it within one year. Prevalence of tuberculosis co-infection among the patients was 8.1% with male preponderance. Being a civil servant was the only patient characteristic that significantly influenced or predicted the time to undetectable viral load among the cohort.

Conclusion

At a median time of 12.5 weeks, the study showed a very good survivorship as it takes a shorter time for those on treatment from the UNTH Enugu programme to achieve undetectable viral load than those from previous studies. The cohort performance also showed that nearly every patient that achieved viral load undetectability at the Enugu programme did so within one year of starting the drug treatment. Prevalence of HIV/TB co-infection at the study site was relatively low at 8% compared to results obtained from other parts of the country and only occupation appeared to improve the speed of achieving undetectable viral load among patients receiving treatment from the Enugu programme. Further studies will be required to determine the sustainability of patients' undetectability status by assessing the proportion of RNA viral load rebound among the cohort.

Key words: HIV/AIDS, UNTH Enugu, Kaplan-Meier estimate, Cox's regression model, CD4 count

CHAPTER ONE
INTRODUCTION

1.1 Background

In a recent global report, UNAIDS stated that although HIV prevalence is much lower in Nigeria than in many other African countries such as South Africa and Zambia, the large size of Nigeria's population meant that by the end of 2007, there were an estimated 2,600,000 people infected with HIV and approximately 170,000 people died from AIDS in that year alone. Another 2007 estimate put life expectancy at birth in Nigeria at 50 years for women and 48 years for men, attributing the decline in life expectancy in Nigeria largely to the scourge of HIV/AIDS (NDHS, 2008).

At the time HIV/AIDS was first recorded in Nigeria in the mid 1980s, those living with the virus, like their HIV victims globally, faced the challenge of poor availability of effective drugs to treat the scourge (Pierre, 2002). However, use of highly active antiretroviral therapy (HAART) for the management of patients infected with HIV commenced in 1996 and has remained the standard of therapy for the infection since then (Carpenter et al, 1996). A three drug combination of two nucleoside reverse transcriptase inhibitors (NRTIs) e.g. Lamivudine and Stavudine (or Zidovudine) with either a protease inhibitor (PI) e.g. Ritonavir or Saquinavir or a non nucleoside reverse transcriptase inhibitor (NNRTI) like Efavirenz or Nevirapine, has remained the first line standard of care. Efficacy of these three-drug regimens in the achievement of viral load suppression as well as their effect on mortality and morbidity has been previously demonstrated in randomized controlled trials (Hammer et al, 1997).

As drug availability for HAART became resolved, the challenge of affordability of those drugs for HIV/AIDS care and treatment became the next burden for those living with the virus as the drugs were at that time considered too costly and consistent treatment became unsustainable for most victims due to payment of user fees by those patients. That development influenced the launching in November 2004 of the "Free by 5" campaign for universal, free antiretroviral therapy (Whiteside and Lee, 2005). Harvard PEPFAR's treatment intervention in Nigeria and some other countries was in apparent response to the "Free by 5"campaign for a sustainable treatment of people living with HIV/AIDS.

HIV type-1 plasma RNA viral load measurement has been employed as a benchmark for defining the success or failure of an antiretroviral treatment and current guidelines indicate that the main goal of therapy is to obtain and maintain viral

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load (VL) undetectability. VL undetectability is defined as a plasma VL below the cut-off lower limit of detection (LLD) of routine VL assays, previously <400 copies per ml but currently put at <50 copies/ml (Geretti et al, 2008). The cut-off point of this undetectability depends on the sensitivity and brand of a particular machine in use. In a European collaborative study among HIV-infected pregnant women, it was observed that 57.8% of non-black women and 66.3% of black women with baseline HIV- RNA viral loads of ≥ 4 $\log_{10}$ (10,000) copies/ml achieved an undetectable viral load at 10 weeks, while 64.1% of non-black women and 79.3% of black women achieved an undetectable viral load at 15 weeks, suggesting possible race-associated differences (Giaquinto et al, 2007). In this study, threshold for undetectable viral load was defined as HIV-1 RNA viral load of <200 copies/ml.

The net benefit of HAART for the HIV-infected persons, however, is dependent not only on the efficacy of HAART but also on a large proportion of those persons receiving and remaining on their medications for an extended period (Cunningham et al 2000). A nationally-funded antiretroviral treatment programme began in Nigeria in February 2002 in 25 treatment centres across Nigeria's six geopolitical zones. By 2004, the Harvard School of Public Health through the US President's Emergency Plan for AIDS Relief (PEPFAR) commenced partnership with teaching hospitals, research institutes, federal medical centres and other institutions to scale up HIV care and treatment activities in nine states in Nigeria. University of Nigeria Teaching Hospital (UNTH) is one of such PEPFAR partner sites and is the only such main site in South-east Nigeria (Harvard PEPFAR/ APIN-Plus, 2009). APIN-Plus/Harvard PEPFAR intervention is widely understood to have expanded access to antiretroviral therapy in sub-Saharan Africa.

1.2 Rationale

Hitherto, research on HAART for HIV-positive patients in developing countries especially Nigeria has been largely donor-driven and centred on clinical outcomes like reconstitution of the patients' immune system as evidenced by increase in plasma CD4 count or overall clinical well being of patients. Knowledge of chance of survival for HIV-infected patients on treatment will no doubt help programme managers to improve HIV/AIDS patient care. Few studies had explored such patients' chance of survival after initiation of antiretroviral treatment, let alone understand the proportion of the patients on treatment who attain undetectable RNA viral load at specific time periods. A good understanding of the factors that influence patients' virological response to antiretroviral drugs is also important in order to enable stakeholders to use such evidence to improve clinical management of patients infected with HIV (Paredes et al, 2000). In other words, knowledge of factors other than antiretroviral drugs which influence the survivorship of HIV-infected patients on treatment will go a long way to enabling program managers and funders to promote such determinants for optimal

program outcomes thereby achieving the overall objective of halting by 2015 and beginning to reverse the incidence of HIV in Nigeria in line with the Millennium Development Goals.

Nevertheless, there is no published data from local studies bordering on patients' time to achieving undetectable RNA viral load or the major factors that influence or predict that treatment outcome or the proportion of those on treatment achieving viral load undetectability at specific time periods after commencement of treatment in Nigeria. This study therefore aims to bridge that knowledge gap by seeking to determine Nigerian HIV patients' survival probability through establishing the median time to achieve undetectable viral load following initiation of antiretroviral treatment among a cohort of patients.

Findings from this study will be useful to the programme managers for evaluation of the HIV treatment programme in UNTH Enugu, similar PEPFAR-funded HIV treatment sites and a number of other HIV/AIDS treatment programmes in Nigeria in which measurement of RNA viral load is employed. Furthermore, in the possible event of withdrawal of donor funding for HIV care and treatment in Nigeria, results from this study will also form part of evidence base for estimating the proportion of patients failing to respond to first-line drugs used in treatment, information that will serve as a further guide in the assessment of financial and other resource needs for the sustainability of Nigeria HIV/AIDS control and treatment programme.

1.3 Objectives

The broad objective of the study was to assess the median time to achieve undetectable viral load after initiation of antiretroviral treatment in an HIV treatment cohort as well as those factors that influence that treatment outcome among adult HIV-positive patients on highly active anti-retroviral therapy (HAART) in a treatment programme in Nigeria.

1.3.1 *Specific Objectives*

i) To determine the median time to achieve undetectable viral load in the treatment cohort.

ii) To determine the proportion of the cohort who attained undetectable viral load by the twelfth, twenty fourth and fifty second weeks' periods after commencement of highly active antiretroviral therapy (HAART).

iii) To assess the prevalence of HIV/tuberculosis co-infection in the cohort.

iv) To assess the factors that may influence or predict the time to achieve undetectable viral load in the cohort.

CHAPTER TWO
LITERATURE REVIEW

Median time to achieve UVL

In a prospective cohort study to assess treatment outcomes in the first 12 months among HIV-positive patients managed with a combination of nevirapine + stavudine+ lamivudine under the current national antiretroviral (ARV) programme in Nigeria, Idigbe and co-investigators concluded that there was an effective suppression of viral replication, the reconstitution of the immune system, and improvement of the physical well-being of the study population (Idigbe et al, 2006). On the other hand, a retrospective study to compare the time to achieve a viral load<50 copies/ml from >5.3 log_{10} (~200,000) copies/ml among ART naïve patients within the first 16 weeks after the commencement of treatment between 1 January 1999 and 1 January 2004, Manavi and Scott found that the median time to undetectable viral load was achieved at 13.5 weeks for both three and four drug regimens (Manavi and Scott, 2006).

In a European prospective multicentre cohort study – the celebrated Euro SIDA study involving fifty-two clinical centres in 17 European countries – to assess the predictors of virological success and ensuing failure in HIV-positive patients starting HAART in from August 1996 to April 1999, Paredes and colleagues found out that the median time to achieve UVL was 16 weeks (Paredes et al, 2000). However, this was based on undetectable RNA viral load threshold of <500 copies/ml.

In another European collaborative study among HIV-infected pregnant women using 200 copies/ml as the cut-off point for RNA viral load undetectability, it was observed that 57.8% of non-black women and 66.3% of black women with baseline HIV RNA viral loads of ≥4 log_{10} (10,000) copies/ml achieved an undetectable viral load at 10 weeks, while 64.1% of non-black women and 79.3% of black women achieved an undetectable viral load at 15 weeks, suggesting possible race-associated differences (Patel et al, 2007).

In a retrospective cohort study on HIV-infected patients who received HAART between January 1999 and December 2003 at a university hospital using an undetectable viral load cut-off of <50 copies/ml, Sungkanuparph et al noted that the median time to achieve undetectable RNA viral load was 4.3 months (17.2 weeks) in patients with persistent low-level viraemia and 3.6 months (14.4 weeks) in patients without persistent low-level viraemia (Sungkanuparph et al, 2006). However, there is ample evidence that viral replication continues in patients with undetectable viraemia but achieving and maintaining this undetectablity is associated with long term virological suppression and sustained immunological and clinical benefit (Palmer et al, 2008; Mocroft et al, 2007).

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Proportion of the patients achieving UVL at different time periods.

In a cohort study conducted at Bamrasnaradura Infectious Diseases Institute, Nonthaburi, Thailand involving a four-year follow-up of 244 patients using <50 copies/ml as cut-off for undetectability, Soe and colleagues reported that 84.6% of the cohort achieved virologic success at the end of the follow-up period (Soe et al, 2010).In a Swiss HIV cohort study on suppression of viral load and progression to AIDS or death involving 2674 outpatients who started HAART in 1995–98 using Kaplan-Meier analysis, Ledergerber and co-investigators noted that an estimated 90.7% of treatment-naïve patients achieved undetectable viral load of <400 copies/ml by the twelfth month following commencement of anti-retroviral treatment (Ledergerber et al, 1999).

In a prospective cohort study to determine the predictors of a viral response and subsequent virological treatment failure among 243 HIV-positive patients receiving anti-retroviral treatment in a Southern Alberta Clinic in Canada, it was found that 52.8% of the patients had achieved an undetectable viral load by 24 weeks after first exposure to treatment (Mocroft et al, 1998).

Prevalence of HIV co-infection with tuberculosis

At the Aminu Kano Teaching Hospital, (AKTH), Kano, Nigeria, Iliyasu and Babashani did a study on the prevalence and predictors of tuberculosis co-infection among HIV-seropositive patients attending the hospital's specialist clinic for HIV-positive patients in 2006 and found out an HIV-tuberculosis co-infection prevalence of 10.5%. This is much lower than the 40% and 32.8% prevalences of active TB earlier reported among HIV seropositive patients in the Nigerian cities of Ilorin and Ibadan, respectively (Iliyasu et al 2009). However, in a recent six-month prospective study to explore the sensitivity of direct smear microscopy for the diagnosis of TB in high HIV prevalent population at the Nigerian Institute of Medical Research, Yaba, Lagos, Nigeria, HIV/TB co-infection rate was 38.1% (Onubogu et al, 2012).

In a study in Brazil published by de Carvalho et al in 2008, 3.7% of all registered new cases of tuberculosis in 2006 were HIV/TB co-infected patients (de Carvalho et al, 2008). In another cohort study in Thailand in 2004 to assess early viral suppression predicting long-term treatment success among HIV patients commencing NNRTI-based antiretroviral therapy, Soe and colleagues found that tuberculosis was the commonest pre-ART opportunistic infection,

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involving 39.3% of all HIV-positive patients (Soe et al, 2010). In a review of all pulmonary tuberculosis (TB) cases diagnosed by Ziehl-Neelsen staining technique and extra-pulmonary TB diagnosed by tissue histology between January 2000 and December 2004 at the University of Ilorin Teaching Hospital, HIV/TB co-infection was found to occur in 40% of the 744 new cases of TB seen at the site in five years (Salami and Katibi, 2006). In another study to establish the prevalence of active pulmonary TB in HIV sero-positive adult patients in University College Hospital (UCH), Ibadan, a co-infection burden of 32.8% was found (Awoyemi et al, 2002).

Predictors of time to achieve UVL

Several patient and treatment characteristics had been found to influence the time it takes a patient on antiretroviral treatment to achieve undetectable viral load. Considering the impact of age on time to achieve undetectable viral load, it appears that the effect of age is prominent at the two extremes of life. This was confirmed in a prospective study to evaluate epidemiological and clinical features, response to HAART, and survival in elderly HIV-infected patients with regard to younger HIV-infected patients in Barcelona, Spain, between 1998 and 2003 in which the investigators found out that not only greater number of older patients developed full blown AIDS, but also that disease progression and mortality following HIV diagnosis were faster in older patients (Nogueras et al, 2006).

In another study to evaluate the effect of age, CD4 percentage (CD4%) and plasma HIV-1 RNA on response to highly active antiretroviral therapy (HAART) in previously untreated children in UK and Ireland, the investigators discovered that children generally respond immunologically to HAART irrespective of pre-HAART HIV-1 RNA or clinical status. However, immunological response is better in younger children and those with lowest CD4%, although they have poorer virological response, increasing the risk of resistance (Walker et al, 2004).In a report of a prospective European collaborative study involving 240 HIV-1–infected women starting HAART during pregnancy who were enrolled in the study from 1997 through 2004, it was found that Nevirapine-based HAART, western African origin, and lower baseline viral load were associated with shorter time to achieving viral suppression (Patel et al, 2007).

Adherence has also been noted as a significant predictor of survival in patients receiving antiretroviral treatment. In a study to evaluate the association between HAART adherence – as estimated by pharmacy claims - and survival in HIV-1 infectedSouth African adults enrolled in a private-sector AIDS management programme between 1999 and 2004, Nachega and co-

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investigators found out that adherence < 80% was associated with lower survival and when medication adherence was divided into 5 strata with a width of 20% each, each stratum had lower survival rates than the adjacent higher-adherence stratum, indicating a dose-response relationship between adherence and patient survival (Nachega et al, 2006). In a different study to ascertain the impact of ART adherence on survival in HIV-infected patients in a single HIV care unit setting in Barcelona, Spain, de Olalla et al observed that a non-adherent patient on triple therapy is 3.87 times more likely to die than an adherent patient on the same therapy (de Olalla et al, 2002).

CHAPTER THREE

METHODOLOGY

3.1 Study Site

The study was conducted at the University of Nigeria Teaching Hospital (UNTH), Enugu, Enugu State, South-east Nigeria. The UNTH Enugu was established by the Federal Government of Nigeria in July 1976 through decree 23 of 1974 and had since inception served as the flagship of tertiary health care delivery in the South-eastern part of Nigeria, occasionally as far as parts of South-south and North-central Nigeria. Formerly located close to Ogbete area of Enugu near the Ogbete Main market and Enugu prisons in Enugu, capital of Enugu State, the hospital took off from the then existing Enugu General Hospital which itself formerly served, since the early 20th century, as the standard General Hospital for Africans built by the colonial administrators. It later became Enugu General Hospital in the 1960s after the attainment of Nigeria's independence in 1960. The Enugu General Hospital had since 1976 therefore served as a temporary site for the UNTH Enugu, one of the flagship tertiary health care facilities in Nigeria and a national centre of excellence in cardiothoracic surgery. It is fully owned by the federal government of Nigeria. The hospital is principally dedicated to the delivery of clinical services to patients, basic and health systems research as well as the training of human resources for health. It has 21 clinical departments, 10 schools, 6 care centres and also involved in postgraduate medical training in several medical and surgical specialties– the residency training programme. On 8 January, 2007, the hospital moved to its present location at Ituku-Ozalla, near Enugu, which presently serves as its permanent site (www.unthenugu.org/about_unth/history.html).

The APIN-Plus/ Harvard PEPFAR programme is one of the research programmes which UNTH Enugu is engaged in, among other clinical outcome research projects. The programme itself was at inception intended to build upon the foundation established by the AIDS Prevention Initiative in Nigeria (APIN) which was originally funded by the Bill and Melinda Gates Foundation. Involved in the APIN-Plus/ Harvard PEPFAR programme in Nigeria are thirty-five institutions, comprising nine university/ medical research institutions (including the University of Nigeria Teaching Hospital, Enugu), twenty-two hospitals and four non-governmental organisations (NGOs) all spread across ten of the thirty-six States of Nigeria.

The UNTH Enugu treatment site commenced the determination of RNA viral load as part of the steps for the monitoring of progress of her programme patients in May, 2008. This study therefore covered patients that started accessing antiretroviral drugs at the centre from June 1, 2008.

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Patients usually get recruited into the UNTH Enugu treatment programme after testing positive to HIV screening done either as part of case finding during clinical work-up within the hospital (UNTH) or through HIV counselling and testing (HCT) done at the study area or any of the peripheral health facilities. Effort is made by the programme to do a thorough work-up of every patient presenting for treatment such that all clinical and laboratory records of the patient meet the criteria to commencement of treatment. Part of that workup is recording of patient's baseline data, including socio-demographic characteristics of, clinical records, CD4 count and RNA viral load. Immunological records like baseline RNA viral load, virologic records like baseline CD4 count as well as clinical records like blood pressure and status of co-infection with tuberculosis are all required to enable the clinicians to assign a stage to the HIV infection of the patient.

Where the outcome of the work-up favours commencement of treatment, patient is further made to go through sessions of adherence counselling, a process that enables care counsellors to offer psychological support to the patient while emphasizing the imperatives for continuous and consistent use of the dispensed drugs in addition to regular clinic visit based on scheduled clinic appointments. Each patient is enabled, through the counselling sessions, to understand the factors that may enhance or worsen the prognosis of the patients' and that treatment is for life (Harvard PEPFAR/APIN-Plus, 2009).

Drug combination in use

The first line drugs in use for all treatment-naive patients accessing treatment at the UNTH Enugu programme are three drug combinations from two classes of ARVs. The two drug classes are nucleoside reverse transcriptase inhibitors (NRTIs) and non nucleoside reverse transcriptase inhibitors (NNRTIs). Two drugs are selected from the NRTI class to constitute the highly active anti-retroviral therapy (HAART) and include Zidovudine and Lamivudine. Only Nevirapine is chosen from the NNRTI class to make up the three drug combination HAART used at the centre (Harvard PEPFAR/APIN-Plus, 2009).

