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Short Communication *Biochemistry*

Relationship between mean corpuscular haemoglobin concentration and placental alkaline phosphatase activity among pregnant women in Nigeria

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ABSTRACT
The relationship between mean corpuscular haemoglobin concentration (MCHC), a maker of folate status in our environment and placental alkaline phosphatase (pALP) activity value, and hence, birth weight measures was investigated. One thousand and three pregnant women between 15-22 week gestation age were selected from some clinics in Delta state. The study area covered the six major tribes in the state. The women who were monitored throughout the period of gestation were between 18-35 years of age. The apparently healthy subjects were classified into two groups depending on the MCHC value at term. Group A (low MCHC:280-329 g/L; n=551) and group B (normal MCHC: 330-370 g/L; n=451). Results show that MCHC level relates positively with pALP activity and birth (placental and neonatal) weight measures. Socio-demographic data implicate illiteracy, low household income, poor diet and inadequate antenatal care for the observed reduction in MCHC among the group A subjects. Thus, MCHC and pALP can be used to monitor fetoplacental health. Albeit, the public should be educated on the importance of healthy diet and good antenatal care.

Key words: Placental alkaline phosphatase, Folate, Haemoglobin, Illiteracy, Gestation.

INTRODUCTION

Pregnancy is defined as the condition where an embryo and then foetus is carried in a women's uterus for a period of approximately thirty-eight weeks from conception to delivery [1]. In pregnancy the nutritional status of the mother including the bioavailability of folate is completely changed and this varies with the stage of gestation [2]. Pregnancy reduces folate absorption and increases its blood clearance rate [3]. It has been reported that the demand for folate doubles towards the beginning of the third trimester period [4]. Poor dietary intake (of especially folate and reduced supplementation during pregnancy) could put such pregnant population at risk of developing symptoms characteristic of folate deficiency which include reduced rate of proliferation of actively dividing cells and tissues, which can culminate in megaloblastic anaemia. The placental tissue which represents the link between the mother and the foetus is synthesized during pregnancy. It plays crucial roles that support the growth of the foetus and this include the secretion of alkaline phosphatase involved in the transport of basic nutrients across the trans-placental membrane to the foetus [5]. Albeit, the effect of folate deficiency on the rate of placental tissue proliferation and development among Nigerian pregnant women is yet to be documented. This paper thus attempts to report the relationship between socio-demographic data and mean corpuscular haemoglobin concentration (MCHC) to placental alkaline phosphatase and birth weight measures at term. MCHC is commonly used as an index of folote status in our environment, and so this investigation highlights the effect of income, education and diet on MCHC, placental alkaline phosphatase activity and birth weight at term.

MATERIALS AND METHODS

Subjects: One thousand and three pregnant women were selected from the tribal groups in Delta state at 15-22 week gestation age and monitored to term. The subjects (18-35 years of age) were screened and selected from maternities located in thirteen rural communities and twenty-seven urban centres in Delta state. The women were then grouped into two depending on their MCHC values at delivery. Group A: low MCHC (280-329 g/L; n=552) and group B: normal MCHC

(330-370 g/L; n=451). Information on socio-demographic data were obtained via interviewer's administered questionnaire. Informed consent was sought and obtained from the subjects and management of the maternities. The investigation was approved by Faculty of Medical Sciences' Research and Ethics Committee.

Collection of blood specimens : The normal process of venepuncture with the aid of a syringe and needle was used as previously described [6]. From each subject, 4.0ml of fasting whole blood was collected and divided into two portions of 2.0ml each. One portion was emptied into plain, sterile collection bottle, allowed to clot and then centrifuged at 1,200 x g for 5min at room temperature to get approximately 1.0 ml of serum. The serum obtained was decanted into bijou bottle and heated at 65^{0}C for 7 min. The heating exercise denatures the other isoforms of serum alkaline phosphatase possibly derived from the bone, liver, spleen, kidney and intestine, with the exception of the placental isotype which is heat-stable [7]. The recorded activity therefore, corresponds to the level of the enzyme secreted by the placenta. The second 2.0 ml portion was dispensed into potassium ethylene diamine tetra-acetic acid (K-EDTA) for the estimation of MCHC.

Analysis of specimens: The activity value of alkaline phosphatase in the heated serum was determined by the p-nitrophenylphosphate/ Diethanolamine (pNPP/DEA) method [8]. The reagents were supplied in a commercial test kit by Randox Laboratories, Ltd, United Kingdom. The reagents were reconstituted and allowed to stabilize at room temperature for about 10 min before use.

Estimation of MCHC: The K-EDTA blood sample for full blood cells count was almost immediately transferred to the electronic multi-parameter blood cell counter – Beekman Coulter Act Diff Haematology autoanalyser (Miami, Florida, USA). The MCHC was then calculated from the values obtained for PCV and Hb concentrations using appropriate formula:MCHC (g/L) = $\frac{Hb(g/dL)}{PCV(\%)} x100$ as previously stated [9].

Measurement of placental and neonatal birth weight at term: The weights of the placenta and neonate were determined between 0-30min after birth using weighing scale.

Statistical analysis : The data obtained were analyzed by repeat measure analysis of variance (ANOVA) followed by Dunnett's post hoc test for multiple comparisons using SPSS 10.0 computer software package (SPSS Inc., Chicago, U.S.A.). Statistical significant difference was established at the 5% probability level ($P < 0.05$).

