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Cytochemical studies of peripheral blood leucocytes in pregnancy

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Summary

Three enzymes (alkaline phosphatase, acid phosphatase and peroxidase) and two metabolites (glycogen and lipids), were studied in the circulating leucocytes of pregnant subjects attending the Antenatal Clinic, Ile-Ife State Hospital, during their second and third trimester of pregnancy. Values obtained were compared with those of a non-pregnant control group in the same locality.

Leucocyte alkaline phosphatase, glycogen and lipid levels were found to be significantly elevated, while acid phosphatase could not be demonstrated in the study group. Peroxidase levels decreased in the second trimester, only to rise again in the third trimester. Peroxidase had a highly negative correlation with birth weight ($r = -0.96$) and was, therefore, suggested as a possible prognostic indicator of birth weight. Glycogen and lipids correlated significantly and positively with the Apgar score, and therefore could possibly be useful in antenatal assessment of foetal health status.

The necessity for further investigations and confirmation of the possible clinical uses of these parameters in pregnancy has been highlighted.

Résumé

Trois enzymes — alcaline phosphatase, acide phosphatase et peroxydase aussi bien que deux métabolites-glycogène et lipides — ont été étudiés dans les leucocytes circulaires des sujets enceintes qui fréquentaient la clinique obstétrique, L'hopital d'état d'Ile-Ife pendant le deuxième et le troisième trimestre de la grossesse. On a comparé les résultats obtenus avec ceux d'un groupe de contrôle non-enceinte que vient de la même région.

Les niveaux de leucocyte alcaline phosphatase, de glycogène et de lipide sont considérablement élevés tandis que l'on ne trouve pas d'acide phosphatase dans le groupe. Les niveaux de peroxydase baissent pendant le deuxième trimestre mais s'élèvent pendant le troisième trimestre. Le peroxydase a manifeste une corrélation négative avec le poids du nouveau-né $r = -0.96$ ce qui fait que l'on l'identifie comme un indicateur prognostique éventuel du poids du nouveau-né. Le glycogène et le lipide sont entrés en corrélation importante et positive avec Apgar, ce qui montre qu'ils peuvent éventuellement aider la vérification de l'état de santé du foetus.

On a attiré l'attention sur la nécessité de poursuivre d'autres investigations et d'autres confirmations des usages cliniques éventuels de ces paramètres dans la grossesse.

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Introduction

Several investigators, [1,2], have recorded a rise in the number of circulating neutrophil polymorphonuclear cells during pregnancy. Furthermore, the activity of leucocyte enzymes, especially leucocyte alkaline phosphatase (LAPA), has been reviewed in detail [3].

Alkaline phosphatase is a hydrolytic enzyme capable of splitting phosphate esters in an alkaline medium. It appears to be involved in glycogenesis [4]. Leucocyte alkaline phosphatase has also been studied by Pritchard [5], and an increase was noted in pregnancy.

On the other hand, leucocyte acid phosphatase is capable of hydrolysing phosphate esters in an acid medium. It appears to have been less studied in pregnancy, although [5] using biochemical methods, there was observed no change in its level of activity during pregnancy. Peroxidase has a protein moiety attached to a prosthetic iron-porphyrin complex. To the best of our knowledge, its role in pregnancy does not appear to have been studied.

Dempsey and Wislocki [6] had commented on the possible inter-relationship between glycogen and alkaline phosphatase. They observed that the highest unit cell LAPA was associated with neutrophilic leucocytes, in which glycogen level was equally elevated.

Lipids are a heterogenous class of compounds which include phospholipids, neutral fats, lipoproteins and cerebrosides. Recent studies by Corberand [7] showed elevated leucocyte levels of lipids in new born infants. The objectives of this study were:

(a) To establish normal values for the parameters investigated in normal Nigerian pregnant women;
(b) To relate the values of these enzymes to placental weight, birth weight and Apgar score;
(c) To relate these values to the prognosis of the foetal well-being.

Materials and methods

A total of 44 patients attending the Antenatal Clinic at the Ife State Hospital were investigated. Fresh smears of peripheral blood obtained by means of a thumb-prick were used.

Routine examination

Freshly prepared air-dried smears were fixed in absolute methanol for about 10 min, stained in Giemsa solution for a further 10 min, and then washed in tap water for 5–10 min. They were then mounted in a neutral medium.