Health management information system

The programme's treatment database is built as patients present for treatment and includes their socio-demographic characteristics and baseline clinical and laboratory records. Follow-up data on patients are updated at subsequent visits. The programme database also contains the drugs and other clinical interventions made available to those patients in the course of their treatment. In order to maintain confidentiality for the patients, a special PEPFAR identification number code (called PepID) is used to replace patients' names in the database.

During the study time that spanned two years – from June 1, 2008to May 31, 2010 – a total of two thousand and forty one patients were recruited into the programme, comprising patients transferred from other treatment sites and those without any previous treatment experience.

3.2 Study Population

This is comprised of HIV-infected adults attending the APIN-Plus/ Harvard PEPFAR clinic of the University of Nigeria Teaching Hospital (UNTH) Enugu. They come from both within and outside Enugu metropolis as far as and sometimes beyond the five States of the present-day South-east Nigeria comprising Abia, Anambra, Ebonyi, Enugu and Imo States. They are predominantly of the Igbo ethnic group of Nigeria. The predominant faith in the region is Christianity with few traditional religion adherents while the principal occupation is trading, with the traders operating within the numerous major markets in the South-eastern States. , ranging from Ogbete main market, Enugu; Onitsha main market, Onitsha; Ariaria main market, Aba; Nkwo market in Nnewi; Ochanja, Bridge head and Ose markets all in Onitsha plus other major ones located at Ugwu Agba Obosi; Nkpor, Owerri; Abakaliki; Umuahia and others. The region also accommodates one of the major divisions of the Nigerian Army – the 82 Division at Enugu – with satellite battalions at Onitsha (Anambra State) and Owerri (Imo State) as well as Naval and Air Force Bases in Abia and Enugu States respectively. A zonal command of the Nigerian Police Force – Zone 9 - is also located within the region at Umuahia (Abia State) which serves the five States' police commands. Agriculture and civil service are also prominent occupations among the people of the South-east Nigeria, a people collectively reputed for their fiercely enterprising and republican spirit. The population is a mixed type, males and females of different age groups coming from different educational, occupational and marital backgrounds.

Included in this study are patients who were HIV- positive, determined through ELISA test and confirmed by Western blot technique; aged 15 years or older; antiretroviral treatment-naïve at the commencement of antiretroviral therapy at the study site; had spent at least twelve weeks in the study and also had earlier signed a written informed consent for possible use of their personal treatment records for purposes of research. However, pregnant patients on antiretroviral drug regimens for purposes of preventing mother-to-child transmission (PMTCT) of HIV and who discontinued treatment postpartum were excluded from the study

3.3 Study Design

This is a retrospective review of records of a cohort of patients from a hospital-based programme database.

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3.4 Sample Size

All patients receiving antiretroviral treatment from the study site who met the inclusion criteria within the two-year study period were included in the study. Of the two thousand and forty one (2041) patients that were recruited into the programme at the time, only eight hundred and ninety (890) who were treatment-naive at first contact with the treatment site were selected for the study.

3.5 Data Extraction

Pre-approved data extraction proforma were used to extract the following independent variables from the clinic records of those patients that met the inclusion criteria.

3.5.1 Socio-demographic characteristics

Socio-demographic characteristics present in the patients' records and available for use for the study include age, gender, marital status, level of education and occupation.

Age:

Patients' individual ages were recorded in the programme database as year of birth. From this, age was defined as the difference between the year of commencement of HAART and the patient's year of birth. This was then grouped into six different age categories with ten-year intervals starting with the treatment entry age of fifteen (15) years. That is, 15 – 24 year age group, 25 – 34 year age group, 35 – 44 year age group, etc. Age was further grouped into three major categories based on age of employability or economic activity of the Nigerian environment. The three new categories were: the young population aged 15 – 24years who are usually either in school or still learning one trade or the other; the economically active category aged between 25 – 60years who are usually engaged in either formal employment or in the informal sector of the economy; while the last category was those aged above 60 years who are ordinarily expected to have retired from formal employment or any meaningful informal economic activity. Age was categorised further into two major groups – those aged thirty eight years or younger and those aged above thirty eight years. The two-group categories were based on patients' mean age of 37.6.

Marital status:

The study patients' marital status in the programme database was primarily adjudged as single, married, separated, divorced or widowed. In addition to that categorization, patients in this study were further grouped in two clear categories based on either having a companionship at the time of study or not. As such, those who were never married (single),

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separated, divorced or widowed were categorized as 'without companion' while those in marriage during the study period were classified as 'with companion'.

Educational status:

Educational status was assigned to each patient by the programme based on the highest level of education completed/attained or actively engaged in by the patient at the time of enrolment in the programme. In line with that, patients were categorized into: no formal education; primary; secondary and tertiary educational attainment. For further exploration, educational status of the patients was further categorised into: no formal education; primary education and beyond primary education.

Occupational status:

From the more than fifteen different occupations contained in the patients' records at the programme site, seven different occupational categories were derived and included: the civil servants - those in paid employment in the public sector including teachers, nurses, lecturers, police, military, medical practitioners and youth corps members. Those classified as students in the programme were mainly those who were either in the secondary or tertiary institutions. Drivers were those in commercial vehicle operations and included: the taxi drivers, bus drivers, long distance truck drivers (who were few in the study) and also commercial motorcycle operators. Traders included different areas of trading or business activity as well as petty trading. In the study, artisans were the group of persons in different forms of crafts and private informal sector jobs like carpenters, plumbers, hair dressers, tailors, welders, electricians, butchers, masons, auto-mechanics and vulcanizers. Farmers were another sizeable occupational group among the patients in the study. Other poorly defined groups like housewives, the unemployed, retirees, security guards, receptionists in private concerns, cleaners, clergy/pastors and sales girls/boys were all categorized in the study as 'others'. For further exploration, patients' occupational status was further categorised into: civil servants; business persons and others. The study site, however, did not document records on patients' ethnicity and religion as part of the socio-demographic variables and as such and those variables were therefore not included in this study.

3.5.2 Clinical characteristics

The main clinical characteristics in the cohort were the patients' blood pressure (BP) and patients' status of co-infection with tuberculosis.

Blood pressure (BP):

Patients' BP were initially recorded separately as systolic BP and diastolic BP. These were then further categorized as hypotensive or low BP; normotensive or normal BP or as hypertensive or high BP. In this study, a patient's blood pressure

was classified as hypotensive if both systolic and diastolic blood pressures (BPs) were less than 90/60 millimetres of mercury (mmHg) or if the numerator (the systolic BP) is <90 mmHg, irrespective of the value of the denominator (the diastolic BP); or where the denominator is <60 mmHg, irrespective of the value of the numerator. All values of BP between 90/60 mmHg and 139/89 mmHg (both values inclusive) were considered normotensive in the study. On the other hand, a patient's BP in the study was classified as being in the hypertensive category if it was of the value ≥140/90 mmHg or if the numerator is ≥140 mmHg, irrespective of the value of the denominator; or where the denominator is ≥90 mmHg, irrespective of the value of the numerator. From the foregoing, patients could be found to be hypertensive based on both the systolic and the diastolic BPs being above the upper limit of normalcy, that is, >139/89 mmHg or where only the systolic was above normal while the diastolic was normal. This is termed isolated systolic hypertension. On the other hand, situations arise whereby the systolic BP would be found to be within the normal limit while the diastolic BP would be above the normal limit of 89 mmHg. Patients with such BP records were classified as having isolated diastolic hypertension. The patient's blood pressure of interest in the study was the baseline blood pressure taken from the patients on the day of first clinic visit as part of baseline patient's clinical records.

Tuberculosis co-infection:

Decision on patients' co-infection with tuberculosis in the programme was determined based on results from three assessments: initial clinical assessment at presentation conducted by the attending clinicians; laboratory-based, three-sample sputum acid-fast bacilli test (AFB x 3) and radiological assessment by way of chest X-ray. A final decision based on the three results was then used to classify each patient as either co-infected or not co-infected with tuberculosis. Patients with co-infection were assigned "yes" while those without co-infection were assigned "no". Only the patients' baseline co-infection status was used in this study. Data on patients' adherence to HIV treatment was not recorded by study site as part of its programme database. Therefore, patients' adherence to treatment was not included in this study.

3.5.3 Virological and immunological characteristics

Immunological characteristics (denoted as the CD4 count, in cells/millilitre) and virological characteristics (denoted as RNA viral load, in copies/millilitre) were each measured and recorded both at baseline and then during patient's subsequent follow-up visits.

Immunological data:

The programme protocol in use by the centre makes use of both CD4 count and clinical state of patients in determining which patient is eligible for commencement of anti-retroviral treatment. Those records are used to grade HIV patients into four stages – I, II, III and IV – I being the earliest stage and IV being the most advanced stage of the infection commonly referred to as AIDS (Acquired Immune Deficiency Syndrome). The CD4 count corresponding to this advanced stage of

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HIV infection is 200 cells/ml or lower and forms a major criterion for commencement of HAART. Patients under care at the site therefore usually have their CD4 monitored from initial contact with the site until it gets down to 200 cell/ml. In situations where the initial measured CD4 count is as low as 200 cells/ml or even lower, the programme still does not see commencement of HAART as an emergency. Rather, some time is usually taken to gather all the other baseline clinical data in addition to two or more treatment adherence counselling sessions before commencing HAART. However, the CD4 count of interest in this study was the baseline value collected at commencement of treatment or just before commencement of HAART. Patients' CD4 count at the site is measured with: ©Partec Cyflow Version 2.4 (Germany).

In this study, baseline CD4 count was therefore defined as the record of patient's CD4 count that was taken on the day of commencement of anti-retroviral drugs or such measurement taken just before commencement of therapy. Treatment adherence, on the other hand, was defined as the sum total of the steps taken by the patient to comply with prescribed medications/drugs in terms of timing and doses as well as follow-up clinic visits.

Virological characteristics:

Of the entire RNA viral load measurements recorded in the programme database, only two of them were of importance to this study and were therefore extracted/obtained for this study. The two records were the baseline RNA viral load and the undetectable viral load. Baseline RNA viral load was in this study defined as the viral load measurement that was obtained on the day of commencement of anti-retroviral therapy or just before the commencement date. Undetectable RNA viral load, on the other hand, was defined as the critical value of RNA viral load at which the machine cannot detect any further value and that level of RNA viral load is 200 copies/ml. This undetectable level was the second RNA viral load of interest in this study. However, it was not the level per se, that was of interest in this study. Rather, the record of interest was the time taken (in weeks) to achieve that undetectable level starting from the date of commencement of HAART. In this study, time origin was defined as the date of commencement of HAART while the failure time or end-point was the date that an undetectable RNA viral load level (of 200 copies/ml) was reached. At the point when a patient achieved undetectable RNA viral load level of 200 copies/ml, the patient was deemed to have attained the main study outcome and no further viral load record was extracted for the patient from the database. This is the patient's "failure time", "death time" or study "end-point" which was recorded in weeks. For a patient who had been in the programme for twelve weeks or longer without achieving an undetectable viral load as at the time the study ended on May 31, 2010; or where a patient failed to attain undetectability before being lost to follow-up, that patient was deemed to have been censored. No entry would be made for that patient under the variable, 'time to achieve undetectable viral load'. Patients' RNA viral load at the study site is measured with: ©Applied Biosystem Model: Geneamp PCR System 9700 (Singapore).

Data schedule for CD4 count and RNA viral load count

From the date of commencement of HAART, a patient's CD4 count and RNA viral load data were measured every twelve weeks for the first twenty four weeks, and then every twenty four weeks thereafter. Extraction of these immuno-virologic data was therefore considered at the following periods of each subject's patient time:

i)   At baseline (inception), either on the day of commencement of therapy or just before that date, and at other specific time periods after commencement of therapy, as follows:

ii)   End of twelfth week (or third month) after commencement of HAART,

iii)   End of twenty fourth week (or sixth month) after commencement of HAART

iv)   Every twenty fourth week thereafter.

That is, in weeks, it is at such intervals as: 0, 12, 24, 48, 72, 96, and 120. For patients failing to achieve undetectable RNA viral load after the twenty four weeks, measurement intervals would remain twelve weekly until such a time patient's RNA viral load profile is re-evaluated for possible resistance to first-line anti-retroviral drugs.

The above-stated patients' socio-demographic and clinical data were extracted from the patient's clinic records with a proforma and each subject was identified by his/her specific code number (PepID) and his/her specific date of commencement of antiretroviral treatment. The socio-demographic characteristics and those clinical data (like tuberculosis co-infection or blood pressure) were each collected only once from baseline documentation and then later assessed for their contribution in predicting the attainment of the main outcome variable, 'time to achieve undetectable viral load'.

3.6 Data Analysis

Data analysis in this study was done using SPSS version 20 (© Copyright IBM Corporation USA, 2011) as the "ten-space" PepID codes could not be accommodated in other SPSS software versions lower than 20, making it impossible to use the PepID codes for differentiating individual patient records. Descriptive statistics was used to present categorical variables like sex, marital status, age groups, occupation and educational status using percentages/proportions, frequency tables and graphs while means, medians, standard deviations and range were used in presenting quantitative variables like age, blood pressure values, baseline CD4 count and baseline RNA viral load. A log transformation of the CD4 count and RNA viral load was done to substantially reduce the level of skewness of those data elements and also help in further exploration of the

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effects of those variables on the main study end-point. Bivariate analyses with Chi-Squared test and Pearson's correlation were also used to determine associations between TB status of patients and patients' socio-demographic and baseline clinical characteristics as well as use of Kaplan-Meier estimator to test the equality of survival distributions for the different levels of the covariates. Tests of hypothesis were based on maximum acceptable error of 5%.

Median time to achieve undetectable viral load: the Kaplan-Meier estimate/analysis

Kaplan-Meier survival analysis derived from the technique published in 1958 by Edward Kaplan and Paul Meier in the journal of American statistical association titled non-parametric estimation from incomplete observations (Cook, 2008; Rich et al, 2010). The Kaplan-Meier (K-M) estimate is the simplest way of computing the survival of members of a treatment sample over time in which some of those members did not reach the study end-point (Goel et al, 2010). It is a method that involves generating tables and plots of the survival or the hazard function for the event history data. According to Chan, itis the usual technique performed to analyze survival-time data (Chan, 2004). It is a kind of explanatory method for the time to event, where the time is considered as the most prominent variable (Lani J, 2009).

The Kaplan-Meier survival estimate is based on three key assumptions:

i) It is assumed that at any time patients who are censored have the same survival prospects as those who continued to be followed.

ii) It is assumed that the survival probabilities are the same for subjects recruited early and late in the study.

iii) It is also assumed that the event happens at the time specified. This creates problem in some conditions when the event would be detected at a regular examination

The censored cases are those cases in which the event of interest has not yet occurred. A censored observation is also a variable of interest in the analysis of survival data. In Kaplan-Meier survival analysis, time is considered as the continuous variable and the initial (first) time of the occurrence of the event is very important and needs to be clearly defined. In this study, the time variable was the "time to achieve undetectable viral load", recorded in weeks. The very first failure time is the time of interest, that is, the time in weeks it took the patient on drug treatment to reach the very first undetectable RNA viral load of 200 copies/ml for those that achieved it. Another variable of interest in Kaplan-Meier survival analysis is the status variable which defines the terminal event – whether the terminal event occurred or did not occur. It is usually a categorical variable. In this study, it was explained as the viral load status, with "1" as event achieved and "0" for censored observation or event not achieved.

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In this study also, both descriptive statistics and Kaplan-Meier estimate were used in estimating the survival probability of the cohort of patients, represented by the cohort's median time to achieve undetectable viral load after initiation of therapy, and the results were presented in tables and graphs - the Kaplan-Meier curves. Kaplan-Meier curves for survival and hazard functions as well as mean and median survival times with their percentiles were also generated for the different covariates in the study, including the socio-demographic variables, baseline clinical data, CD4 count and RNA viral load.

Log Rank test was used to test whether the difference between survival times among the categories in a covariate is statistically significant or not. That is, it was used to test the equality of survival distributions for the different levels of the covariates. The levels of significance, as p-values, obtained from the Log Rank (Mantel-Cox) Chi-Squared tests were used to determine which covariates were eligible for further (multivariate) analysis. P-values of ≤0.20 on Log Rank test were arbitrarily chosen for the covariates to qualify for inclusion for multivariate (Cox's regression) analysis[1].

The covariates found eligible were then fitted into a Cox's regression model for analysis and the ability of each of them to influence or predict the study's primary outcome was determined. In this study, the Kaplan-Meier estimator and the Log Rank (Mantel-Cox) test were used to construct and compare the survival probabilities of the different levels or categories.

Sensitivity analysis

At the UNTH Enugu programme site, the schedule for the measurement of RNA viral load is set/fixed at 0 week (baseline value), 12 weeks, 24 weeks, 48 weeks, 72 weeks, etc. That is, even if RNA viral load undetectability occurred earlier than the due RNA viral load measurement, the time to event for that patient would only be recorded as occurring on the date of that next measurement period. It would therefore appear that the recorded patients' time to end-point would be mostly over-estimated, at least mathematically. This violates a key assumption of the Kaplan-Meier estimate which pre-supposes that the event or end-point is achieved at the time it is specified through measurement to have occurred. To minimize this bias, a sensitivity analysis was done by creating assumed values for the individual patients' time to event. That is, it was assumed that each failure time actually occurred halfway in-between the observed time and the immediate previous RNA viral load measurement. To further increase the robustness of the estimates for time to study end-point, the Life Table method was also used to obtain the cohort's median time to undetectable RNA viral load.

[1] Unpublished lecture notes and personal guidance from Ayeni, O, Professor of Medical Statistics who handles the Survival Analysis course in the Department of Epidemiology and Medical Statistics, Faculty of Public Health, University of Ibadan, Ibadan.

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In summary, the Kaplan-Meier estimate is a clever method of statistical treatment of survival times which not only makes proper allowances for those observations that are censored, but also makes use of the information from these subjects up to the time when they are censored.

Proportion of the cohort of patients attaining undetectable RNA viral load at different time periods.

The individual time taken by each patient to achieve undetectable RNA viral load was categorized into: patients achieving the outcome within twelve (12) completed weeks, categorized as "1"; those that achieved it by (24) completed weeks, categorized as "2"; patients achieving the outcome by fifty-two (52) completed weeks or one year, categorized as "3" and then patients that achieved undetectability beyond fifty second week but within the twenty four months (one hundred and four weeks) of the study time, categorized as "4". Frequencies were thereafter generated from those four categories to determine the proportion of the patients that achieved the primary outcome at different periods within the study time of two years.

Prevalence of tuberculosis co-infection

The status of tuberculosis (TB) co-infection among the patients was recorded in the programme database as a dichotomous variable– yes or no – such that those with TB in the cohort were marked "yes" under TB and those without TB at entry into the treatment programme were marked "no". From that record, frequencies were generated to determine the proportion of the cohort who had co-infection with TB.

