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THE LEVEL OF HEAT-STABLE ALKALINE PHOSPHATASE IN SERUM OF SOME NIGERIAN PREGNANT WOMEN

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ABSTRACT: This study was conducted to monitor the changes in the level of heat-stable alkaline phosphatase, hsALP (principally secreted by placenta) in maternal serum during pregnancy, and to relate results to placental and birth weights. Sera samples used for the assay were collected at the 15-22, 23-30 and 31-37wk gestational age from the same set of 411 consenting 'normal' pregnant women in apparently good health. Two hundred and thirteen pregnant women with low mean corpuscular haemoglobin, MCH (< 27 pg) were included as control group. The subjects were drawn from seven tribal groups in Delta State. The results indicated that the percentage proportion of hsALP activity – the placental isotype in the maternal serum – was highest during the 31-37 gestational age (53%) when compared with the 15-22 (7.6%) and the 23-30 wk (40%) gestational values. The respective values for the low MCH group were 4.6%, 22.7% and 36.8%. Neonatal birth weight mean values (kg) for 'normal' and low MCH pregnancy are: M=3.4±0.6, F=3.0±0.8 vs M=2.7±0.7, F=2.6±0.6, respectively. For each parameter determined, tribal variations were minimal. The demonstrated trend suggests that changes in heat-stable alkaline phosphatase level support pregnancy. Thus, the established level of hsALP in maternal serum could be diagnostic in assessing foetoplacental development and maternal health among the Nigerian pregnant population.

Key words/phrases: Heat-stable alkaline phosphatase; Neonates; Nigeria Placenta; Pregnancy.

INTRODUCTION

The closest possible interaction between mother and foetus is provided by the placenta (WHO, 2001). The placenta is a complex organ and may exceed the liver in its multiplicity of roles. It produces hormones, transports nutrients, allows diffusion of gases and is rich in enzymes, which appear in the maternal blood. Alkaline phosphatase (ALP) is one of the enzymes associated with placental function, although it is an ubiquitous enzyme with several isoenzyme forms derived from the bone, liver, kidney, intestine, spleen and placenta (Ahmed, 1996). However, the placenta isoform differs from the other isotypes by being heat-stable (Bajoria *et al.*, 2001). The heat-

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stable alkaline phosphatase, originates from the placenta and this augments the maternal total serum ALP activity reported during pregnancy (Mode and Rosalki, 1963; Fishman, 1974; Bloom *et al.*, 2001).

For many years now, researchers have been searching for reliable methods to diagnose failing placenta function and foetal well being. Estimation of urine oestriol is sometimes used as a marker of placental function but this hormonal estimation is of little value in deducing foetal wastage (Goldenberg and Jobe, 2001), and it is not popular in our environment. Lately, the use of serum enzymes produced in the placenta is receiving attention, but available reports concentrated on the third trimester period of pregnancy (Vongthavaravat *et al.,* 2000), whereas, abnormalities in foetoplacental development may have originated during the first trimester (Hebisch *et al.,* 2000; Coustan *et al.,* 2001). This study therefore attempts to report the level of heat-stable alkaline phosphatase (hsALP) in maternal serum at different gestational periods, and the relation of the isoenzyme value to placental and birth weights at term. The study aims at deriving reference range for hsALP which could be used to monitor foetoplacental health and development.

SUBJECTS AND METHODS

Subjects

Four hundred and eleven women in apparently good health with uncomplicated pregnancies, and who were within 15-22 weeks gestational age were selected from clinics in the three Senatorial Districts in Delta State in order to include almost all the tribes in the state. Their social status, environment, age, previous births, and nutritional background varied minimally. Two hundred and thirteen pregnant women with low mean corpuscular haemoglobin, MCH (<27pg), an index of iron deficiency in our environment, were included as control subjects. Socio-demographic information was obtained from the participants via interviewer's administered questionnaire. Subjects' informed consent was sought and obtained, and Management Committee of the health care providers approved the research.

Collection of blood specimens

Venepuncture technique with the aid of syringe and needle was used to collect 4.0 ml whole blood sample at the 15-22, 23-30 and 31-37 gestational age ranges (weeks) usually after the health screening exercise. Two ml of whole blood sample was collected into a sterile plain bottle and allowed to

clot. This was centrifuged at 1,200 x g for 5min at room temperature. The supernatant (serum) was decanted into bijou bottle and then divided into two portions. The other 2.0 ml whole blood was dispensed into K-EDTA bottle for the estimation of MCH.

