HEAT STABLE ALKALINE PHOSPHATASE ACTIVITY DURING PREGNANCY AND PUERPERIUM IN INDIAN WOMEN

by

E. PETERS,* F.R.C.O.G. (Lond.)

and

TARA PARIHAR**, M.S.

A number of enzymes previously thought to be homogeneous, may be fractionated into components of similar substrate specifications but differing in electrophoretic mobilities. These components have been termed as 'Isoenzymes" (Wroblewski *et al* 1960). One of the isoenzymes of alkaline phosphatase has been found to have a characteristic staining diffusible band situated at front of fast alpha 2 globulin (Meade and Rosalki 1963). This isoenzyme which remains unaffected by heating at 56° C for 30 minutes, is known as heat stable alkaline phosphatase. Mc Master et al (1964), have reported that the level of heat stable alkaline phosphatase in pregnant women progressively increases with advancement of pregnancy while the other fraction of alkaline phosphatase, known as heat labile, which is destroyed on heating at 56° c for 30 minutes, remains within normal range. A survey of literature shows that "Amritmahal *et al* (1950), 'Das and Bhagwanani (1964) and Chowdhury *et al* (1965) have studied the activity of total alkaline phosphatase in Indian pregnant women but none of them has undertaken the study of heat stable alkaline phosphatase.

Young *et al* (1946), report a slower decline in the activity of alkaline phosphatase after delivery. Similar observations have also been reported by Vermehren (1937) and Speert *at* al (1950), while Meranze *et al* (1937) report an abrupt fall in alkaline phosphatase activity to normal value after pregnancy.

Consequent to the conflicting reports regarding the maternal alkaline phosphatase activity after delivery and in view of the fact that heat stable alkaline phosphatase activity has not been studied under Indian conditions, this work has been designed and carried out to establish the normal of serum heat stable alkaline phosphatase level and also to get comprehensive idea about heat stable alkaline phosphatase activity in the maternal blood during pregnancy and puerperium and in the cord blood.

* *Prof. and Head, Dept. of Gynec. and Obst.*

** *Tutor in Gynec. and Obst.*

S. M. S. Medical College, Jaipur.

Received for publication on 11-8-67.

Material and Method

Cases were divided into six groups.

Group I :— 50 normal healthy males between the ages of 22-50 years.

Group II :— 50 normal healthy females between the ages of 22-50 years.

Group III :— 50 cases of normal pregnancy in the third trimester between the ages of 22-50 years.

Group IV :— 25 cases of severe pre-eclamptic toxaemia.

Group V :— 25 cases of mild pre-eclamptic toxaemia.

Group VI :— 25 cases of hypertensive toxaemia.

The subjects in the puerperium were selected from the cases of group III and were divided into two groups, lactating and non-lactating. Blood was drawn by venipuncture while samples of cord blood were drawn from the cord before the separation of placenta. Blood was allowed to clot at room temperature for half an hour. The clotted blood was centrifuged at 3000 r.p.m. for 10 minutes. The serum was separated. The estimation was done on the same day.

Heat labile and heat stable fractions of alkaline phosphatase were estimated by the method described by McMaster *et al* (1964).

Result and Discussion

TABLE I

Normal levels of serum heat stable alkaline phosphatase in Indian males and females

	Heat stable alkaline Phosphatase in K.A. units/100 c.c.
Males	0.66 ± 0.18 (50)
Non-pregnant females	0.63 ± 0.18 (50)

Normal levels

It is evident from Table I that serum heat stable alkaline phosphatase in Indian males and females are 0.66 ± 0.18 K.A. units and 0.63 ± 0.16 K.A. units respectively. The results are more or less in agreement with advocated normal values for western subjects (male = 0.6 ± 0.5; female = 0.7. ± 0.3; McMaster *et al* — 1964). No significant difference has been observed in the levels of heat stable alkaline phosphatase in normal males and normal non-pregnant females. Similar observations have been reported by McMaster *et al* (1964).

Normal Pregnancy

TABLE II

Heat labile and heat stable alkaline phosphatases levels in normal pregnant and non-pregnant women

	Alkaline phosphatase in K. A. units/100 c.c.	
	Heat stable	Heat labile
Non-pregnant women	0.63 ± 0.18 (50)	4.37 ± 0.52 (50)
pregnant women	7.13 ± 1.25 (50)	4.50 ± 0.50 (50)

No significant difference in the levels of heat labile alkaline phosphatase in normal non-pregnant females and pregnant females has been observed (Table II). This shows that heat labile alkaline phosphatase does not undergo any change during pregnancy. Further it is found that during pregnancy, the elevated maternal serum alkaline phosphatase level is entirely due to heat stable

isoenzyme of alkaline phosphatase. Similar findings have been reported by McMaster *et al* (1964).

The heat stable fraction of alkaline phosphatase circulating in the sera of pregnant women resembles placental alkaline phosphatase in its substrate specificity (Sadovsky and Zuckerman 1965), resistant to chemical inactivation (Kitchener *et al* 1965) and reacting with an anti-human placental alkaline phosphatase antibody (Birkett *et al* 1966). Thus it has placental origin. During the present study, the factor for converting the activity of enzyme from Bodansky units to K.A. units for placental alkaline phosphatase and serum heat stable alkaline phosphatase works out more or less the same (2.0 ± 0.1), while the same for serum heat labile alkaline phosphatase comes out to 2.6 ± 0.1. This further confirms the placental origin of heat stable isoenzyme of alkaline phosphatase. Similar substrate specificity of maternal serum alkaline phosphatase has been reported by Sadovsky and Zuckerman (1965). The villi of placenta are very rich in alkaline phosphatase and their number increases with the advancement of pregnancy. These villi lie in close contact with the maternal circulation. This causes an increase of heat stable alkaline phosphatase in sera of pregnant women.