Predictors of time to achieve undetectable viral load: Cox's regression analysis and the concept of Cox's proportional hazards

In addition to the effect of treatment on the survival time of the patients in a survival study, it is also often important to determine which combination of potential explanatory variables affect the hazard function (survival time) as distinct from the effect of treatment. Whereas the Kaplan-Meier method with Log-Rank test is useful for comparing survival curves in two or more groups, Cox's regression (or proportional hazards regression) allows analyzing the effect of several risk factors on survival (http://www.medcalc.org/manual/cox_proportional_hazards.php). The procedure is therefore employed to examine the relationship between survival and one or more predictors, usually termed covariates. The basic model used by the Cox's regression procedure is the proportional hazards model. The model explores the relationship between the survival time and explanatory variables, that is, by typically examining the relationship of the survival distribution to covariates by showing which of those covariates

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significantly influences the survival time. The probability of the endpoint (death, or any other event of interest, e.g. recurrence of disease) is called the hazard, which is modelled as:

$$h_i(t) = h_0(t) \exp (\beta_1 x_{1i} + \beta_2 x_{2i} + \beta_3 x \beta_{3t} \ldots + \beta_k x_{ik})$$

Where;

$h_i(t)$ = hazard of death (or attaining the end-point) at time t,

$h_0(t)$ = the baseline hazard function at time t,

$\beta_1, \beta_2 \ldots$ = the coefficients,

$x_1, x_2, \ldots$ = explanatory variables (the covariates).

The hazard function is a measure of the potential for the event to occur at a particular time t, given that the event had not yet occurred. Larger values of the hazard function indicate greater potential for the event to occur. The Cox's proportional regression model assumes that the effects of the predictor variables are constant over time. Put differently, the model works on the principal assumption that during the modelling process using independent variables, the effects of the different variables on survival are constant over time. Furthermore, there should be a linear relationship between the endpoint and predictor variables. It is a semi-parametric model as it does not assume any particular form of distribution for survival times.

In this study, multivariate Cox's regression model was fitted to determine significance of patients' blood pressure status, status of co-infection with TB and occupation in predicting the time to undetectable viral load. Those three covariates were therefore potential confounders if those covariates were significantly associated with reduced time to achieve undetectable viral load.

3.7 Ethical considerations

Ethical approval for this study was obtained from the Institutional Ethical Review Committee of two institutions -the University College Hospital/University of Ibadan, Ibadan and the University of Nigeria Teaching Hospital (UNTH), Ituku-Ozalla, Enugu/University of Nigeria, Enugu Campus, Enugu. The study was considered a very sensitive one and so, specific steps were taken to ensure that the patients' confidentiality was maintained. Efforts were made to ensure that patients' records used for the study did not bear their names but only special identification codes

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– the PepID. As such, patients in the study were not identified by name. Information on them was therefore handled in a manner that posed no threat to their individual privacy.

The outcome of the study which will be made available to the APIN-Plus/Harvard programme team at the UNTH Enugu is expected to help them in identifying any existing gaps in the treatment programme, thereby serving the best interest of the patients. No harm was inflicted on the patients as there was no contact with any of them during the study period, since only their records were used for data extraction. Only subjects who had earlier signed the written ~~informed~~ consent form at entry into the treatment programme were included in the study, thereby ensuring their voluntariness in being involved in the study.

CHAPTER FOUR
RESULTS

4.1 Univariate analysis

4.1.1 *Socio-demographic characteristics*

A total of two thousand and forty one (2,041) patients were recruited into the UNTH Enugu APIN-Plus/Harvard PEPFAR programme within the study period spanning June 1, 2008 through May 31, 2010. Of that number, eight hundred and ninety (890) patients met the inclusion criteria for the study and were therefore included in the study. The excluded one thousand, one hundred and fifty-one (1,151) patients were those transferred into the UNTH Enugu site for purposes of continuing care either from other APIN-Plus/Harvard PEPFAR sites or other funded anti-retroviral treatment programmes or even those patients who had used anti-retroviral drugs from sources other than funded programmes. Such patients were therefore considered not to be anti-retroviral treatment naive at the time of joining the UNTH Enugu treatment site and as such not eligible for inclusion in this study.

Out of the eight hundred and ninety patients that met the study's inclusion criteria, only eight hundred (800) of them were eventually included for analysis. Ninety (90) other patients either had their key variables missing or had variables that were essentially invalid and therefore unusable.

Age

Of the 800 patients whose records were analyzed in this study, the youngest of them was aged 15 years while the eldest was 74 years with a mean age of 37.6 ± 9.8 (95% CI; 36.9 − 38.3). As much as 64.8% (562) of them were females. Also, 25% of the patients were aged 31 years or younger, 50% of them were aged 36 years or younger, while 75% of them were aged 43 years or younger. Majority of the patients were in the 25 − 34 year age group, constituting 36.5% (292) of the patients while those aged above 64 years were the least, constituting only 2% (16) of the cohort. On further categorization of age into three groups based on working age or age of active economic engagement, it was found that 91.5% (732) of the patients accessing care at the centre were aged between 25 and 60 years. The elderly - above 60 years of age - were only 2.6% (21) while the young persons aged below 25 years were only 5.9% (47). Further grouping the cohort

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of patients into two age categories of either 38 years or younger or above 38 years, it was found that the young population below forty were in the majority with 58% (464) of the patients. This is illustrated in Figure1 below.
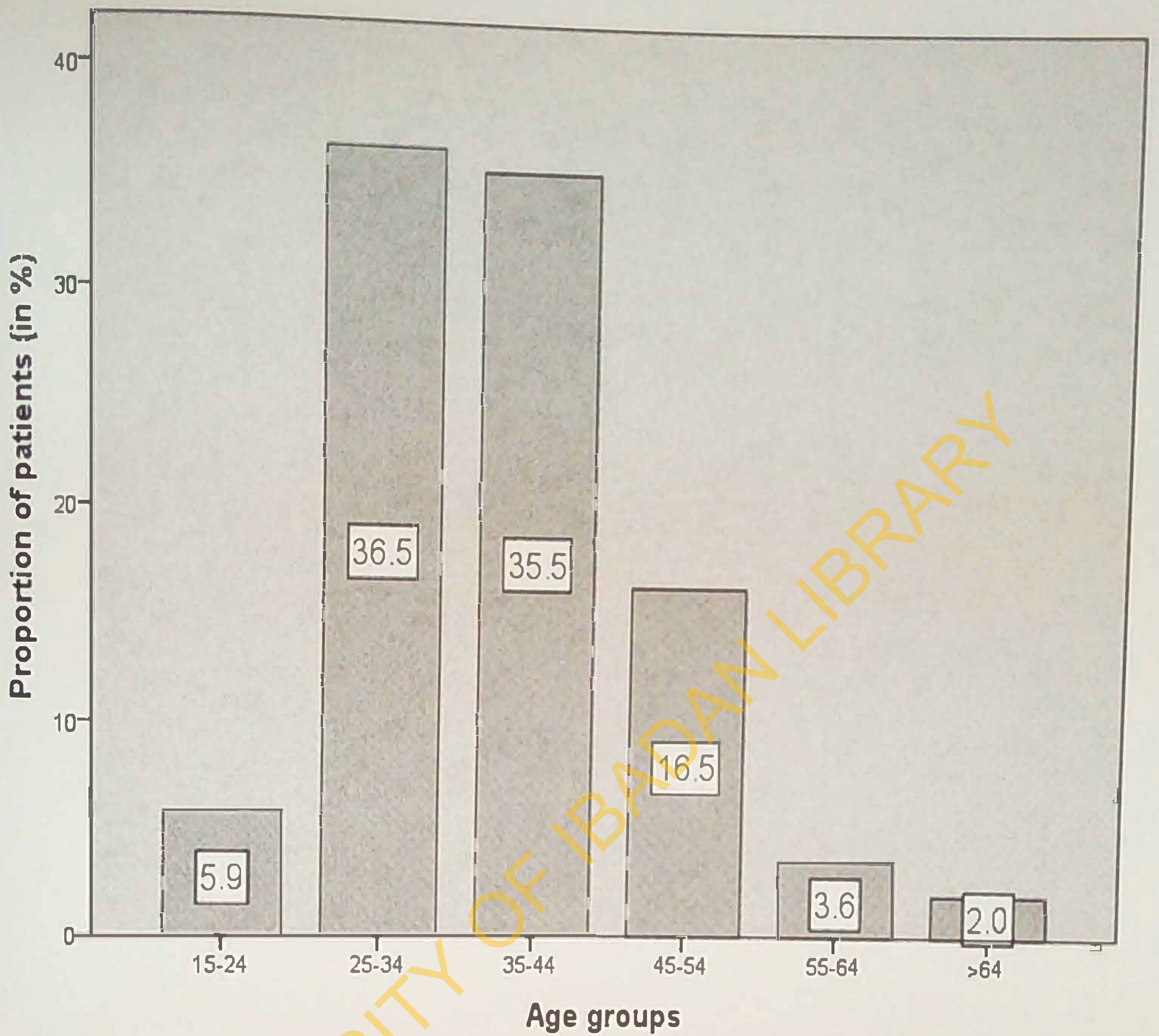
Fig. 1: Distribution of the patients according to age groups

Sex

The cohort was comprised of more females than males, with females accounting for 64.5% (516) of the patients.

Marital status

Majority of the patients were married, accounting for 45% (360) of the patients while those who were divorced were the least with only 1.4% (11) of patients in that category. On further grouping of the cohort in terms of whether a patient was in marriage or not, it was found that those who had no companionship were in the majority with 55% (440) of the patients. This is illustrated in Figure 2 below.
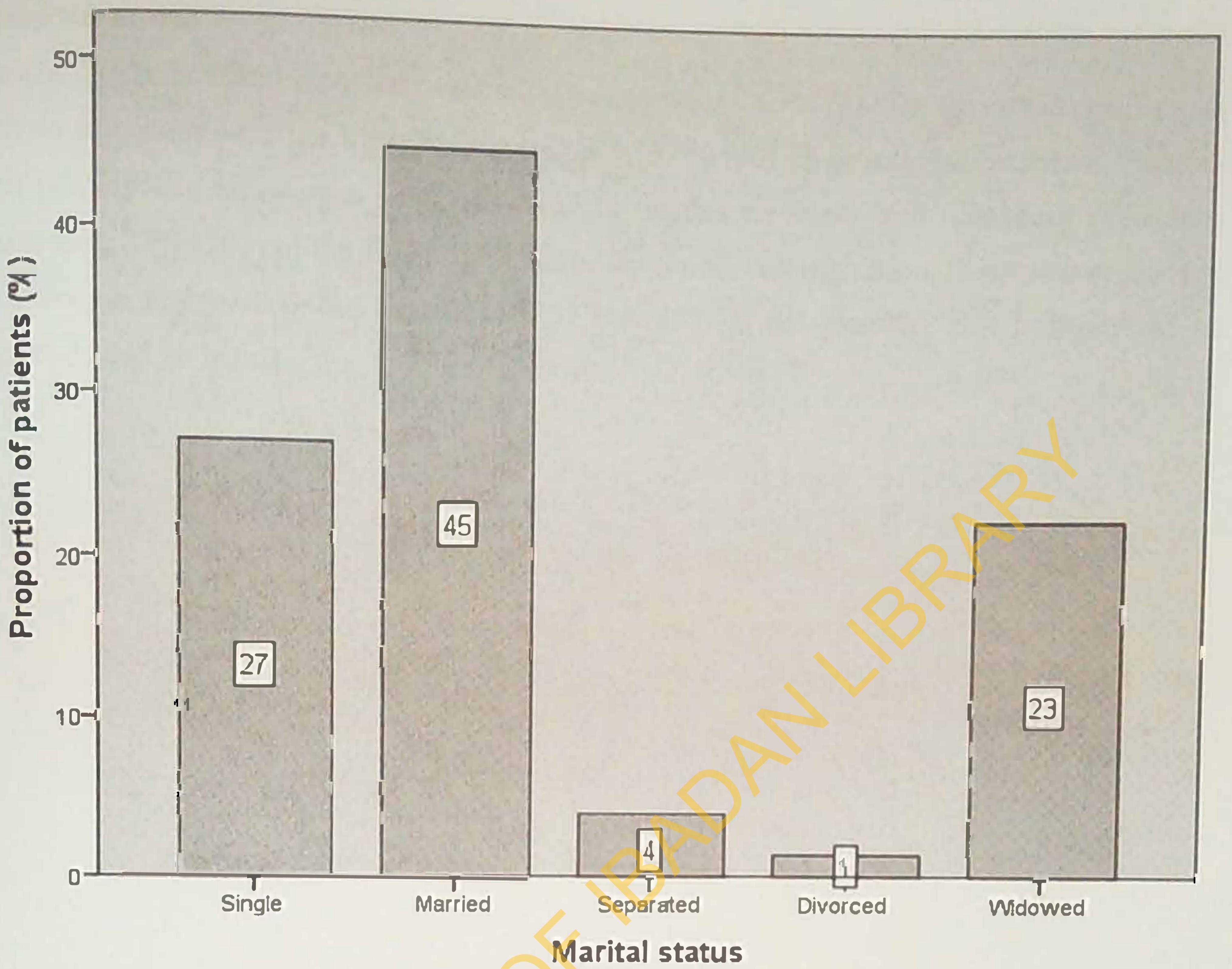
Fig. 2: Distribution of patients according to marital status

Education

Most of the patients had secondary education, constituting 38.9% (311) of the cohort while those with no education were the least, constituting only 5.0% (40) of patients in the cohort. Patients with primary education were nearly as large in number as those with secondary education, constituting 37% (303) of the cohort of patients. On further categorization, those with secondary education or higher were the largest with 57.1% (457) of the patients. This is illustrated in Figures 3a and 3b below.
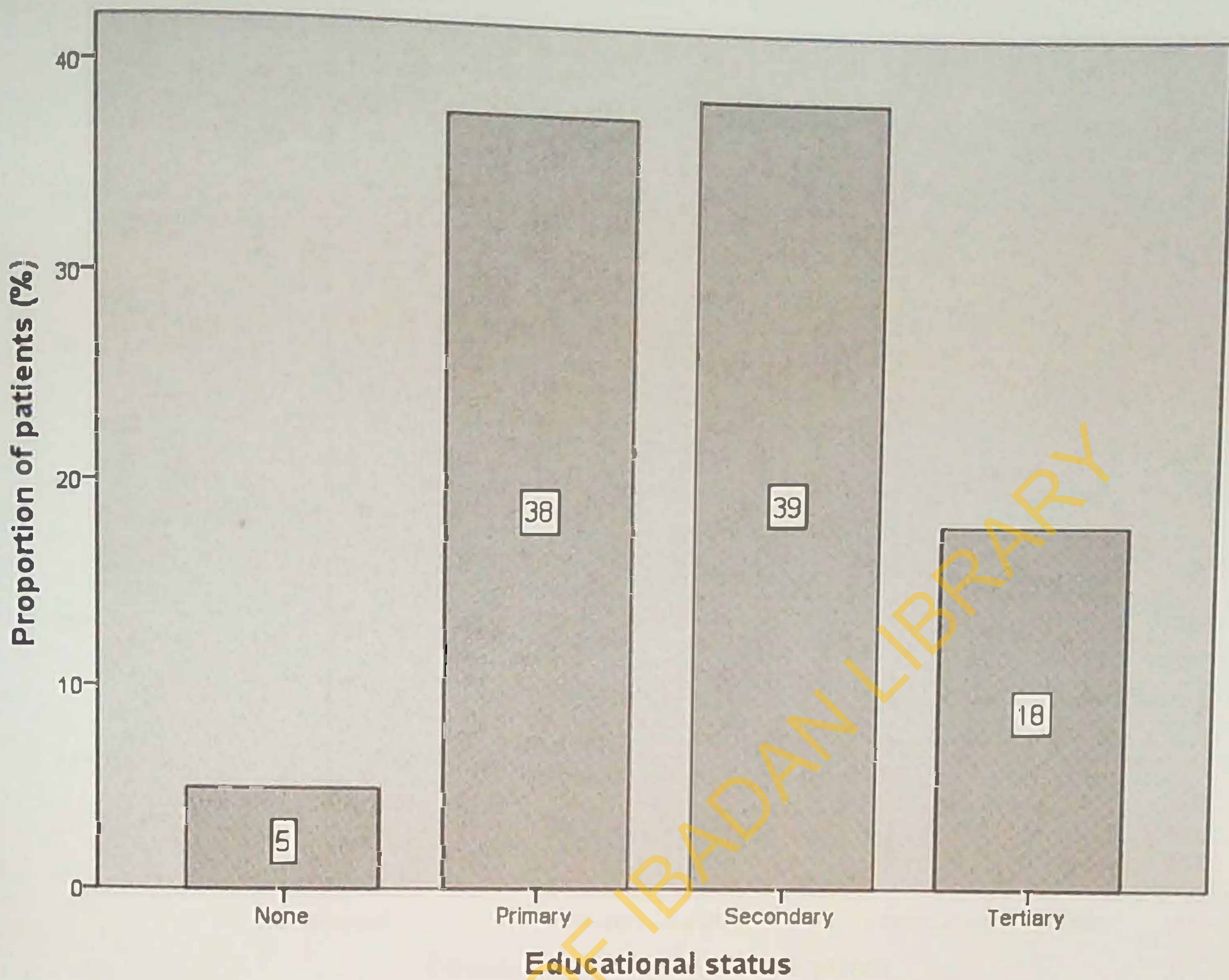
Fig. 3a: Distribution of patients according to educational status
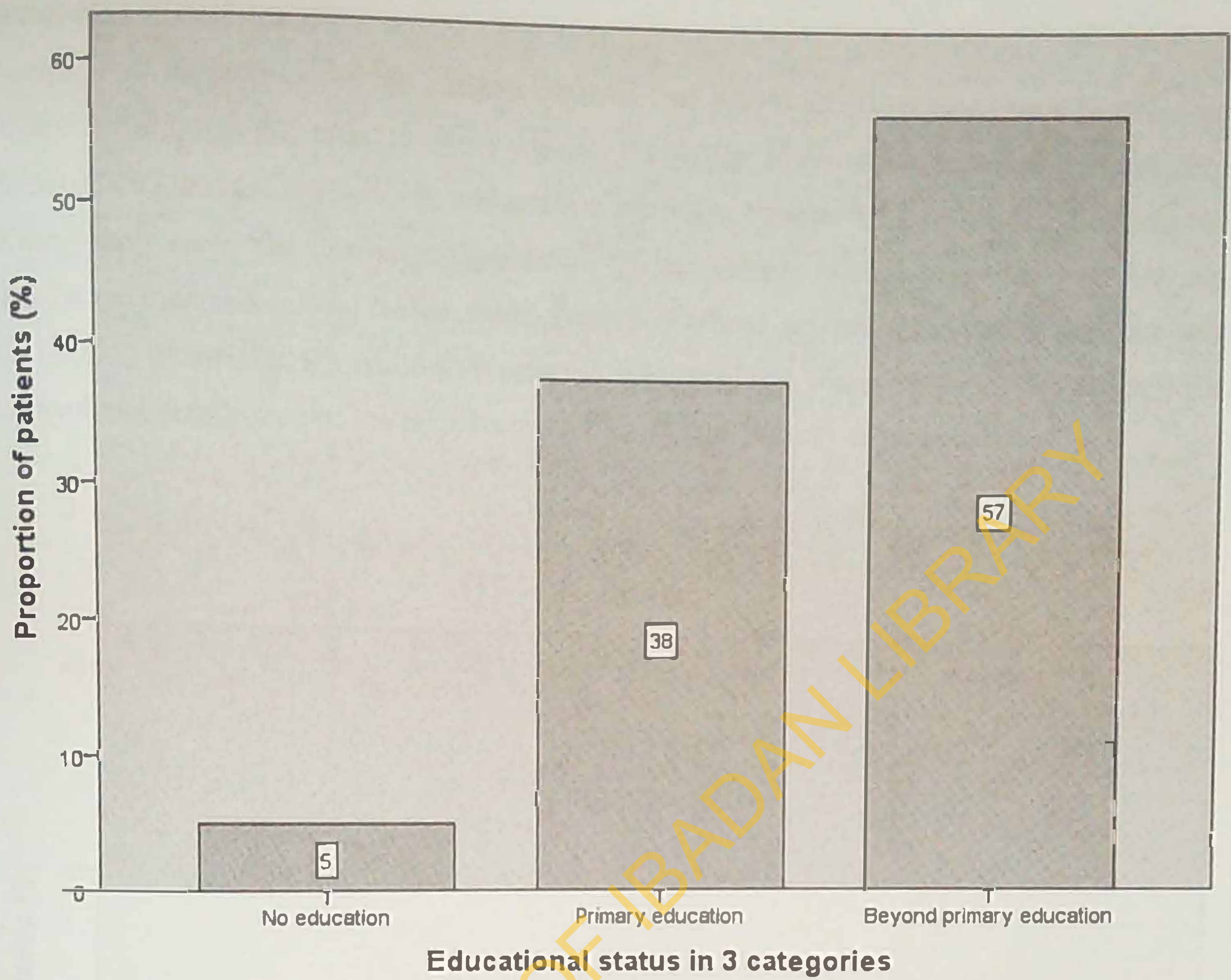
Fig. 3b: Patients' educational status in three categories