Histochemical methods

(a) For lipid: Sudan Black B [8];
(b) For glycogen: PAS procedure [9,10];
(c) For peroxidase [11,12];
(d) For alkaline phosphatase [13];
(e) For acid phosphatase [14];

The method of Kaplow [15] was adopted in assessing the cytochemical results in peripheral blood leucocytes.

Results

Tables 1–4, and Figs 1–4 illustrate the findings in this study.

Table 1 shows the range of scores, Table 2 the mean scores and Table 3 the results of Student's *t*-test at 5% level of significance for the parameters investigated; vis-à-vis the control group. Two enzymes, namely, succinic dehydrogenase and lactic dehydrogenase could not be demonstrated in leucocytes in pregnancy.

Regarding the mean scores, as well as the standard errors of the mean for the cytochemical investigations, alkaline phosphatase, glycogen and lipid levels in the second and third trimesters of pregnancy are significantly higher than levels for the control group.

Table 1. Range of scores for the different parameters investigated

Cytochemical reaction for:	Controls		Pregnancy 2nd trimester		Pregnancy 3rd trimester	
	No.	Range	No.	Range	No.	Range
Alkaline phosphatase	20	84–125	35	150–260	30	177–281
Acid phosphatase	20	0–10	35	—	30	—
Peroxidase	20	270–322	35	204–294	30	213–302
Glycogen	20	170–192	35	242–296	30	271–315
Lipids	20	230–256	35	292–320	30	299–330

Table 2. Mean scores for the parameters investigated as well as their corresponding standard errors of the mean

Cytochemical reaction for:	Controls		Pregnancy			
			2nd trimester		3rd trimester	
	No.	Mean score	No.	Mean score	No.	Mean score
Alkaline phosphatase	20	98.6 ± 14.1	35	186.2 ±43.6	30	222.2 ± 34.5
Acid phosphatase	20	< 4.0	35	—	30	—
Peroxidase	20	289.3 ± 17.3	35	230.2 ± 31.8	30	253.3 ± 52.1
Glycogen	20	180.5 ± 19.5	35	268.2 ± 30.7	30	293.6 ± 48.9
Lipids	20	243.6 ± 15.9	35	303.8 ± 54.6	30	315.6 ± 48.7

Table 3. Results of Student's *t*-test carried out on parameters investigated at 5% level of significance

Cytochemical reaction for:	Student's *t*-test result		
	t_T	t_c	Conclusion
Alkaline phosphatase	2.00	2.73	Significant
Peroxidase	2.00	2.01	Significant
Glycogen	2.00	2.41	Significant
Lipids	2.00	3.30	Significant

t_T = t tabulated (P value).
t_c = t computed (P value).

Peroxidase, however, showed a fall in the second trimester of pregnancy. In the third trimester, its level rose again, but this remained lower than the control group level (Fig. 1). On the other hand, acid phosphatase, lactic dehydrogenase and succinic dehydrogenase were not scored because they could not be demonstrated in the study group.

The Student's *t*-test was applied to the parameters whose levels were altered in pregnancy (Table 3). The results of the analysis indicated that for alkaline phosphatase, peroxidase, glycogen and lipids their pregnancy levels were significantly different from the control levels at 5% level of significance or 95% confidence level.

Table 4 shows how the above four para-

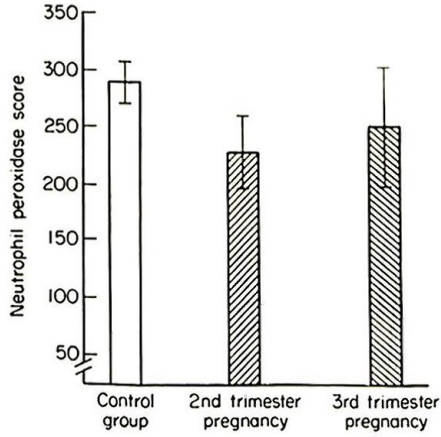

Fig. 1. Mean scores of peroxidase levels during pregnancy and in non-pregnant controls.