Analysis of specimens

One portion of the fresh serum was heated at 65^0C for 7min before assay. This thermal treatment denatures the other isoenzyme forms of ALP derived from other sources except the placental isoform. Heat-stable ALP value therefore, corresponds to the level of ALP from the placenta.

The ALP values in both portions of the sera samples were determined using the p-nitrophenylphosphate (pNPP) and diethanolamine (DEA) method (Bessey *et al.*, 1996). MCH of the K-EDTA sample was determined by haematology autoanalyser (Beekman Coulter Act Diff; Florida, USA). The placenta and neonatal birth weights at term were determined by standard weighing scale designed for such work.

Statistical analysis

Student's *t*-test analysis was used to compare the data obtained from the iron deficient pregnant subjects with those obtained from apparently healthy (non-iron deficient) pregnant individuals.

RESULTS

The results obtained are shown in Tables 1-3. Table 1 shows the changes in maternal serum ALP, level of hsALP in maternal serum, placental and birth weight measures obtained from 'normal' pregnant women across the tribes studied. Table 2 is the same analytical data for iron-deficient pregnant women. These tables show that iron-deficiency in pregnancy (MCH:<27pg; Table 2) lowers ALP, hsALP, placental and birth weights. There was no marked tribal differences ($p>0.05$), suggesting that tribe may not have significant impact on pregnancy outcome. Table 3 shows the socio-demographic information and the pooled analytical data for all the tribes. The table indicates that education, poverty, inadequate health care and poor health practices could contribute to poor dietary intake. Poor diet during pregnancy induces iron-deficiency in pregnancy, and cases are not uncommon especially among the rural dwellers (Table 3). Apart from reducing the proportion of hsALP in maternal serum, iron-deficiency can lead to low birth weight for gestational age (SGA) infants (Table 3).

Table 1 Maternal serum alkaline phosphatase values obtained from 'normal' pregnant women and birth weight measures at term.

Gestation age (wk)	n	Maternal Serum ALP Activity (IU/L)			
		Total ALP	hsALP	% Proportion of hsALP	MCH (pg)
15-22					
Tribe					
Ibo	70	148±9	13±2	8.8	27.8±1.3
Ijaw	50	151±11	10±3	6.6	28.3±3.2
Ika	71	145±8	14±3	9.7	28.7±2.8
Isoko	53	143±12	12±4	8.4	29.2±3.1
Itsekiri	52	138±10	11±5	8.6	28.5±2.7
Ukuani	55	150±11	10±4	6.7	29.3±2.1
Urhobo	60	142±9	9±3	6.3	28.8±2.5
23-30					
Ibo	70	196±14	80±6	40.8	29.3±2.1
Ijaw	50	202±16	86±7	42.6	30.2±3.2
Ika	71	206±12	81±8	39.3	30.0±2.5
Isoko	53	188±13	74±5	39.4	30.5±3.1
Itsekiri	52	181±11	71±7	39.2	29.8±2.5
Ukuani	55	184±14	77±6	41.8	30.2±3.2
Urhobo	60	179±13	73±8	40.8	30.4±2.4
31-37					
Ibo	70	235±15	115 ±17	48.9	29.1±2.3
Ijaw	50	241±13	126±18	52.3	29.8±2.4
Ika	71	243±16	133±11	54.7	30.3±3.1
Isoko	53	209±14	111±13	53.1	30.2±3.2
Itsekiri	52	211±13	109±12	51.7	29.5±2.6
Ukuani	55	219±14	116±13	53.0	30.6±3.3
Urhobo	60	217±16	121±15	55.8	30.2±2.4

	Birth weight measures							
	Neonatal birth weight at term (kg)				Placental weight (kg)			
	Male	n	Female	n	Male	n	Female	n
Ibo	3.7±1.1	28	3.4±0.6	42	0.7± 0.2	28	0.5±0.2	42
Ijaw	3.6±0.8	21	2.8±0.7	29	0.8 ±0.3	21	0.7±0.4	29
Ika	3.1±1.0	30	2.9±0.8	41	1.0 ±0.3	30	0.6±0.4	41
Isoko	2.9±0.5	28	3.0±1.1	25	0.9 ±0.2	28	1.0±0.3	25
Itsekiri	3.6±0.6	31	2.7±0.9	21	1.1 ±0.4	31	0.8±0.3	21
Ukuani	3.8±0.5	25	3.1±0.8	30	0.8 ±0.3	25	0.7±0.3	30
Urhobo	3.2±1.0	24	3.1±0.9	36	1.2 ±0.3	24	0.9±0.4	36

ALP – Alkaline phosphatase
hsALP – Heat-stable alkaline phosphatase
MCH – Mean corpuscular haemoglobin
Standard reference range: Neonatal birth weight (2.5 – 4.0kg)
ALP (30 – 130 IU/L for non-pregnant women)
MCH (≥27pg)

Table 2 Maternal serum alkaline phosphatase values obtained from iron-deficient pregnant women and birth weight measures at term.