Occupation

Occupational distribution of the patients showed that 34.3% (274) of them were traders, with drivers constituting the least category among the major occupations with only 58 members (7.3%) of the cohort. Civil servants and artisans were also sizeable with 12.9% (103) of members of the cohort each. On further categorization of the patients into 3 groups of civil servants, business persons and others, it was found that the business persons were in the majority with 66.5% (532) of the patients while civil servants were the least with 12.9% (103) of the patients. Occupational distribution of the patients is as illustrated in Figures 4a and 4b below.
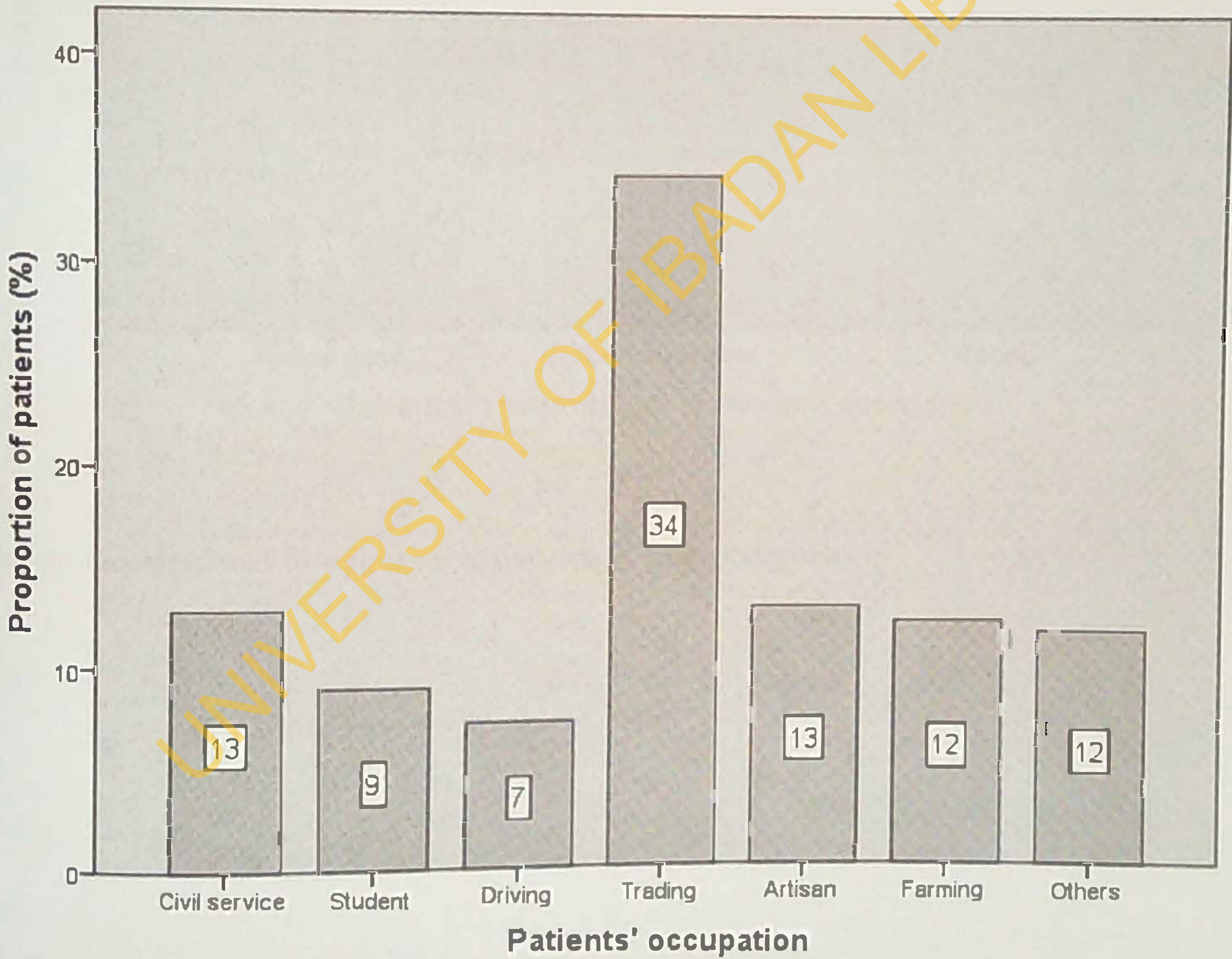
Fig. 4a: Occupational distribution of the patients

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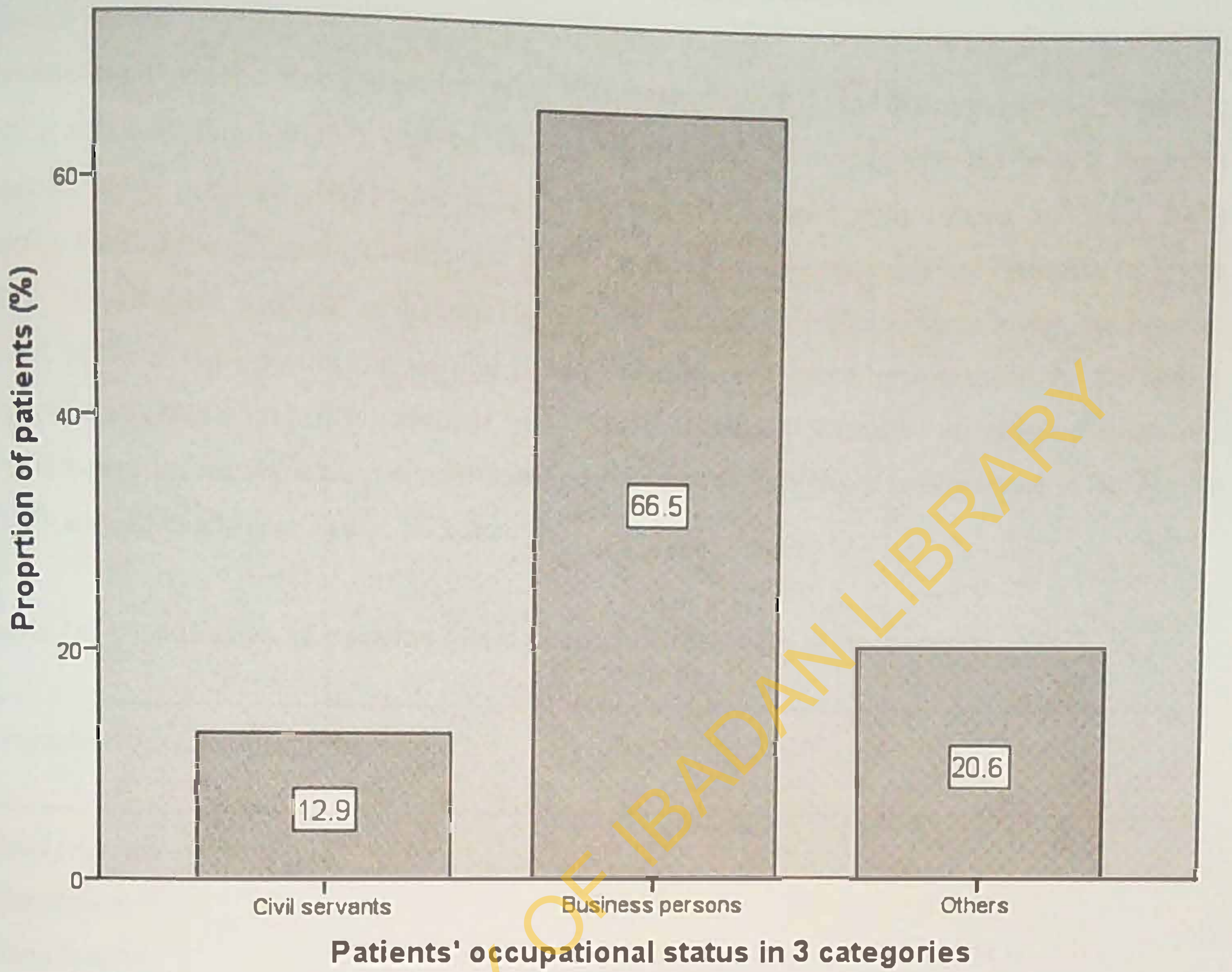
Fig. 4b: Occupational distribution of patients in three categories

4.1.2 Baseline clinical characteristics, CD4 count and RNA viral load

Blood pressure

The minimum systolic blood pressure (sBP) was 80 mmHg while the maximum was 190 mmHg with a mean of 114.5 ± 13.5 mmHg. On the other hand, 50 mmHg was the lowest recorded diastolic blood pressure (dBP) while 120 mmHg was the highest, with a mean of 73.4 ± 10.1) mmHg. Half of the patients had sBP that was 110mmHg or lower and dBP of 70mmHg or lower while 75% of them had sBP of 120mmHg or lower and dBP of 80mmHg or lower. As high as 84.9% (679) of the patients had normal BP while 12.1% (97) were hypertensive. In this study, hypertension was not differentiated into double, isolated systolic or isolated diastolic hypertention. Information on patients' baseline BP records and status is illustrated in Tables 1a and 1b as well as Figures 5a and 5b below.

Table 1a: Distribution of baseline blood pressure (BP) status of the patients

Characteristics	Frequency (n=800)	Proportion (%)
Blood pressure status		
Hypotensive	24	3.0
Normotensive	679	84.9
Hypertensive	97	12.1

Table 1b: Distribution of patients' baseline BP records (in mmHg)

Characteristics	Minimum	Maximum	Mean	Standard Deviation
Systolic BP	80	190	114.5	13.5
Diastolic BP	50	120	73.4	10.1
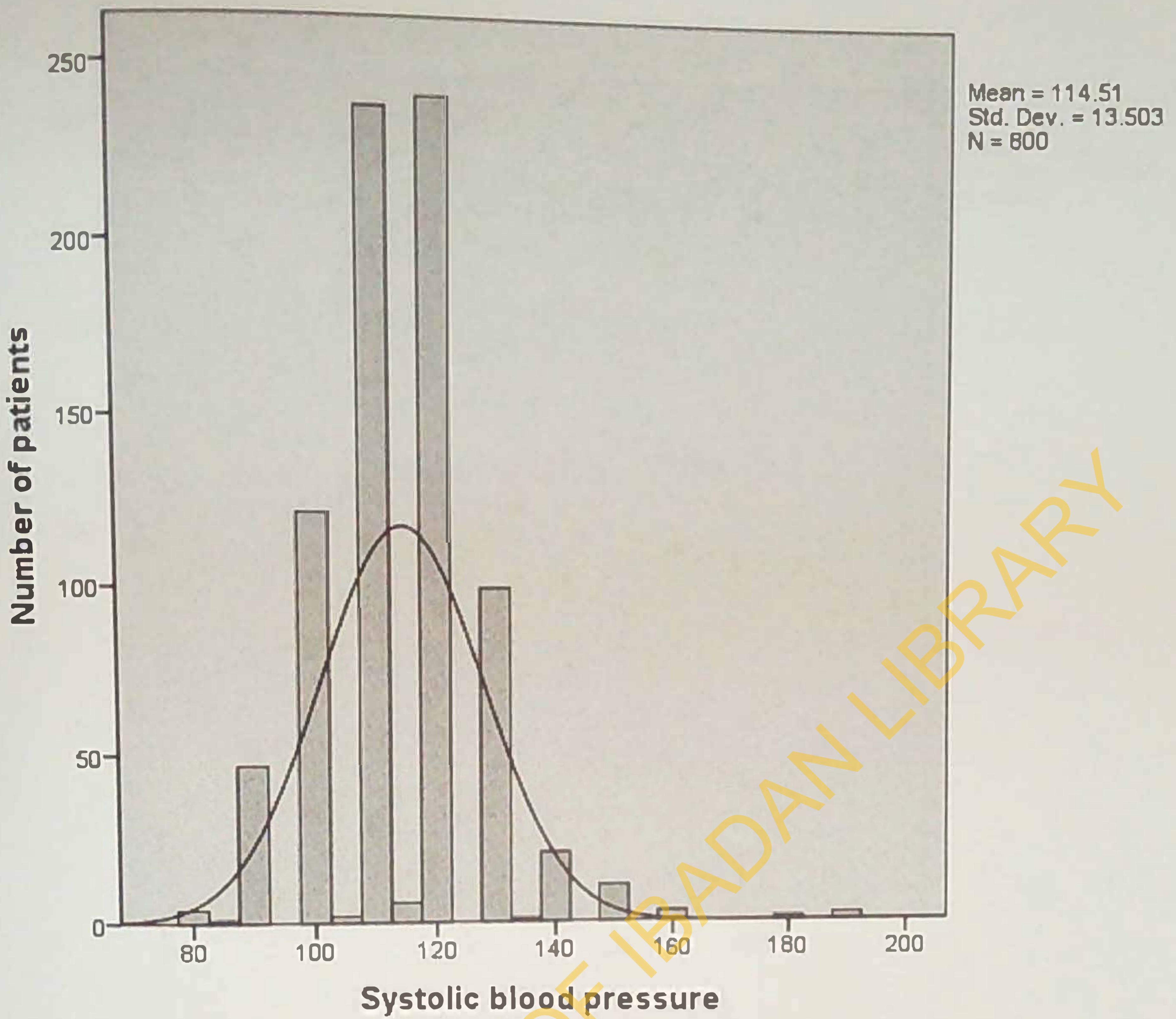
Fig. 5a: Distribution of systolic BP among the patients

Mean = 73.4
Std. Dev. = 10.094
N = 800
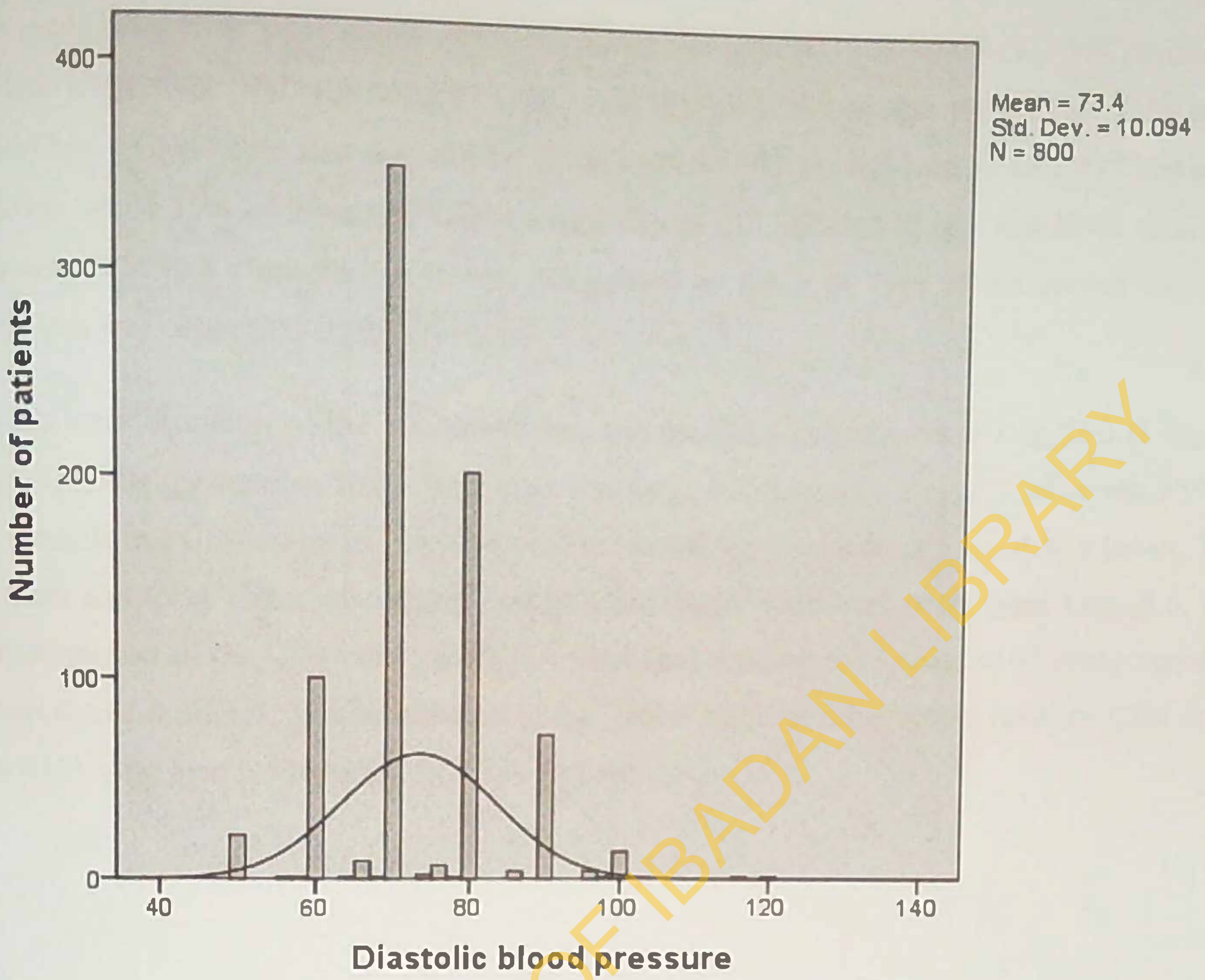
Fig.5b: Distribution of diastolic BP among the patients

CD4 count and RNA viral load

The median baseline CD4 count recorded among the patients was 150.5 (9 – 992) while the median RNA viral load was 153,007 (355 – 26,761,862). It was also shown that 25% of the cohort had a CD4 count that was at least 86 cells/ml and RNA viral load of 46,790.5 copies/ml or lower while 75% of them had CD4 count that was 211 cells/ml or less and RNA viral load that was 385230.8 copies/ml or lower. Altogether, as much as 71% of the cohort had CD4 count that was either 200 cells/ml or less.