Table 4. Correlation coefficients between 3rd trimester levels of parameters studied and Apgar score, birth weight and placental weight

Parameter	Correlation coefficient with Apgar score	Correlation coefficient with birth weight	Correlation coefficient with placental weight
Alkaline phosphatase	+0.41	−0.057	+0.21
Peroxidase	−0.004	−0.96	+0.009
Glycogen	+0.79	−0.27	−0.20
Lipids	+0.67	+0.07	+0.14

meters in the third trimester of pregnancy correlate with the Apgar score, birth weight and placental weight. Third trimester values were used as some of the subjects in the second trimester were lost to follow-up or did not reach delivery stage.

Lipids and glycogen showed high positive correlation (r +0.79 and +0.67, respectively) with the Apgar score (Figs 2 & 3).

Alkaline phosphatase showed weak positive correlation (r = +0.41) with the Apgar score while peroxidase correlated very poorly with it (r = −0.004). With birth weight, only peroxidase appears to be significant; it had a very high negative correlation with it (r = −0.96) (Fig. 4). Others correlated rather poorly with birth weight. None of the parameters was found to correlate significantly with placental weight.

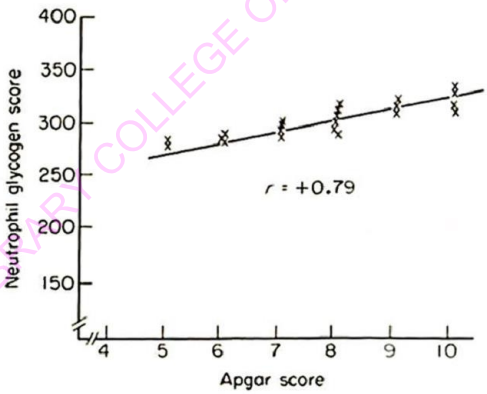

Fig. 3. Relationship between 3rd trimester glycogen levels and Apgar score, showing high positive correlation.

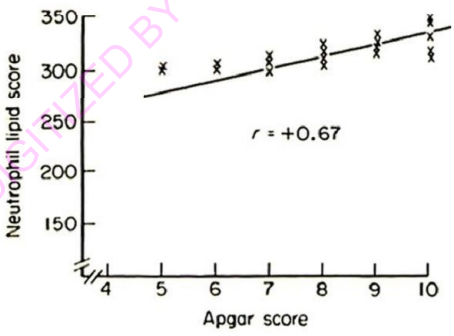

Fig. 2. Relationship between 3rd trimester lipid levels and Apgar score at 1 min, showing high positive correlation.

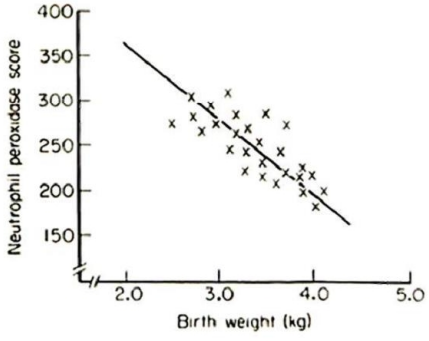

Fig. 4. Relationship between 3rd trimester peroxidase scores and birth weight, showing significant negative correlation.

It is, however, noteworthy that lipids correlated positively with all the three indices — Apgar score, birth weight and placental weight. Glycogen correlated only positively with the Apgar score, and negatively with others. Peroxidase correlated only positively with placental weight and negatively with the rest. Alkaline phosphatase, however, correlated positively with the Apgar score and placental weight, but negatively with birth weight.

Discussion

A great deal of evidence is now available indicating that pregnancy creates its own homeostatic laws [16]. From our findings, some aspects of the cytochemistry of leucocytes from pregnant women in our locality (Ile-Ife) will be discussed, emphasis will be laid on the normal values for both pregnant and non-pregnant women obtainable here (Ile-Ife).

The method of scoring adopted in this study is a semi-quantitative assessment based on that proposed by Kaplow [15]. The method is, however, associated with some disadvantages such as possible statistical and observer errors due to inconsistency in rating the cells, by the experimenter. However, Kaplow [15] and Shousha *et al.* [17] have found this method of assessment consistent and acceptable.