Gestation age (wk)	n	Maternal Serum ALP Activity (IU/L)			
		Total ALP	hsALP	% Proportion of hsALP	MCH (pg)
15-22					
Tribe					
Ibo	36	118±6	5±3	4.2	25.6±2.3
Ijaw	28	121±8	4±2	3.3	26.3±2.1
Ika	32	126±7	6±3	4.8	27.3±2.5
Isoko	26	119±6	5±2	4.2	26.7±2.4
Itsekiri	29	123±5	6±2	4.9	26.5±3.1
Ukuani	38	125±6	7±3	5.6	26.1±2.0
Urhobo	24	122±7	6±2	4.9	25.9±1.8
23-30					
Ibo	36	126±8	27±5	21.4	24.4±1.3
Ijaw	28	131±6	30±6	22.9	25.2±1.6
Ika	32	138±7	35±6	25.4	24.8±1.2
Isoko	26	128±7	29±5	22.7	25.6±2.1
Itsekiri	29	136±8	32±4	23.5	25.3±2.3
Ukuani	38	134±6	30±5	22.4	24.5±2.4
Urhobo	24	137±7	28±6	20.4	24.9±1.7
31-37					
Ibo	36	144±10	55±8	38.2	24.6±1.2
Ijaw	28	148±12	48±6	32.4	23.5±1.1
Ika	32	156±14	58±7	37.2	23.1±2.4
Isoko	26	161±13	63±9	39.1	23.9±2.6
Itsekiri	29	159±11	57±7	35.9	23.1±1.7
Ukuani	38	168±12	61±8	36.3	23.1±2.2
Urhobo	24	170±13	66±9	38.8	23.4±1.3

	Birth weight measures							
	Neonatal birth weight at term (kg)				Placental weight (kg)			
	Male	n	Female	n	Male	n	Female	n
Ibo	2.8±0.8	15	2.5±0.6	16	0.5±0.1	15	0.4±0.1	16
Ijaw	3.0±0.9	12	2.6±0.7	18	0.6±0.2	12	0.5±0.1	18
Ika	2.6±0.1	17	2.6±0.5	20	0.7±0.1	17	0.7±0.2	20
Isoko	2.7±0.8	13	2.5±0.8	17	0.6±0.1	13	0.6±0.1	17
Itsekiri	2.5±0.5	10	2.8±0.4	15	0.9±0.2	10	0.5±0.1	15
Ukuani	2.9±0.6	12	2.7±0.6	18	0.6±0.1	12	0.8±0.2	18
Urhobo	2.6±0.5	11	2.6±0.5	19	0.7±0.1	11	0.7±0.2	19

ALP – Alkaline phosphatase
hsALP – Heat-stable alkaline phosphatase
MCH – Mean corpuscular haemoglobin
Standard reference range: Neonatal birth weight (2.5 – 4.0kg)
ALP (30 – 130 IU/L for non-pregnant women)
MCH (≥27pg)

Table 3 Socio-demographic information of subjects and pooled experimental data

	PERCENTAGE DISTRIBUTION OF PREGNANT WOMEN	
	'Normal pregnant women (n=411)	Iron-deficient pregnant women** (n=213)
Education		
None	-	23.9
Primary	12.2	39
Secondary	48.7	37.1
Tertiary	39.2	-
Household income (N)/month		
<10,000	-	32.9
10,00 – 49,999	26	43.7
50,000 – 99,999	46.2	23.5
100,000 and above	27.7	-
Diet		
Poor	2.7	47.4
Fairly adequate	24.3	43.7
Adequate	73	8.9
Settlement		
Rural	-	75.1
Suburban	29.4	24.9
Urban	70.6	-
Antenatal care		
Traditional	2.7	71.4
Orthodox	97.3	28.6
Previous birth(s)		
0	23.6	23.5
1	26.8	26.3
2	29.7	29.6
3	20	20.6
4	-	-
5	-	-
Age (yr)		
20-25	12.2	44.6
30-35	73.5	49.8
36-40	14.4	5.6
	***Analytical data	
Maternal Serum		
15-22 wk Gestation age	145±10(135-155)	122±6(115-130)
23-30 wk Gestation age	191±15(175-205)	133±7(125-140)
31-37 wk Gestation age	225±15(210-240)	158±12(145-170)
Maternal hsALP(IU/L)		
15-22 wk Gestation age	11±4(7-15)	6±2(4-10)
23-30 wk Gestation age	77±8(70-85)	*30±5(25-35)
31-37 wk Gestation age	119±13(105-135)	*58±8(50-65)
Proportion of hsALP in maternal serum (%)		
15-22 wk Gestation age	7.6±2.4(5-10)	4.6±1.8(2.5-6.5)
23-30 wk Gestation age	40±5(35-45)	22.7±2.7(20.0-25.5)
31-37 wk Gestation age	53±7(55-60)	36.8±3.3(33.5-40.0)
MCH (pg)		
15-22 wk Gestation age	28.7±2.5(25-31)	26.3±2.3(24.0-28.5)
23-30 wk Gestation age	30.1±2.6(27-33)	25.0±2.0(23.0-27.0)
31-37 wk Gestation age	30.5±2.8(27-33)	*23.5±1.8(21.0-25.5)
Neonatal birth weight (kg)		
Male	3.4±0.6(2.8-4.0)	2.7±0.7(2.0-3.5)
Female	3.0±0.8(2.2-3.8)	2.6±0.6(2.0-3.2)
Placental weight (kg)		
Male	0.9±0.3(0.6-1.20	0.7±0.1(0.6-0.8)
Female	0.7 0.3(0.4-1.0)	0.6±0.1(0.5-0.7)