On log transformation of the two covariates, the median CD4 count was $Log_{10}2.2$ ($Log_{10}1$ – $Log_{10}3$) while the median RNA viral load was $Log_{10}5.2$ ($Log_{10}5$ – $Log_{10}7$). Whereas 25% of the patients had CD4 count of $Log_{10}1.9$ or lower and RNA viral load of $Log_{10}4.8$ or lower, 75% of them had CD4 count of at least $Log_{10}2.3$ and RNA viral load of at least $Log_{10}5.6$. Log transformation of the CD4 count and RNA viral load reduced the skewness of those variables to less than 1.0 (unity). The distribution of the 'plain' and Log-transformed baseline CD4 count and RNA viral load is illustrated in Table 2below.

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Table 2: Distribution of baseline CD4 count and RNA viral load

Statistics	CD4 count (cells/ml)	RNA viral load(copies/ml)
Minimum	9	355
Maximum	992	26,762,217
Median	150.5	153,007
Range	983	26,761,862
Skewness	1.97	18.26
25%	86	46,790.5
75%	211	385,230.8

	CD4count (Log_{10} cells/ml)	RNA VL (Log_{10} copies/ml)
Minimum	1	3
Maximum	3	7
Median	2.2	5.2
Range	2	5
Skewness	-0.8	- 0.6
25%	1.9	4.8
75%	2.3	5.6

4.1.3 Distribution of the median time to achieve undetectable viral load among the patients

A total of 634 (79.3%) of the patients achieved undetectable viral load within the one hundred and four weeks (or twenty four months) study time, while the other 166 (20.7%) were censored. For those 634 patients that achieved undetectable RNA viral load, frequency distribution showed a median time of 12.5 weeks (4 – 80 weeks). This is illustrated in Table 3 below.

Table 3: Frequency showing median time to undetectable viral load among those patients that achieved it

Statistics	Time (in weeks)
Minimum	4
Maximum	80
Median	12.5
Range	76
Skewness	2.1

4.1.4 Proportion of the patients achieving undetectable viral load at different time periods

Findings from the study showed that a total of 79.3% (634) of the patients in the study achieved undetectable viral load during the study time of two years (or twenty four months), leaving 20.7% (166) of them as censored observations. Out of those that achieved the study end-point, 47.9% (304) of them achieved it within the first twelve weeks of commencement of treatment while additional 31.9% (202) of the cohort achieved undetectability by the twenty fourth week. That is, of those that achieved the study end-point (undetectable RNA viral load) within the study time of 104 weeks, 79.8% (506) 0f them had achieved it by the twenty fourth week (or sixth month) of commencement of treatment. However, by the fifty second week, that is, one year after commencement of treatment, an additional 19.4% (123) of the patients had attained undetectable RNA viral load.

Altogether, among the 79.3% (634) of the cohort that achieved undetectable RNA viral load within the study time of two years, 79.8% (506) of that number achieved it within 24 weeks or 6 months of commencing anti-retroviral drugs while 99.2% (629) of them achieved it by the fifty second week or one year after commencement of medications. In other words, 629 (78.6%) of the entire cohort of patients achieved an undetectable RNA viral load within the study time of two years and of the entire number that made that achievement within two years, 99.2% of them made it within one year. The information is illustrated in Table 4 below.

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Table 4: Proportion of the patients achieving UVL at different time periods among those that achieved the end-point

Time (in weeks)	Frequency (n=634)	Proportion (%)	Cumulative proportion (%)
12	304	47.9	47.9
24	202	31.9	79.8
52	123	19.4	99.2
>52	5	0.8	100

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4.1.5 Prevalence of co-infection with tuberculosis among the patient

Frequency distribution showed that only 8.1% (65) of the patients in the treatment cohort had co-infection with tuberculosis, as illustrated in Table 5 below.

Table 5: Prevalence of tuberculosis (TB) co-infection among the cohort of patients

Status of co-infection (Yes or No)	Frequency (n=800)	Proportion (%)
Yes	65	8.1
No	735	91.9

4.2 Bivariate analysis

4.2.1 Factors associated with TB status of patients

Significant association was observed between patients' gender and co-infection with tuberculosis (p = 0.007). Further analysis showed that the odds of co-infection with TB among male patients were about twice that of the female patients (OR; 1.99). However, marital status, age (both in 6, 3 and 2 categories), occupation and educational status of patients did not show any significant association with patients' co-infection status. Significant negative correlation was observed between Log-transformed baseline RNA viral load and status of patients' co-infection with TB (p = 0.045), but no significant correlation was found between Log-transformed CD4 count and TB co-infection (P = 0.60). Associations between patients' TB status on one hand and other covariates on the other hand are illustrated in Table 6 below.

Table 6: Chi-Square tests showing associations between patients' TB co-infection status with covariates (n=800)

Outcome measure (number with/without TB co-infection)

Yes	No	Total	p-value
Exposure factors			
*(Gender)*Male 33	251	284	
Female 32	484	516	0.007
(Marital status)			
With companionship 28	332	360	
No companion 37	403	44	0.75
(Occupation)			
Civil servants 9	94	103	
Business persons 45	487	532	0.74
Others 11	154	165	
(Age, in years)			
15 – 38 42	425	467	
>38 23	310	333	0.18

2.2 Kaplan-Meier estimate of time to undetectable RNA viral load

Using Kaplan – Meier estimate, the median time to achieve undetectable RNA viral load by the cohort of patients was 12.5 weeks (CI = 12.4 – 12.6) while the mean time was 17.1 weeks (CI = 16.4 – 17.8). These are illustrated in Tables 7a, 7b and Figure 6 below.

Table 7a: Overall mean and median survival times from Kaplan-Meier analysis

Estimate (in weeks)	95% Confidence interval (in weeks)
Median 12.5	12.4 -12.6
Mean 17.3	16.6 – 18.1

Table 7b: Overall mean and median survival times in quartiles/percentiles

Percentiles (%)	Estimate (in weeks)
25	23.0
50	12.5
75	12.0
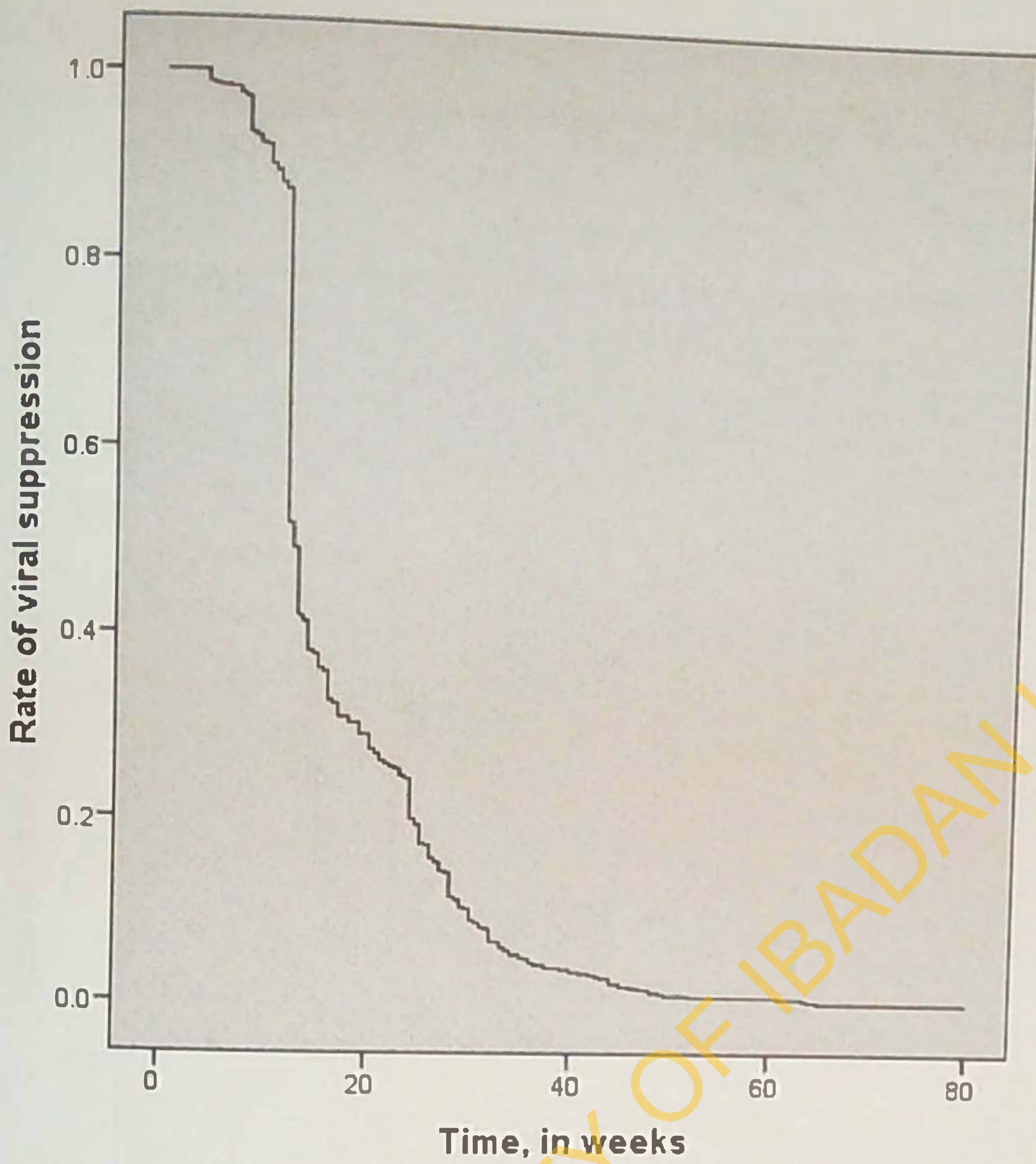
Fig. 6: Kaplan-Meier survival plot with no specified factor variable

The survival curve above was used to depict the shape of a typical survival plot from the cohort of patients involved in the study, without particular reference to any independent variable.

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The Kaplan-Meier survival plots for the different covariates with their accompanying Log Rank (Mantel-Cox) Chi-Square tests are illustrated in Figures 7a – 7i below.
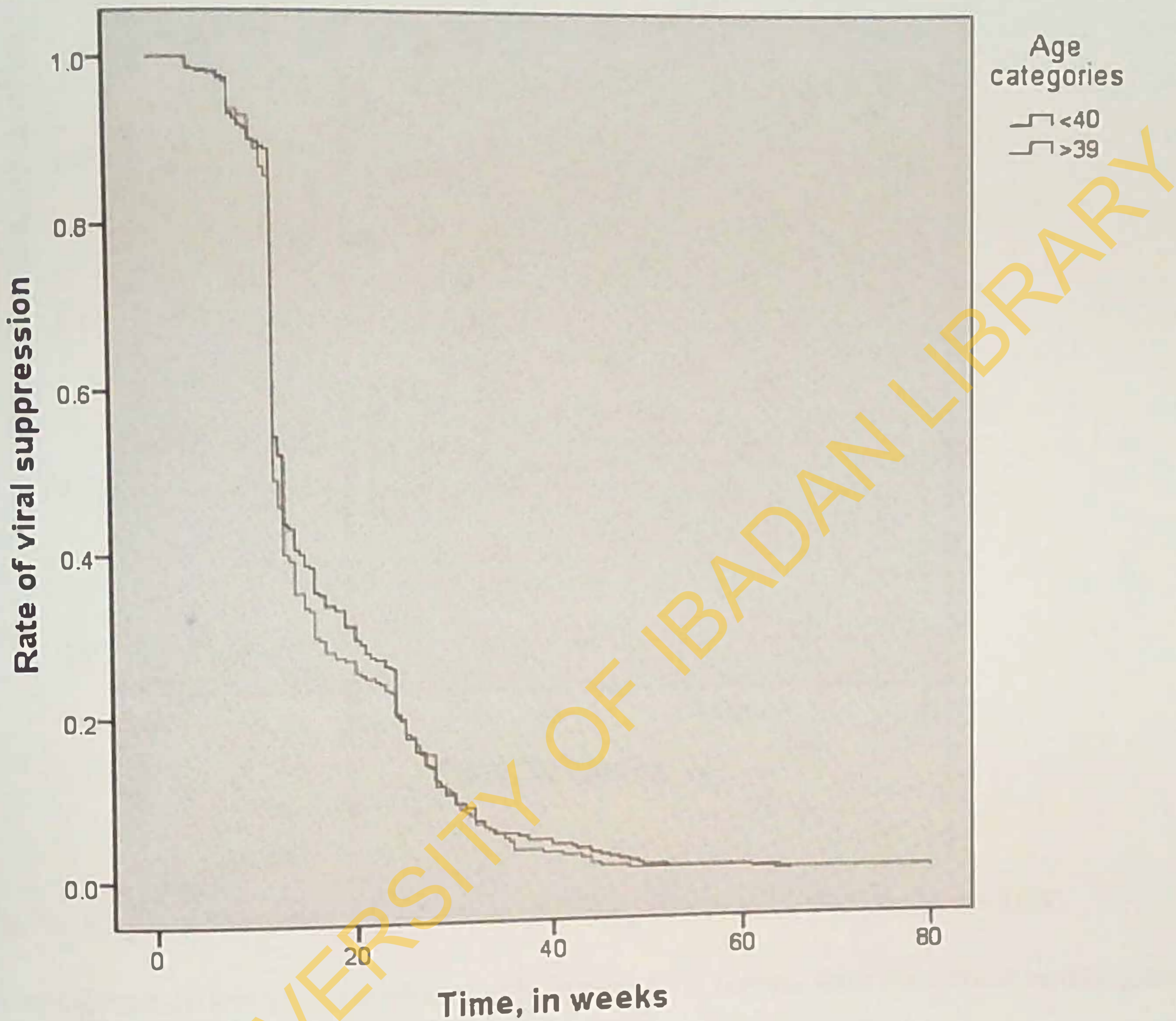
Fig.7a: Kaplan-Meier survival curve for patients' AGE GROUPS

On visual comparison of the two survival curves, the curves crossed each other and no observable significant difference is evident from the two curves, with the Log Rank (Mantel-Cox) Chi-Square test showing p-value =0.36.

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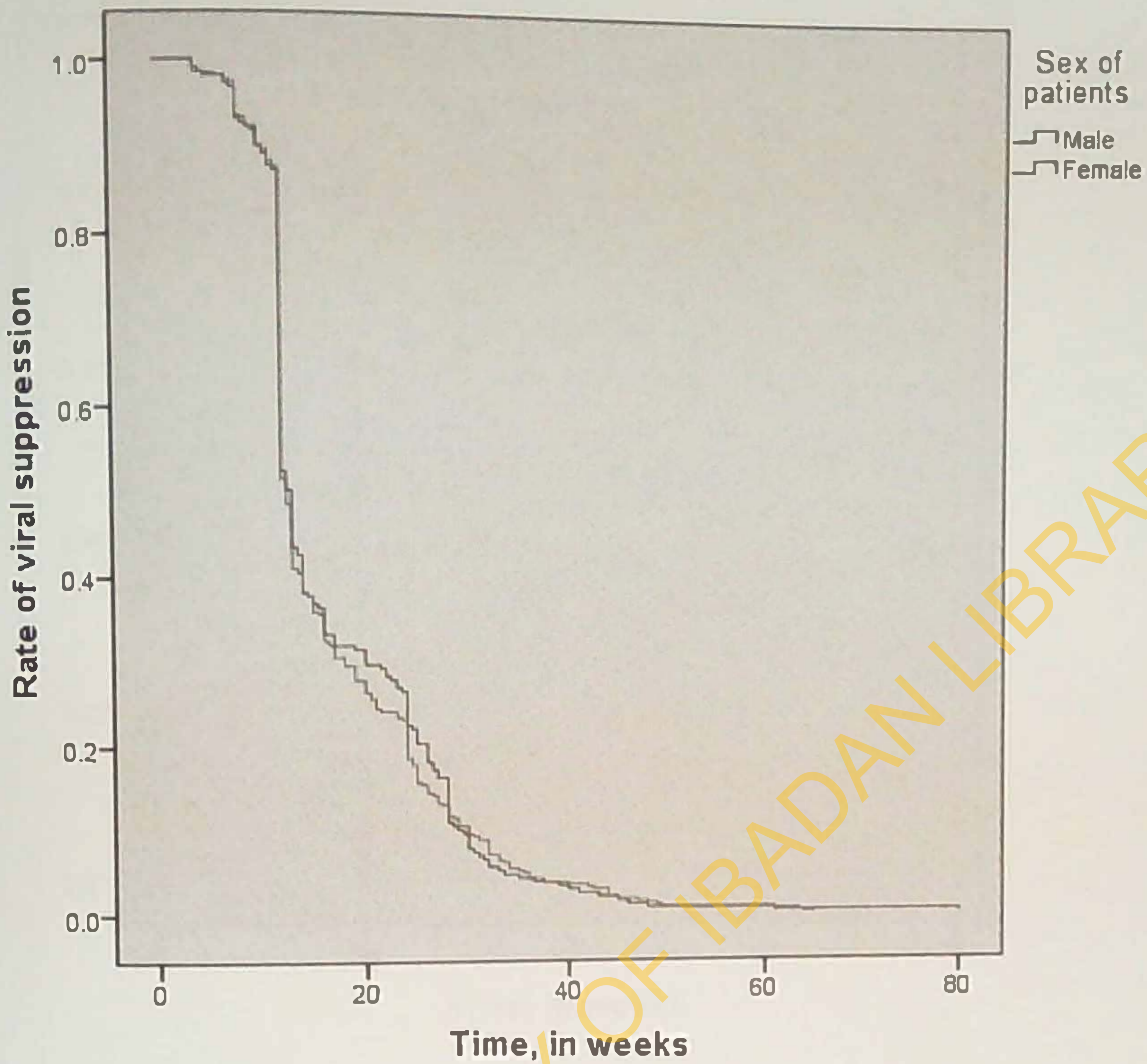
Fig.7b: Kaplan-Meier survival curve showing differences for patients' SEX