RESULTS AND DISCUSSION

Table 1 shows that the percentage number of subjects selected from each of the six tribal groups into the two experimental classes (Group A: low MCHC:280-329 g/L; n=552; and group B: normal MCHC:330-370 g/L; n=451) varied minimally. The information gathered also, indicate that the level of education may have influenced household income, dietary intake and the centre patronized during antenatal care. Consequently, income and diet – products of education level, affected MCHC level which bears positive relationship to pALP and birth weights (both placental and neonatal) at term. The MCHC and pALP values obtained from the group B subjects at 31 – 37 week gestation age were significantly ($P<0.05$) higher when compared with the corresponding values among the group A women (Table 2), and these influenced the placental and Nonatal birth weights at term (Table 3), though not to be a significant level ($P>0.05$). It can be observed that as pregnancy advances, the difference in the activity value of placental alkaline phosphatase (pALP) for group A and group B subjects progressively widens, suggesting that as the gestation age increased, the effect of low MCHC on the marker enzyme, pALP becomes more severe (Table 2).The level of education, income and diet have been implicated as these bear positive relationship to MCHC distribution. The bioavailabillity of folate-derived coenzymes has been reported to be beneficial during rapid cell division [10]. It follows that deficiency could slow down such rapid process resulting in a wide array of complications including the improper development of the cells and tissues concerned. Although, serum folate was not directly measured during the study, the marker – MCHC demonstrates that low MCHC and hence folate deficiency could reduce the progressive growth rate of the placental tissues as evidenced by the pALP activity. This appeared to further confirm previous reports among the Egyptian subjects [7]. Folate deficiency inhibits DNA synthesis due to decreased availability of purines and deoxythymidine monophosphate (dTMP) leading

to arrest of cells in S-phase, the phase that prepares the cell for mitotic division by duplicating the genetic (DNA) material. This in turn, causes megaloblastic changes in the size and shape of rapidly dividing cells, that results in giant, immature cells with fragile membrane [4]. Data indicate that low MCHC level could reduce the placenta's ability to secret an important enzyme, alkaline phosphatase, required for pregnancy increase. This resulted to low placental weight and low neonatal birth weight at term (Table 3). Determination of pALP activity during pregnancy can be used to monitor the progress in placental tissue growth and foetal development. This is imperative since pregnancy complicates the metabolism and the bioavailability of nutrients especially folate [11, 12]. Apart from establishing ranges for pALP in health and disease (low MCHC) which could be important for clinical diagnosis, this study suggests that poor diet possibly caused by low income and illiteracy, may be a strong factor determining the progress of pregnancy. We therefore, advocate that the lay public be educated on the importance of healthy diet and proper antenatal care during pregnancy.

Table 1: Information on subjects' socio-demographic data

	Pregnant subjects (n=1003)	
	Group A (n=552)	Group B (n=451)
	Percentage distribution of 'n' pregnant women	
Household income (₦)/month		
Low (5,000 – 20,999)	74.3	-
Average (21,000-49,999)	25.4	11.3
Above average (50,00-199,999)	0.3	67.0
High (200,000 and above)	-	21.7
Household size		
2-5	11.2	58.3
6-10	50.5	34.1
11-15	38.2	7.6
Education		
None	49.3	-
Primary	38.0	11.8
Secondary	12.7	26.6
Tertiary	-	61.6
Diet intake		
Poor	89.1	0.7
Fairly adequate	10.9	35.9
Adequate	-	63.4
Antenatal visit		
Traditional	35.9	1.1
Maternity/PHCC	50.0	22.6
Clinic/Hospital	14.1	76.3
Tribe		
Ibo	46.8	53.2
Ijaw	59.4	40.6
Isoko	48.2	51.8
Itsekiri	63.5	36.5
Ukuani	51.5	48.5
Urhobo	54.6	45.4

Group A: Low mean corpuscular haemoglobin concentration MCHC (280 – 329 g/L) at delivery
Group B: Normal mean corpuscular haemoglobin concentration MCHC (330 – 370 g/L) at delivery
PHCC – Primary Health Care Centre

Table 2: Relationship between mean corpuscular haemoglobin concentration (MCHC) and placental alkaline phosphatase (pALP) activity values at different gestation periods.

Group	A[n = 552]			B [n=451]		
MCHC Reference range (g/L)	280 – 329 [Low]			330 – 370 [Normal]		
Gestation age (week)	15 – 22	23 – 30	31 – 37	15 – 22	23 – 30	31 – 37
MCHC (g/L)	346±21 (9.4)	322±24 (54.5)	307±21 (100)	351±22 (0.2)	349±21 (0.4)	347±17* (0.0)
pALP (IU/L)	9.1±2.2 (11.4)	58.7±11 (58.3)	96.6±18.7 (83.9)	9.7±2.9 (0.7)	64.3±10.1 (1.1)	103.2±15.1* (1.3)
pALP Reference range (IU/L)	8.0 – 15.0	56.0 – 80.0	100.0 – 130.0	8.0 – 15.0	56.0 – 80.0	100.0 – 130.0

Values are expressed as Mean ± SEM for 'n' subjects in each group; Values in parenthesis are percentages of subjects with low values; * Significantly higher when compared with corresponding value in group A subjects ($P < 0.05$)

Table 3: Placental and neonatal birth weights at delivery

Group	A (n = 552)	B (n = 451)
Placental weight at delivery (kg)	0.6±0.2	1.0±0.4
Neonatal birth weight at term (kg)	3.1±0.4	3.6±0.5

Values are expressed as Mean SEM for 'n' subjects per group., Group A – Low mean corpuscular haemoglobin concentration, MCHC (280 – 329 g/L) at delivery.; Group B – Normal mean corpuscular haemoglobin concentration, MCHC (330 – 370 g/L) at delivery.

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