ALP – Alkaline phosphatase
hsALP – Heat-stable alkaline phosphatase
MCH – Mean corpuscular haemoglobin
Standard reference range: Neonatal birth weight (2.5 – 4.0kg)
ALP (30 – 130 IU/L for non-pregnant women)
MCH (>27pg)
*Significantly reduced (P<0.05) when compared with values from 'normal' pregnant women
**Judged by MCH level
***Mean tribe values pooled and mean+SD then determined.

The serum total ALP level was highest and lowest during the third and first gestational age ranges, respectively. The hsALP mean at the third gestational age range for 'normal' pregnant women was significantly ($p<0.05$) higher than the iron-deficient pregnant subjects (Students *t*-test), and these appear to be influenced by the MCH (Table 3). Changes in MCH and hsALP affected birth weights.

DISCUSSION

The increase in serum ALP values obtained during pregnancy may be likely due to the synthesis of the placental isoform. Values of ALP in the sera of pregnant women were found to be statistically higher than the values of normal non-pregnant women (Ghosh and Fishman, 1969). Gosh and Fishman proposed that the enhanced level of the pregnant women could be due to the enrichment of the circulation with an isoenzyme of placental origin. Present observation (Table 1) further confirms earlier reports (Mode and Rosalki, 1963), and the results (Table 1) compare well with Caucasian values (Ghosh and Fishman, 1969; Vongthavaravat, *et al.*, 2000).

In this study, serum maternal heat-stable ALP level gradually rose to the peak during the third gestational age range (31 – 37 weeks). The low hsALP value in maternal serum observed during the first gestational age range (15 – 22 weeks) could be attributed to the degree of the enzyme's secretion, which usually begins late in the first trimester period (Lederman *et al.*, 1993). This has been claimed to be due to the presence of a macromolecule with the characteristics of a glucocorticoid receptor found in the human placental cytosol (Speeg and Harrison, 1979). However, in the second (23–30 weeks) and third (31–37 weeks) gestational age ranges, hsALP activity progressively increased in order to possibly meet the rising demands of the developing foetus. hsALP has been observed to contribute to pregnancy increase and maintenance (Mode and Rosalki, 1963). hsALP has been speculated to be involved in the transplacental transport of nutrients to the foetus for proper development. This study revealed that iron-deficiency anaemia (judged by MCH levels), significantly reduced the value of hsALP in maternal serum at the third (31 – 37 weeks) gestational age range and this appeared to affect neonatal and placental weights at term (Table 3). Our study indicated that settlement (rural, suburban and urban) and household income level influence diet (poor, fairly adequate or adequate), literacy level and antenatal care and these bear relationship to pregnancy outcome. These identified constellation of factors leading to poor antenatal care and poor dietary intake during pregnancy deserve attention because low birth weight

infants are more likely to have medical complications than normal weight infants (Worthington-Roberts and Williams, 1997), which experts say needs urgent address (Allen, 1997).

The demonstrated level of hsALP in maternal serum hereby established for some healthy ('normal') pregnant women in Nigeria could be useful in monitoring foetoplacental development and maternal health.

However, the socio-demographic factors now recognized to contribute to iron deficiency and hence low birth weight, should be further investigated in our environment in order to curb the complications associated with low birth weight arising from iron deficiency.

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