No consistent difference was observed between the 2 curves, with the curves crossing each other and a p-value = 0.75 on Log Rank test.
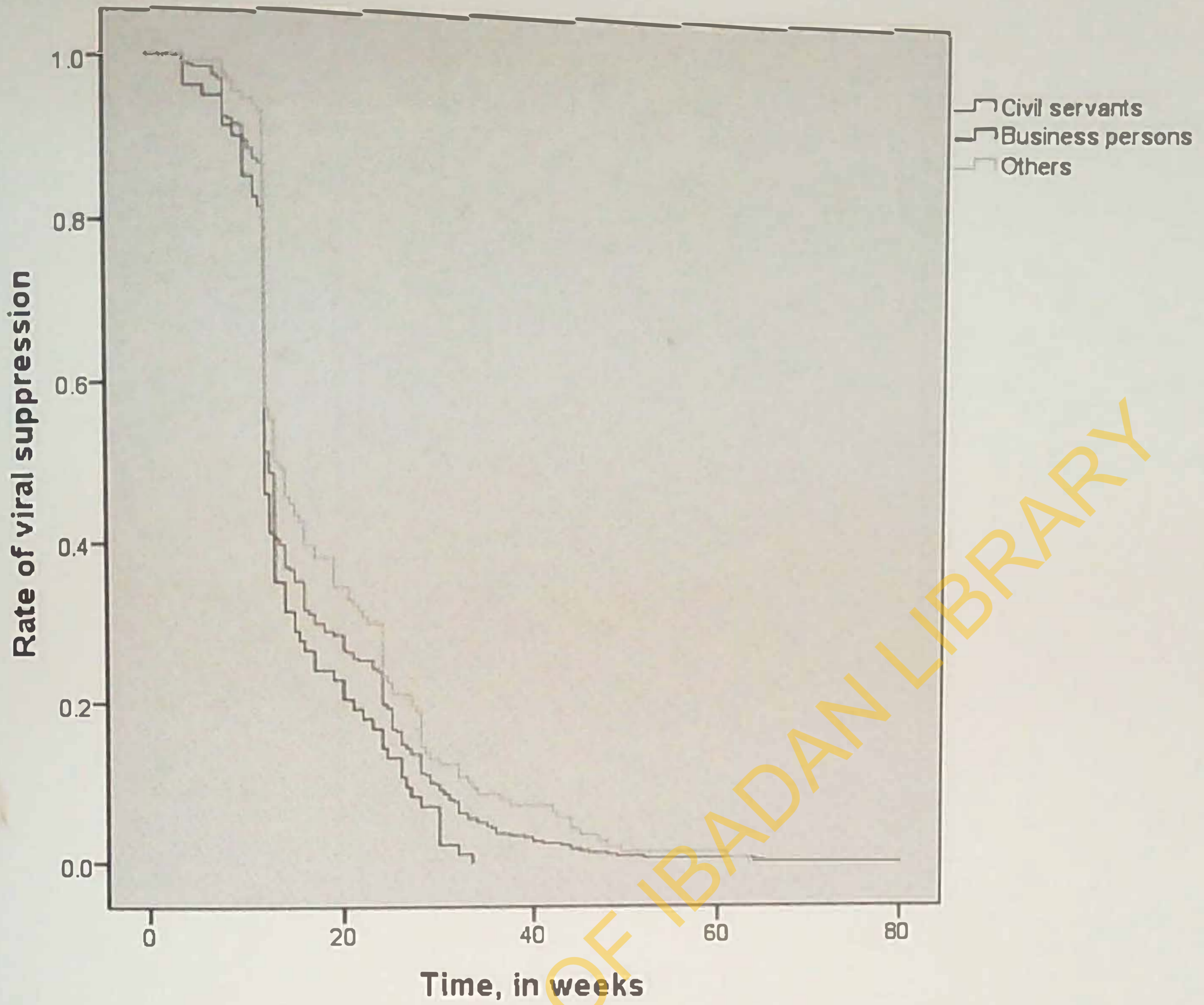
Fig. 7c: Kaplan-Meier survival curve for differences in patients' OCCUPATIONS

Visual comparison indicated a faster time to event for civil servants especially at the origin and tails of the curve, with a p-value = 0.005 on Log Rank test.
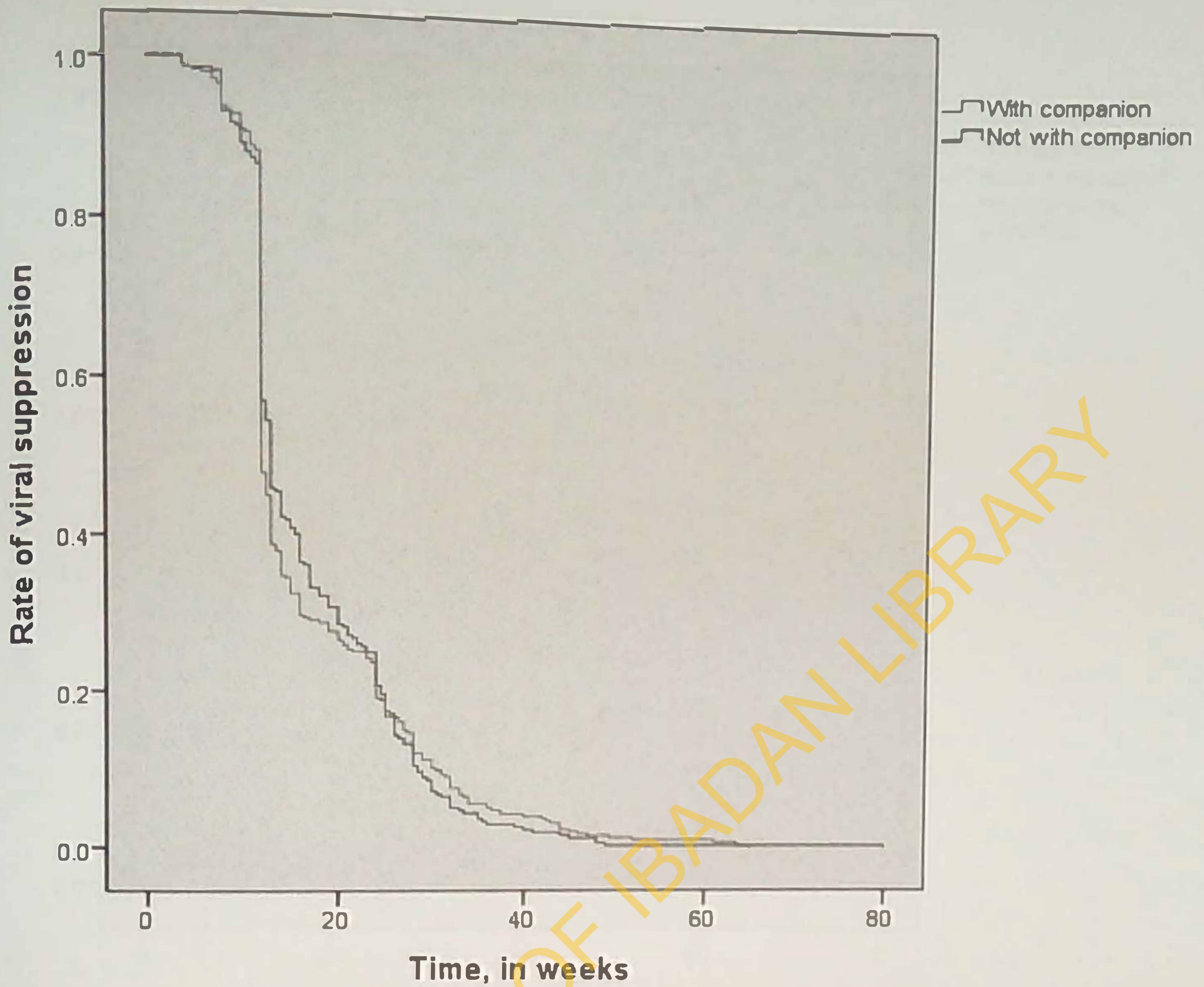
Fig. 7d: Kaplan-Meier curve for differences in patients' MARITAL STATUS

There appeared not to be any consistent difference in the shape of the two curves, with Log Rank (Mantel-Cox) Chi-Square test showing a p-value = 0.98.
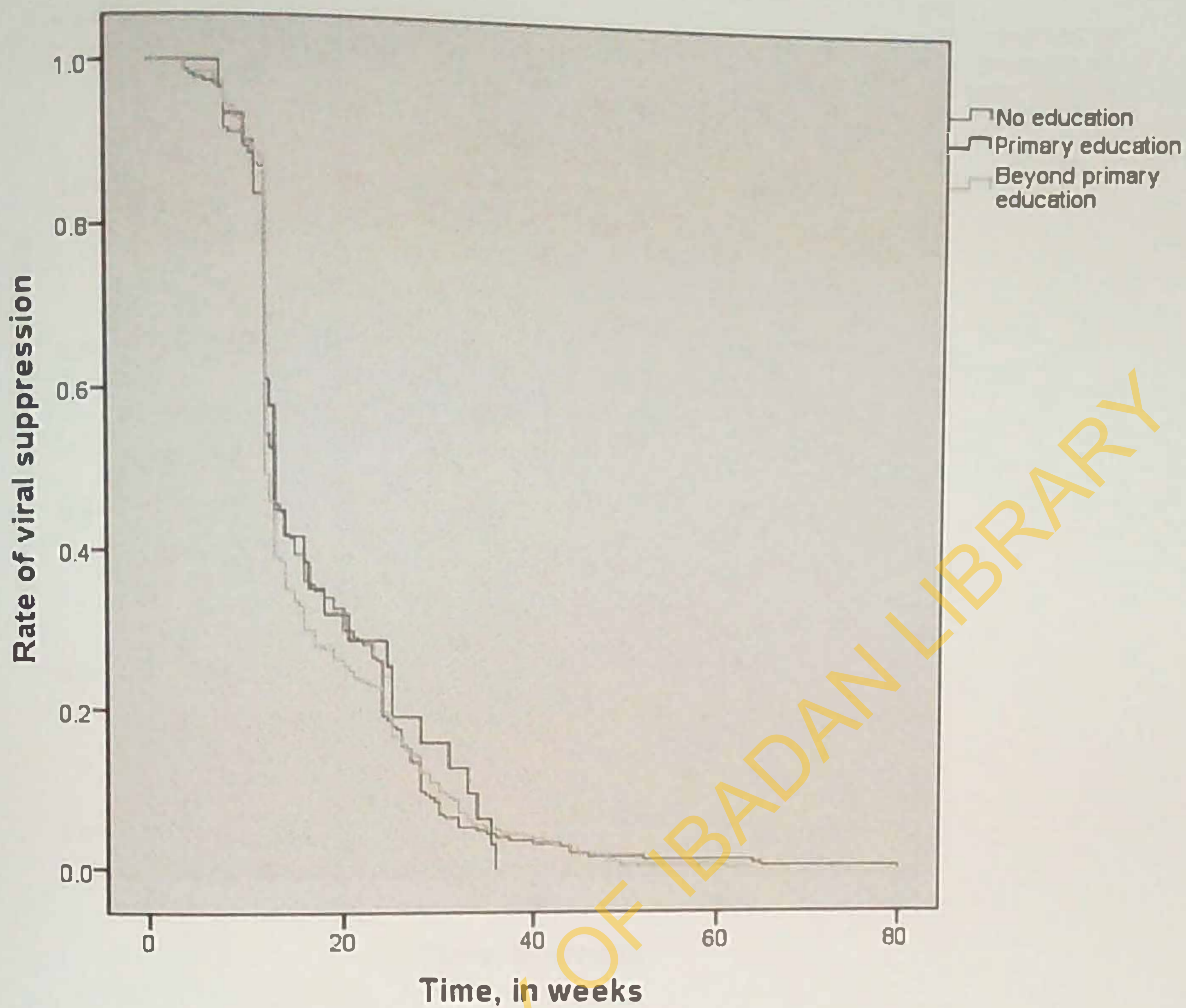
Fig.7e: Kaplan-Meier survival curve for patients' EDUCATIONAL STATUS

No remarkable difference was observed among the three categories of education in the plot, with Log Rank test giving a p-value = 0.81.

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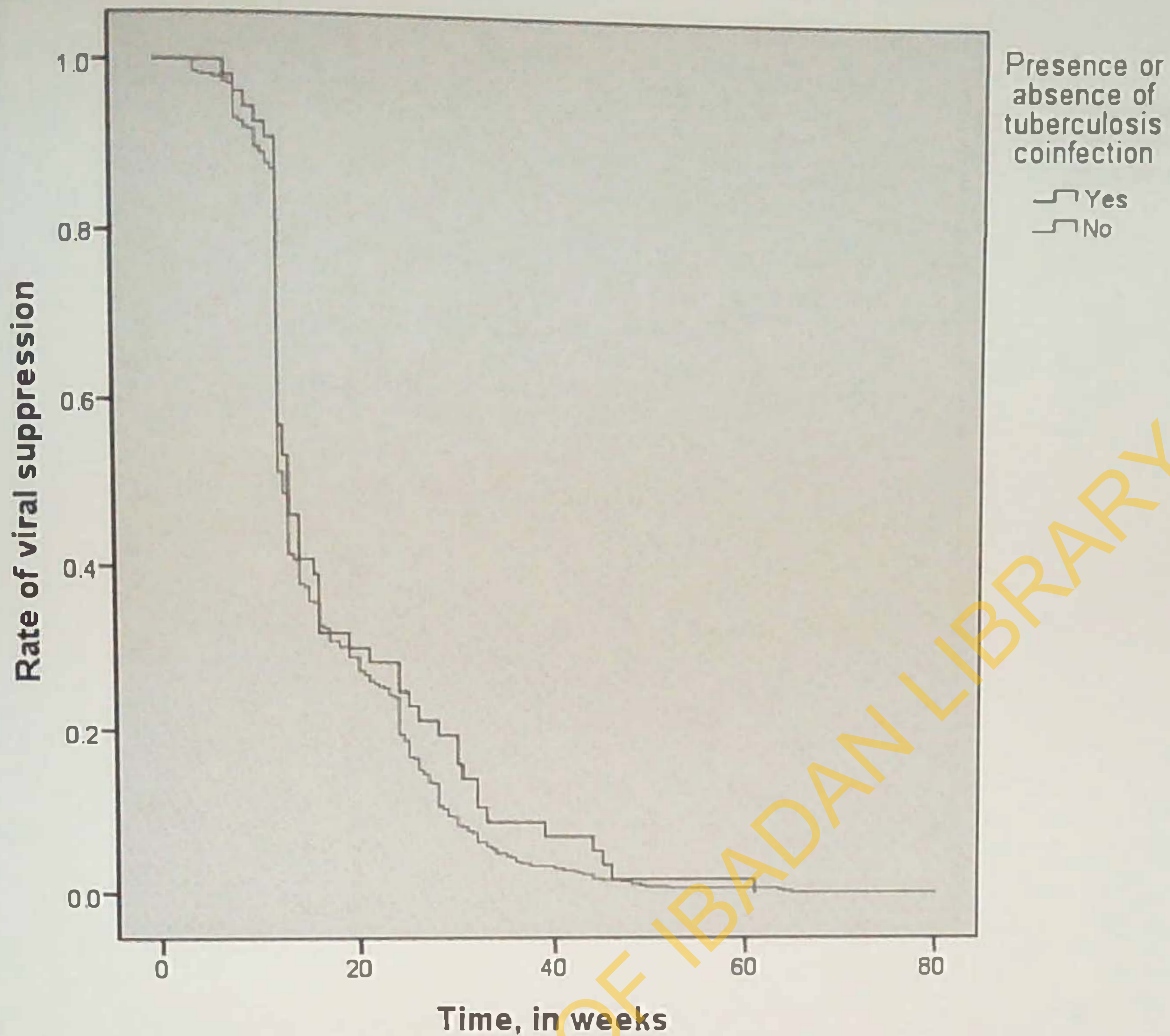
Fig.7f: Kaplan-Meier survival curve showing differences for patients' TB CO-INFECTION STATUS

The curves crossed each other at about the 15 weeks point, although indicating that those without TB co-infection demonstrated a faster time towards the head and tail of the two curves. P-value on Log Rank test = 0.19
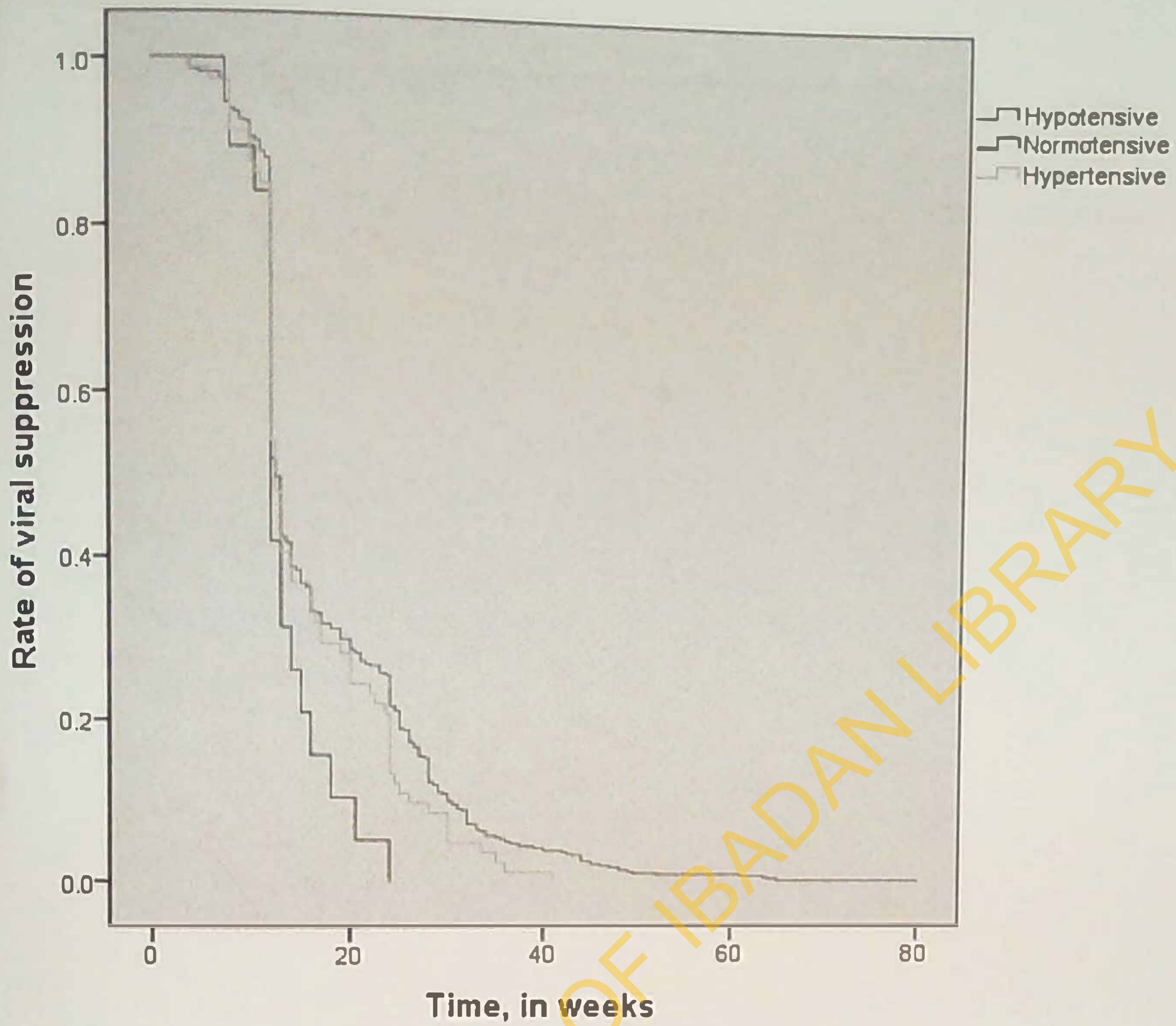
Fig.7g: Kaplan-Meier survival curve for differences in patients' BLOOD PRESSURE STATUS