Alkaline phosphatase

For this enzyme, a score range of 84–125 and a mean and standard deviation of 98.6 ± 14.1 was obtained for the non-pregnant (control) group. This rose to 150–260 and a mean and standard deviation of 186.2 ± 43.6, respectively in the second trimester. In the third trimester, the values rose to 177–281 and mean 222.2 ± 34.5. Kaplow [15] had reported a range of LAPA scores of 10–100 for a group of 479 clinically healthy subjects of both sexes, a range of 130–150 in the first and second trimesters of pregnancy and values of 180 and above in the third trimester. Kaplow [15] did not, however, give any mean scores. It is significant to note that values obtained in this investigation for our local population appear to be slightly higher than Kaplow's figures. A number of factors could account for these differences. Kaplow [15] used human subjects of both sexes while the present investigators used only female subjects. However, as Ray and Pinkerton [18] have reported significantly higher LAPA values in females than in males after puberty, the values obtained for our control group are understandably higher than those of Kaplow [15], who used both human sexes. The slightly higher scores in the second and third trimesters we obtained, viewed in the light of Kaplow's values, may also not be unconnected with increased stress in our local population, such as mild or subclinical infectious inflammatory conditions, and nutritional and environmental conditions. Kaplow [15] had reported a rise in LAPA in inflammatory conditions especially those caused by gram-positive pyogenic organisms. Other forms of stress such as strenuous exercise and those leading to the production of corticosteroids have also been associated with elevated LAPA levels [19].

It is not quite clear why alkaline phosphatase levels become elevated in pregnancy although in the light of what is now known about the activity of the enzyme, a few suggestions may be made to explain this phenomenon. Wiltshaw and Moloney [4] suggested that the enzyme is involved in glycogenesis and nucleic acid formation. It is conceivable then, that LAPA's increased levels in pregnancy may be associated with the greater need for nucleic acid and glycogen synthesis in the pregnant mother in order to meet the demands of foetal development.

LAPA scores in the third trimester of pregnancy correlated positively with the Apgar Score; and negatively and poorly with birth weight. These findings, suggest that LAPA assessments in the third trimester of pregnancy may not be a strong prognostic indicator of foetal well-being or of the health status of the new born.

The poor correlation with placental weight is to be expected, as the placenta is probably not the source of LAPA, but the poor negative correlation with birth weight is difficult to explain. It may, however, suggest that extremely high LAPA scores in the third trimester of pregnancy should be watched carefully.

Therefore, in the light of previous findings it would seem obvious that a normal or decreased LAPA score is indicative of absence of normal

or even extrauterine pregnancy, whereas an elevated LAPA score is probably not a reliable indicator of pregnancy [15,20]. Also, further work is indicated, with respect to the use of LAPA assays in the diagnosis of threatened, missed or incomplete abortions.

Acid phosphatase

Acid phosphatase activity could not be demonstrated at any stage in the second and third trimester of pregnancy in the present study. However, a very weak activity of the enzyme was observed in the non-pregnant control group providing a range of scores 0–10 and a mean value of less than 4. Apparently, little or no work has been reported on the cytochemistry of leucocyte acid phosphatase activity in the second and third trimesters of pregnancy. It was not possible to monitor first trimester levels of the enzyme because our local women do not attend antenatal clinics before their 3rd month of pregnancy. However, the very poor results we obtained even in the non-pregnant group raises some doubt as to the diagnostic usefulness of this enzyme in pregnancy. Pritchard [5] using biochemical methods observed that the level of activity of this enzyme did not change during pregnancy and puerperium.

Peroxidase

The results of peroxidase assessment in this investigation appear interesting. A range of scores of 270–322 and a mean score and standard deviation of 289.3 ± 17.3 was obtained for the control group. The second trimester scores were 204–294 and 230.2 ± 31.8, while in the third trimester the values rose to 213–302 and 253.3 ± 52.1. Thus, the control group had the highest scores, followed by the score for the third trimester group and then the score for the second trimester group. If the first trimester values had been available, perhaps a better and clearer picture could have emerged. From this study, it would appear that peroxidase activity as reflected by these values is low in the early phases of pregnancy (at least up to the second trimester and thereafter begins to show an upward trend (i.e., from about the third trimester). Anger [21] was, however, able to demonstrate that leucocyte verdoperoxidase in the presence of hydrogen peroxide could detoxify tetanus and diphtheroid toxins. Also, Avila *et al.* [22] postulated that peroxidase in conjunction with hydrogen peroxide forms an effective antibacterial system. In the light of these earlier reports, therefore, it would seem that increased levels of leucocyte peroxidase is associated with increased antibacterial performance and vice versa. In the context of our findings it would seem that the second trimester subject has a lower leucocyte antibacterial activity compared to the third trimester subject. Due to the higher leucocyte peroxidase levels in the control group, it would appear that the members are equipped with leucocytes most active against bacteria and their toxins.