Patients with hypotension appeared to achieve faster undetectable viral load within the study time than those with hypertension or normal BP. Log Rank test showed p-value = 0.03.
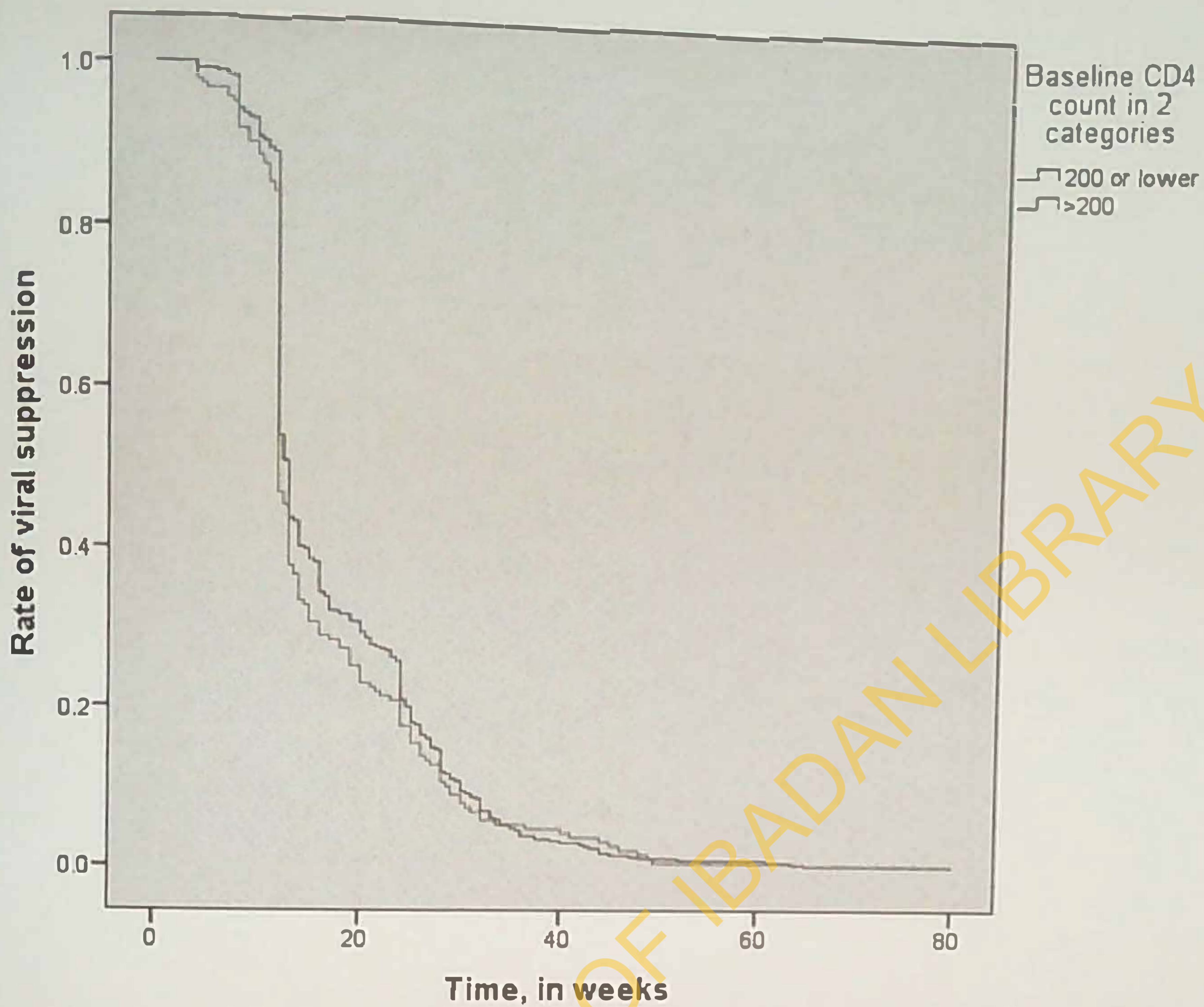
50
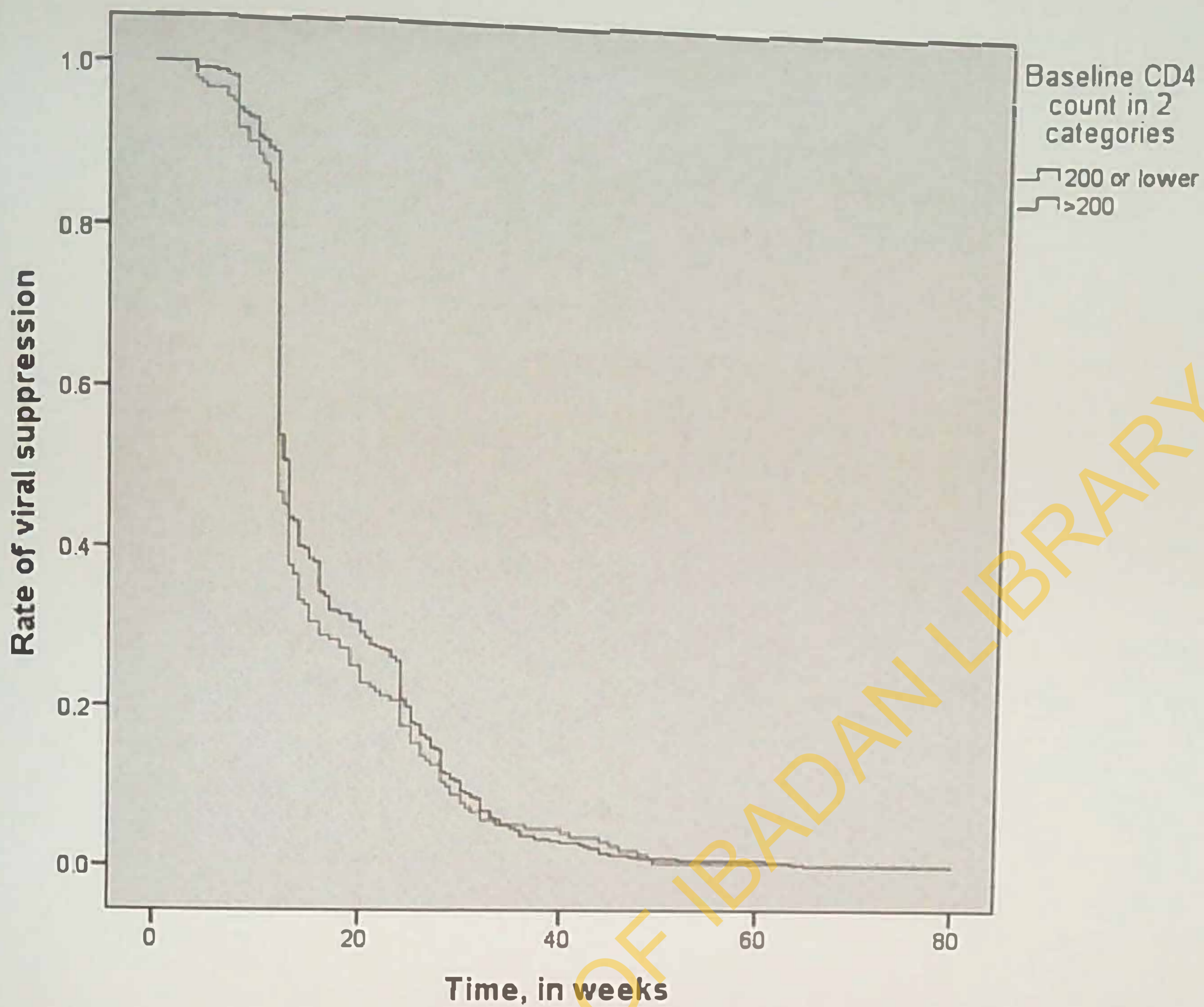
Fig.7h: Kaplan-Meier survival curve for differences in patients' BASELINE CD4 COUNT

On visual inspection, there is no obvious difference between the two curves, with a crossing of the curves at about 33 weeks. P-value from Log Rank test = 0.25

Fig.7h: Kaplan-Meier survival curve for differences in patients' BASELINE CD4 COUNT

On visual inspection, there is no obvious difference between the two curves, with a crossing of the curves at about 33 weeks. P-value from Log Rank test = 0.25

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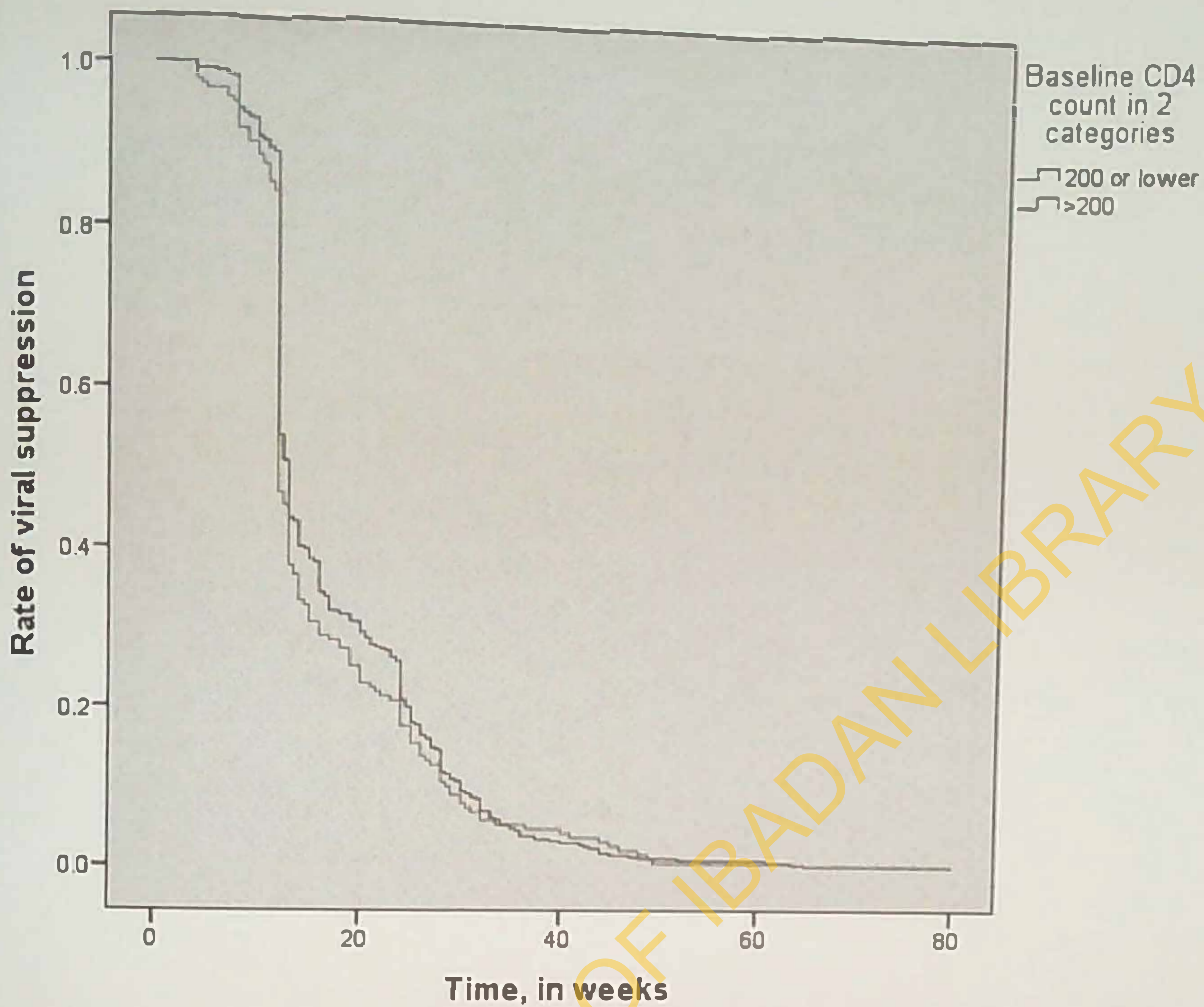
Fig.7h: Kaplan-Meier survival curve for differences in patients' BASELINE CD4 COUNT

On visual inspection, there is no obvious difference between the two curves, with a crossing of the curves at about 33 weeks. P-value from Log Rank test = 0.25
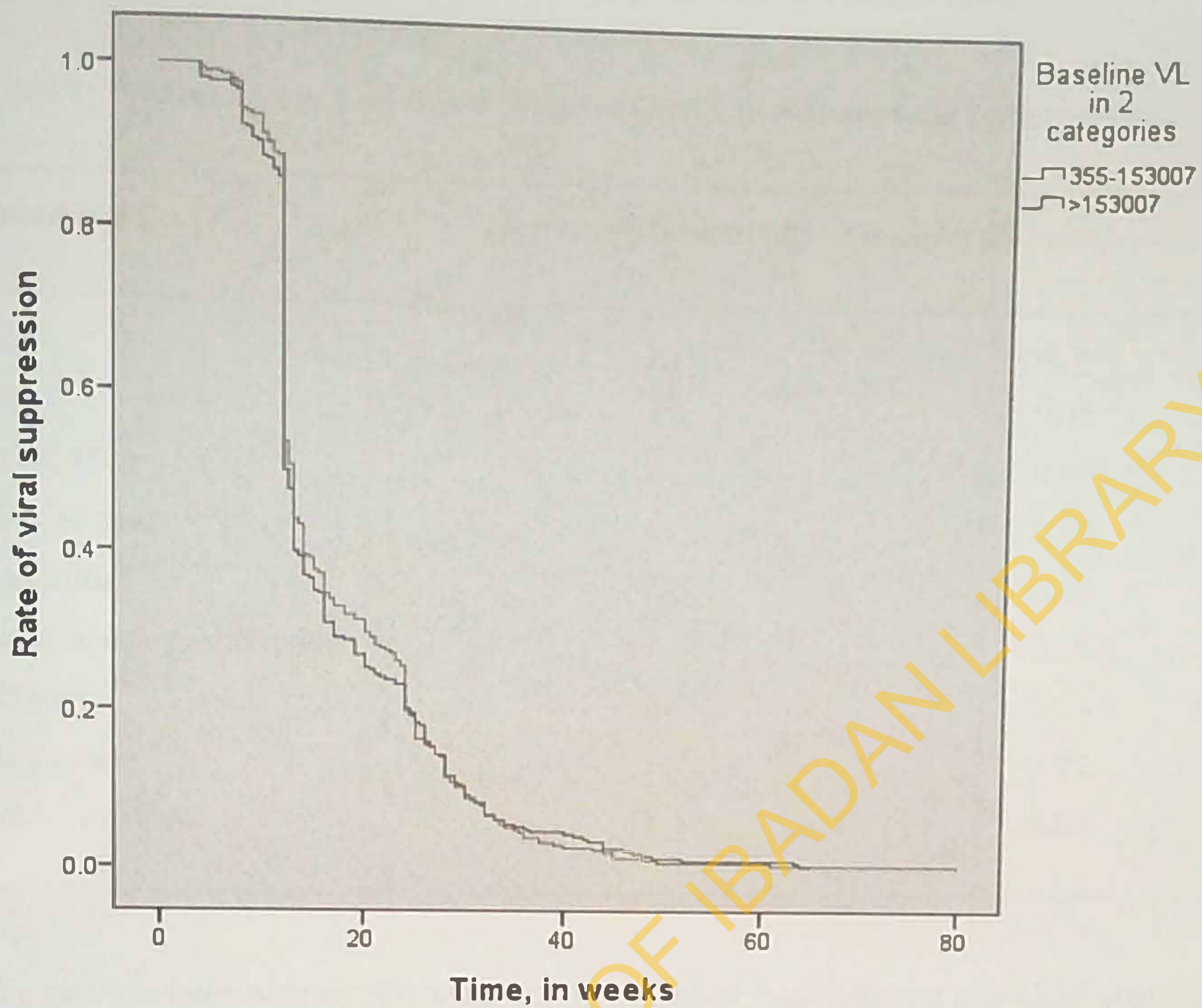
Fig.7i: Kaplan-Meier survival curve for patients' BASELINE RNA VIRAL LOAD

No significant difference was noted in the two RNA viral load categories as they cross each other, with p-value from Log Rank test=0.89

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Summary of P-values from Log Rank test is presented in Table 8 below.

Table 8: P-values from Log Rank (Mantel-Cox) Chi-Square tests for covariates

Covariates	Degree of freedom (df)	Sig. level (P-value)
Age	1	0.36
Sex	1	0.75
Occupation	2	0.005 *
Marital status	1	0.98
Education	2	0.81
Tuberculosis co-infection	1	0.19 *
BP status	2	0.03 *
CD4 count	1	0.25
RNA viral load	1	0.89

*Those covariates with significance level (p-value) on Log Rank test and which met the criterion for fitting into the Cox's regression model.

General note

For all covariates, the survival curves showed much clustering of the rate of viral suppression (a proxy for cumulative survival probabilities) between about 0.54 and 0.86 probability marks, corresponding to the survival time of 12 weeks on the X-axis. That indicates that a large proportion of the patients achieved their undetectable RNA viral load at twelve (12) weeks after commencement of antiretroviral treatment.

Sensitivity analysis results

It was also noted that results from the sensitivity analysis using Kaplan-Meier estimator for assumed failure times yielded 6.50 weeks as the median time to event; while using the Life Table estimate for the un-adjusted patients' time to event variable yielded a median time of 14.3 weeks.

4.3Multivariate (Cox's regression) analysis

4.3.1 Predictors of time to achieve undetectable viral load using Cox's regression analysis

Based on the results of the Log Rank (Mantel-Cox) Chi-Squared tests (see Table 6), only three covariates – occupation, blood pressure status and tuberculosis co-infection status – had p-values$\leq$0.20 and therefore merited inclusion for a multivariate analysis.

Hence, tuberculosis co-infection status, blood pressure status and patients' occupation were fitted into a Cox's model and used for regression analysis. From the analysis, it was observed that being a civil servant (an occupational category) significantly shortened a patient's time to achieve undetectable RNA viral load among HIV-infected adults receiving anti-retroviral treatment through the University of Nigeria Teaching Hospital (UNTH) Enugu in South-east Nigeria (OR = 1.5; 95% CI = 1.13 – 1.98; p = 0.004).It was further observed that being a business person or having a hypotensive blood pressure were associated with shorter time to achieve undetectable viral load but those associations were not statistically significant (OR =1.2; 95% CI = 0.99 – 1.46; p = 0.07 and OR = 1.5; 95% CI = 0.9 – 2.5; p = 0.11, respectively).

However, <u>normotensive</u> blood pressure status and co-infection with tuberculosis were associated with longer time to achieve undetectable viral load but the associations were not statistically significant (OR =0.9; 95% CI = 0.7 – 1.1, p = 0.4 and OR = 0.8, 95% CI = 0.6 - 1.11, p = 0.2, respectively).

The result of Cox's regression analysis is illustrated in Table 9 below, with the last categories of the covariates serving as the reference category for each of the three covariates.

Table 9: Cox's regression analysis showing the effect of patient characteristics on time to achieve undetectable viral load among the cohort

Patient characteristics	Odds Ratio (Confidence interval)	P-value
Blood pressure status		
Hypotensive	1.5 (0.9 – 2.5)	0.11
Normotensive	0.9 (0.7 – 1.1)	0.3
Hypertensive	1	
Tuberculosis co-infection status		
Co-infected	0.8 (0.6 – 1.1)	0.2
Not co-infected	1	
Occupation		
Civil servants	1.5 (1.1 – 2.0)	0.004 *
Business persons	1.2 (0.99 – 1.5)	0.07
Others	1	

CHAPTER FIVE
DISCUSSION

This study had involved the use of survival analysis techniques, although with a peculiarity. In most survival analysis studies, the main outcome of interest is the time to death or time to recurrence of disease from a disease-free state (http://www.dbcg.dk/Foredrag/11%20O verlevelseskurver%20MBJ.pdf). However, in this study, the true event of interest was time to "true survival", that is, time from a period of "severe state of illness" to "a state of wellness and true survival". This is so because the inclusion criteria for this study were, among other things, that the patients should have RNA viral loads that were high (bad) enough and CD4 counts that were low (bad) enough to make the use of antiretroviral medications inevitable.

The study assessed the probability of survival over a two-year period for a cohort of HIV-infected adults in a PEPFAR-funded antiretroviral treatment programme in South-east Nigeria, using patients' median time to achieve undetectable RNA viral load as proxy for survival. It considered as well the proportion of the cohort of patients achieving the primary study outcome at different specific timed periods following commencement of drug treatment, the prevalence of HIV/TB co-infection among the cohort and also examined those patient characteristics that significantly influence the time to achieve undetectable RNA viral load.

Socio-demographic characteristics

Previous studies on HIV/AIDS in adults show that the disease affects mostly people in the young and productive age group. In this study, 72% (576) of the patients fell within the 25 – 44 year age group. This agrees with a large longitudinal cohort study conducted in 2010 to assess patient retention and adherence to antiretroviral treatment at different HIV/AIDS treatment sites in Nigeria in which 78% of the patients were noted to fall within the age range of 25 – 45 years (Charurat et al, 2010). The mean age of 37.6 (95% CI = 36.9 – 38.3) years for patients on antiretroviral therapy at the study site also agrees with findings in another study conducted to determine the long-term benefits of highly active antiretroviral therapy among 176 HIV-infected adult patients in Senegal in which the median age of participants was 38 years (Laurent et al, 2004).

The proportion of the participants in this study within the age of active economic engagement – 25 to 60 years – should be a source of great concern for a low income country like Nigeria considering the economic implications of the impact of HIV/AIDS morbidity and mortality with respect to workforce productivity. With regard to gender, having greater proportion of the cohort of patients from this study as females agrees with findings from the Senegal study in which 52.3% (92) of their 176 patients were females (Laurent et al, 2004).However, there is a significant difference between the gender distribution in this study and that observed in a prospective study in Switzerland in 1999 in which only 27.3% of the 2674-member cohort of patients were females (Ledergerber et al, 1999).

Considering that trading is a predominant occupation for people of South-east Nigeria, it is therefore not a surprise that majority of patients in this study were traders, followed closely by civil servants and artisans. The proportion of the patients in this study who completed more than primary education also agrees with result from the large, longitudinal multicentre study in Nigeria to assess patient retention and adherence to anti-retroviral treatment in which 60% of their patients completed secondary education or higher (Charurat et al, 2010).

Median time to achieve undetectable viral load

Different brands of machines with varied sensitivities or lower limits of detection (L.L.D) had been in use by different anti-retroviral treatment programmes for measuring RNA viral load. Measurements with those machines had put the cut-off limits for undetectable viral load at levels between < 50 copies/ml and < 500 copies/ml. Some of such machines include, but not limited to: the Roche Amplicor v. 1.5 assay (Roche, Alameda, CA, USA) which uses an L.L.D of 400 copies/ml; the Chiron Quantiplex bDNA 2.0 assay (Chiron, Emeryville, CA, USA) using an L.L.D of 500 copies/ml and also Chiron 3.0 assay which uses an L.L.D of 50 copies/ml (Frater et al, 2002).Consequently, there is no strict uniformity in the cut-offs used for undetectable RNA viral load by different researchers whose works involved measurement of undetectable RNA viral load. Findings from this study have therefore been compared with those of other centres based purely on achievement of undetectable viral load by patients in those other studies without strict recourse to the threshold or cut-off used by them for determining undetectable levels of RNA viral load. As such, differences in cut-off points had been ignored in making comparisons in this study.

The median time to undetectable RNA viral load observed from this study appeared to indicate a better cohort performance than the one found in a comparative retrospective study in Edinburgh, Scotland between January 1999 and January 2004 in which the median time was 13.5 weeks (Manavi and Scot, 2006).Result from this study also appears significantly better the median time of 16 weeks observed in the much celebrated prospective multicentre Euro SIDA study in which the authors used RNA viral load of 500 copies/ml as their cut-off LLD. The main outcome measure (median time) from this study also showed a much better cohort performance than the one earlier obtained from a retrospective cohort study among HIV-positive patients treated in a University hospital between 1999 and 2003 in which a median time of 17.2 weeks was observed for patients with persistent low-level viraemia, using an LLD of 50 copies/ml (Sungkanuparph et al, 2006).

Several reasons could have been responsible for the failure of the other 166 patients to achieve the main study outcome of undetectable RNA viral load within the study time of two years. Their failure to attain the desired end-point might have been due to drug resistance, poor adherence to treatment or loss to follow up as a result of death, loss of interest in the treatment, or even relocation away from the study area.

Proportion of the cohort achieving UVL at different time points
Findings from this study showing 78.6% overall cohort success within twelve months or one year appear not to measure up with the Switzerland cohort study in which an estimated 90.7% of the entire cohort achieved undetectable viral load of <400 copies/ml by 12 months (Ledergerber et al, 1999).It is possible that differences in race, socio-economic status and adherence to antiretroviral treatment by patients in the two study cohorts might partly explain the observed significant differences in one year outcome from the two studies. However, in a study to compare the response of African HIV-1-infected individuals to HAART using Kaplan-Meier estimates, investigators observed that the proportions achieving undetectability by 3, 6, 9 and 12 months were 56, 81, 89 and 91% for the European group and 60, 81, 86 and 91% for the African group (Frater et al, 2002). Although there were no significant differences in outcome in that comparative study attributable to race, findings from that study indicated better overall performance compared to our 3, 6 and 12 months cohort performance. It is therefore suggested that a local study done here in Nigeria or at least on Africans living in Africa may be needed to

validate the European comparative study by Frater and colleagues. On the other hand, the six-month cohort performance of 63.3% observed in this study fared a little better than the result from a prospective multicentre, 17-countryEuroSIDA in which 60.4% of the cohort achieved RNA viral load undetectability at six months (Paredes et al, 2000).

It is therefore envisaged that the proportions obtained at the different time periods in this study can form a reference point for those other HIV/AIND treatment programmes in Nigeria that use the same protocol in assessing their programme performance.

Prevalence of tuberculosis co-infection in the cohort

Only 8.1% (65) of the patients in this study had a co-infection with tuberculosis which is fairly comparable to the 10.5% obtained by Iliyasu and Babashani in Kano, North-west Nigeria (Iliyasu and Babashani, 2009)but significantly differs from the 39.3% noted by investigators in Thailand (Soe et al, 2010). On the other hand, the result of tuberculosis co-infection from this study appeared much higher than the 3.7% obtained by researchers in Brazil in 2008 (de Carvalho et al, 2008).The prevalence observed among the cohort of patients in this study also came significantly lower than the32.8% earlier observed in Ibadan (Awoyemi et al, 2002) and the 40%from Ilorin (Salami and Katibi, 2006) or the 38.1% observed from a recent study at the Nigerian Institute of Medical Research, Yaba, Lagos (Onubogu et al, 2012). It is also important to note that the prevalence of HIV/AIDS/TB co-infection found in this study is less than half of the national median of 17% (Odaibo et al, 2006). Whether this low rate of co-infection is due to method of assessment of effectiveness of Isoniazid preventive (prophylactic) treatment of ART-ineligible HIV-infected patients needs to be further explored.

The male predominance found among the TB co-infected patients in this study is similar to the observation made in an earlier study in Kano in 2009 by Iliyasu and Babashani but different from the finding by investigators in South Africa where female preponderance of 57% for HIV/TB co-infection was observed (O'Donnell et al, 2009).

Predictors of time to achieve undetectable viral load

It was observed from this study that being a civil servant appeared to be the only factor significantly associated with shorter time to achieve undetectable RNA viral load (OR; 1.5; CI =1.1 – 2.0; p = 0.004). In specific terms, being a civil servant reduces the time to achieve undetectable viral load by one

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and a half times the period normal for others within the cohort. Therefore, among the civil servants attending the UNTH Enugu treatment programme, their occupation is a confounder to the anti-retroviral drugs they receive in the course of their treatment. However, this is different from the findings from a study in which being of West African origin and having lower baseline RNA viral load were associated with shorter time to achieving viral suppression (Patel et al, 2007). In their own study, Nogueras and fellow researchers found age as a major predictor by observing that disease progression and mortality following HIV diagnosis were faster in older patients (Nogueras et al, 2006). It is important to note that the determination of the key predictors of time to undetectable RNA viral load in this study might not have been sufficiently robust as the study site did not document some key patient variables like ethnicity, religion and treatment adherence in their programme database. This might have narrowed the number of covariates showing significant differences in their factor levels on Log Rank test and then the number eventually qualifying for multivariate (Cox's regression) analysis.

CHAPTER SIX
CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

At a median time of 12.5 weeks, the study showed that it takes a relatively short time for patients receiving anti-retroviral treatment from the UNTH Enugu APIN-Plus/Harvard PEPFAR programme to achieve undetectable RNA viral load. The proportion of the cohort achieving undetectable viral load at different time periods indicated that nearly every patient that achieved undetectable RNA viral load at the Enugu programme do so within one year of starting drug treatment.

It is also evident from the study that the prevalence of HIV/TB co-infection at the Enugu treatment programme was relatively low at 8% compared to results obtained from other parts of the country and also compared to the national median prevalence. Curiously, only occupation appeared to improve the speed of achieving undetectable viral load among patients receiving treatment from the Enugu programme.

6.2 Recommendations

There is a need for studies similar to this present one to be conducted in at least one treatment centre in each of the six major zones of Nigeria. Findings from such studies will help to develop an important database for use by government and policy makers to plan ahead for sustainability of HIV/AIDS control program in Nigeria. Further research is also needed to obtain local data on the size of the burden of relapse or RNA viral load rebounds in patients that had earlier achieved undetectable RNA viral load. As rebound in RNA viral load primarily results from resistance to a particular antiretroviral drug or set of drugs, findings from such study will help programme managers, funders and Government of Nigeria in estimating the size of that burden and then serve as an important tool for estimating the long-term funding need for second-line anti-retroviral drugs which those patients need or depend on.

In order to further improve the operational performance of the Enugu and similar HIV/AIDS treatment programmes in Nigeria, the observed, comparatively low proportion (78.6%)of patients achieving undetectable RNA viral load in one year tends to suggest the need to explore some innovative measures that may be used to have an improvement in this figure.

6.3 Limitations

Measurement bias is a major threat to the validity of findings and conclusions from this study. The lack of uniformity in measuring or calibrating the cut-off lower limits of detection (L.L.D) for undetectable level of RNA viral load by different HIV/AIDS treatment sites across the world limits strict comparability of findings. This can be illustrated by the fact that whereas some centres use machines that stop detecting RNA viral load in a patient at a level as high as 500 copies/ml,

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some other centres use machines calibrated for detection of RNA viral load to a point as low as 50 copies/ml – a 10 times difference.

It is known that the more frequently RNA viral load is measured, the earlier one is likely to observe a patient's achievement of undetectable RNA viral load. At the study site, RNA viral load is measured in the following intervals (in weeks): 0, 12, 24, 48, 72, 96, etc. This implies that any undetectablity that occurs immediately after a particular interval will only be recorded after measurement of the next fixed interval. Time to event in that case will be over-estimated and that violates a key assumption of Kaplan-Meier estimation in which it is assumed that the event happens at the time it is specified to have happened. This study will therefore appear, at least mathematically, to have over-estimated the median time to event.

Sensitivity analysis

In order to assess the effect of the 'mathematical' over-estimation of the median time, a sensitivity analysis was done to explore the robustness of the estimates and subsequent validity of conclusions drawn from those estimates. The median time obtained from the assumed time-to-event variable using Kaplan-Meier estimate was found to be far too low while the one obtained from the un-adjusted time-to-event variable using the Life Table method was higher than the earlier observed, supposedly over-estimated value. It was therefore safe to conclude, compared to observations from studies in the developed countries, that the cohort's median time obtained from this study using the un-adjusted Kaplan-Meier estimate represents a reliable estimate.

Case ascertainment bias also poses a major issue in the procedure for assigning TB co-infection status to patients at the treatment site. Assignment of TB status to anti-retroviral treatment-naive patients in the programme was based on three criteria that include the attending physicians' clinical evaluation; a chest radiograph and sputum microscopy. However, recent evidence indicates that diagnosis of tuberculosis (TB) amongst HIV patients poses a great challenge due to the low density of acid fast bacilli (AFB) in such patients' sputum – the AFB that forms the basis for sputum microscopy among TB patients (Onubogu et al, 2012). This implies an under-estimation of TB in HIV-positive patients in centres where TB screening and diagnostic tests rely heavily on sputum microscopy, as is the case in the study site Possibility of false-negative results therefore existed in patients in this study. Sputum culture in Lowenstein-Jehnsen's medium which serves as the gold standard for TB diagnosis in HIV/AIDS patients was not in use at the study site during the period covered by the study.

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   HIV/AIDS. *Science.* 301. 1534 – 1537.

Assessment of median time to achieve undetectable viral load after initiation of antiretroviral treatment among HIV-infected adults in Enugu, South-east Nigeria

Proforma for Data Extraction from Programme Database (Socio-demographics, Adherence, Tuberculosis, Baseline BP)

S/No	PepID	Date of starting HAART	Age (in years)	Gender 1 = Male 2 = Female	Marital Status 1=Single 2=Married 3=Cohabiting 4=Divorced 5=Widowed	Level of Education 1=None 2=Primary 3=Secondary 4=Tertiary	Ethnicity 1=Hausa 2=Igbo 3=Yoruba 4=(Other, specify)	Occupation	BP 1=Hypotensive 2=Normal 3=Hypertensive	Tuberculosis co-infection 1 = Present 2 = Absent	Mean Adherence (%) 1=≥95 2=<95

Assessment of median time to achieve undetectable viral load after initiation of an ~~antiretroviral~~ treatment among HIV-infected adults in Enugu, South-East Nigeria

Proforma for Data Extraction from Programme Database (CD4 count and Viral load)

CD4 count measurements (cells/ml)						Viral load measurements (copies/ml)					
Baseline/ date	1st 12weeks/ date	2nd 12weeks/date	48th week/ date	72nd week/ date	96th week/date	Baseline/ date	1st 12weeks/ date	2nd12weeks/ date	48th week/ date	72nd week/ date	96th week/date

03 August 2011

Dr. Obioma Obiora Obikeze
University College Hospital (UCH)
Ibadan
NIGERIA

Dear Dr. Obikeze,

Thank you very much for completing the Secondary Use of Data Application for the use of the Harvard PEPFAR Database and Repository Bank. We have reviewed your application and approve your use of data and samples for the following study:

Assessment of time to achieve undetectable viral load after initiation of ant-retroviral treatment among HIV-infected adults in Enugu, South-East Nigeria.

Should your study undergo any changes in protocol or require additional data and/or samples from the Harvard PEPFAR Database and Repository Bank that are not already indicated in your completed application, please be sure to contact Dr. Phyllis Kanki (pkanki@hsph.harvard.edu) or myself as soon as possible.

Once again, thank you very much for your application and best of luck as you begin your research.

Sincerely,

Marykate O'Malley
Data, Training and Education Coordinator

CC: Phyllis Kanki DVM SD

UI/UCH EC Registration Number: NHREC/05/01/2008a

NOTICE OF FULL APPROVAL AFTER FULL COMMITTEE REVIEW

Re: Assessment of Time to Achieve Undetectable Viral Load after Initiation of Antiretroviral Treatment among HIV-Infected Adults in Ibadan South-West, Nigeria.

UI/UCH Ethics Committee assigned number: UI/EC/11/0136

Name of Principal Investigator: **Dr. O. O. Obikeze**

Address of Principal Investigator: Department of EMSEH
College of Medicine,
University of Ibadan, Ibadan

Date of receipt of valid application: 03/06/2011

Date of meeting when final determination on ethical approval was made: 25/08/2011

This is to inform you that the research described in the submitted protocol, the consent forms, and other participant information materials have been reviewed and *given full approval by the UI/UCH Ethics Committee.*

This approval dates from 25/08/2011 to 24/08/2012. If there is delay in starting the research, please inform the UI/UCH Ethics Committee so that the dates of approval can be adjusted accordingly. Note that no participant accrual or activity related to this research may be conducted outside of these dates. *All informed consent forms used in this study must carry the UI/UCH EC assigned number and duration of UI/UCH EC approval of the study.* It is expected that you submit your annual report as well as an annual request for the project renewal to the UI/UCH EC early in order to obtain renewal of your approval to 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 conduct compliance visit to your research site without previous notification.

Prof. A. O. ....
Director, IAMRAT
Chairman, UI/UCH Ethics Committee
E-mail: uiuchirc@yahoo.com

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Chief Medical Director

Dr. C. C. AMAH, MBBS, FWACS, FICS, MNIM, MNIPR
Chairman, Medical Advisory Committee

Our Ref............**UNTH/CSA.329/VOL.5**

Date............**October 26, 2011**

NHREC/05/01/2008B

ETHICAL CLEARANCE CERTIFICATE

TOPIC: THE ASSESSMENT OF TIME TO ACHIEVEMENT OF UNDETECTABLE VIRAL LOAD AFTER INITIATION OF ANTIRETROVIRAL TREATMENT AMONG HIV-INFECTED ADULTS IN ENUGU, SOUTH-EAST NIGERIA.

BY: DR. OBIKAEZE, OBIOMA OBIORA

FOR: A DISSERTATION FOR A MASTER OF SCIENCE (MSc) DEGREE IN EPIDEMIOLOGY AND MEDICAL STATISTICS OF THE DEPT. OF COMMUNITY MEDICINE, COLLEGE OF MEDICINE, UNIVERSITY OF IBADAN

This research project on the above topic was reviewed and approved by the University of Nigeria Teaching Hospital Health Research Ethics Committee.

This certificate is valid for **one** year from date of issue.

Prof. R.E. Umeh
Chairman
Health Research Ethics Committee

Date: 31/10/11