Peroxidase levels in the third trimester of pregnancy also correlated very significantly (although negatively) with birth weight ($r = -0.96$). It, therefore, appears to be a better prognostic indicator of birth weight. Hence, out of all the parameters we investigated, peroxidase appears to be the most useful tool in predicting the weight of the new born. The implication of a very significant negative correlation with birth weight is that very high peroxidase activity scored in the third trimester of pregnancy may suggest low birth weights for such pregnant subjects. On the other hand, very low scores of the activity of this enzyme in this stage of pregnancy predict high birth weights. This is obviously interesting and could prove useful to the obstetrician, and therefore requires further investigation.

Glycogen

The results of this investigation showed that the neutrophils of the control group recorded a glycogen score range of 170–192 and a mean score of 180.5 ± 19.5, while the second and third trimester scores were 242–296, 268.2 ± 30.7 and 271–315, 293.6 ± 48.9, respectively. Earlier studies have demonstrated moderate to intense glycogen staining in healthy human peripheral blood neutrophils [23]. Glycogen is an important macromolecule; the form in which polysaccharides are stored by animal tissues. Valentine *et al.* [24] noted that the cells in which glycogen are stored served as 'store houses' of energy. A possible inter-relationship existing

between glycogen and alkaline phosphatase was reported [6] where both levels were elevated in cases of neutrophilic leucocytosis. The present investigators also noted that both levels were elevated in this study group. This may further support [4] the idea that alkaline phosphatase is involved in glycogenesis. We also noted that two enzymes involved in the later stages of glycogen breakdown, succinic and lactic dehydrogenases were virtually absent in the later stages of pregnancy (second and third trimesters). This may suggest diminished glycogen breakdown in these stages of pregnancy, hence the increased levels of glycogen staining which suggests that glycogen is accumulated in pregnancy. If the view of Wiltshaw and Moloney [4] was correct, then alkaline phosphatase may be involved in this glycogenic process. Glycogen thus accumulated could, therefore, be mobilized [24] to meet the metabolic requirements of the individual in carrying out physiological processes in future; probably for the foeto-maternal unit or may be to enable the mother meet the energy requirements of labour.

Glycogen levels in the third trimester of pregnancy was also noted to correlate positively and fairly significantly with the Apgar score, but poorly with either birth weight or placental weight. It, therefore, appears to be more important than the other parameters investigated in predicting the Apgar score of the newborn, which is an indication of its health status at delivery. Hence, the study indicates that the higher the glycogen level in the neutrophils of the peripheral blood in pregnancy, the more likely is the new-born to have a higher Apgar score and vice versa. This may probably be of some clinical diagnostic value, especially as an index of pre-natal assessment of the health status of the new-born to be.

Lipids

Markedly elevated lipid levels were observed in the neutrophils of the study group. The control group had a range of scores of 230–256 and a mean score of 243.6 ± 15.9. Values for the second trimester of pregnancy were 292–320 and 303.8 ± 54.6, respectively, and for the third trimester, 299–330 and 315.6 ± 48.7, respectively. In a recent work [7], it could not be established that any statistical difference occurred between lipid levels of neutrophils in healthy children and those of healthy adults. Elevated levels were however observed in newborns [7]. Shousha *et al.* [17] found a slight mean rise in lipid levels of neutrophils in the peripheral blood of children with kwashiorkor, but they could not establish any statistical difference between these levels at the peak of illness and the levels obtained from a healthy control group. The increase in lipid levels of neutrophils in pregnancy can be justified on the grounds that it agrees well with the principle of minimum depletion of energy reserves, as well as a tendency toward conserving such reserves that was found for glycogen. It is, thus, interesting to note that apart from glycogen, lipid correlates most significantly with the Apgar Score.

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