It is observed that there is a progressive elevation of serum heat stable alkaline phosphatase with the increase of periods of amenorrhoea and the maximum value is reached by 40 weeks of gestation. Table III discloses that after 40 weeks of gestation, no significant change in maternal heat stable alkaline phosphatase takes place with the increase of period of amenorrhoea but the level becomes constant.

TABLE III

The relation between the period of amenorrhoea and serum heat stable alkaline phosphatase in normal pregnancy

Weeks of gestation	Heat stable alkaline phosphatase in K.A. units/100 c.c.
34-35	5.27 ± 0.23 (4)
36-37	6.02 ± 0.086 (5)
38-39	7.05 ± 0.92 (8)
40	7.48 ± .05 (22)
41-42	7.47 ± 0.70 (5)
43-45	7.49 ± 0.60 (6)

Table IV shows that the level of maternal serum heat stable alkaline phosphatase is in no way related to the age, socio economic status and the degree of gravidity of the mother at the same time period of gestation.

It is also evident from Table IV that there is no significant difference between the heat stable serum alkaline phosphatase activity of a woman bearing either male or female foetus at full term. Similar findings have been reported by Meranze *et al* (1937). Beck *et al* (1950), observed a highly significant differences between the total alkaline phosphatase activities of women bearing male children and those bearing female children. This may be due to the difference in placental weights as pointed out by Sinclair (1948).

TABLE IV

Showing the relation between serum heat stable alkaline phosphatase and socio economic status, age and gravidity of mother and sex of child

Variant	Alkaline phosphatase in K. A. units Heat stable			
Weeks: —	36-37	38-39	40-41	42-43
Socio economic Status				
Upper	—	7.53 ± 0.26	7.90 ± 0.18	—
Middle	6.02 ± 1.6	7.77 ± 0.16	7.28 ± 0.8	7.52 ± 0.35
Lower	6.0 ± 1.2	—	7.05 ± 1.2	7.80 ± 0.26
Age Groups				
15-20 Years	—	7.77 ± 1.34	7.75 ± 1.64	7.25 ± 0.15
21-25	6.02 ± 1.5	7.45 ± 0.79	7.48 ± 1.53	8.1 ± 1.2
26-30	—	—	6.17 ± 0.89	7.65 ± 0.15
31-40	—	5.7 ± 1.0	7.57 ± 0.43	7.59 ± 0.87
Gravidity				
Primigravida	7.2 ± 1.2	6.9 ± 0.75	7.66 ± 1.44	7.25 ± 0.05
Multigravida	5.16 ± 0.87	5.9 ± 1.2	7.65 ± 1.37	7.95 ± 0.015
Grand multigravida	6.0 ± 1.2	5.7 ± 0.98	6.08 ± 0.83	7.50 ± 1.2
Sex of child				
Male	6.6 ± 1.52	7.36 ± 1.08	7.45 ± 1.18	7.8 ± 1.2
Female	5.15 ± 0.98	7.45 ± 0.21	6.85 ± 0.87	7.52 ± 0.292

TABLE V

Comparing serum heat stable alkaline phosphatase level in normal, mild and severe pre-eclamptic toxaemia and hypertensive toxaemia

Duration of gestation in weeks	Heat stable alkaline phosphatase in K.A. units/100 c.c.			
	Normal	Mild toxaemia	Severe toxaemia	Hypertensive Toxaemia
34-35	5.2 ± 0.23 (4)	7.15 ± 0.82 (5)	7.45 ± 0.86 (3)	7.33 ± 0.5 (4)
36-37	0.62 ± 086 (5)	7.20 ± 0.71 (5)	7.52 ± 0.71 (4)	7.60 ± 0.42 (6)
38-39	7.05 ± 0.092 (8)	7.35 ± 0.85 (3)	7.47 ± 0.42 (5)	7.61 ± 0.42
40-41	7.48 ± 0.5 (26)	7.38 ± 0.71 (12)	7.58 ± 0.62 (13)	7.51 ± 0.51 (10)

It is evident from Table V that in the cases of both mild and severe pre-eclamptic toxaemia and hypertensive toxaemia, serum heat stable alkaline phosphatase levels in earlier weeks are equal to those found at full-term in normal pregnancy within experimental errors, but it is considerably different when compared with the levels of the same gestation

period. Mukherjee (1951) observed statistically significant differences in the levels of total alkaline phosphatase between normal, mild and severe eclampsia while Meranze *et al* (1937), Das and Bhagwanani (1964); Arthur *et al* (1961) and Curzen (1965) report that no apparent or statistically significant differences exist in the levels in the case of normal, mild and severe pre-eclamptic toxaemia and hypertensive toxaemia. Thus the observations of the present study are in close agreement with the observations of Meranze *et al* (1937), Das and Bhagwanani (1964); Arthur *et al* (1961) and Curzen (1965).

Puerperium

Twenty-five women were selected for the study of heat stable alkaline phosphatase level in puerperium. Fifteen of them were lactating and ten were non-lactating.

In 60 per cent of the cases studied, an abrupt fall of heat stable alkaline phosphatase was observed while in the rest, there was a gradual fall of the level of heat stable alkaline phosphatase. Fig. 1 plots the representative data showing abrupt and gradual fall. Meranze *et al* (1937) observed the abrupt fall while Vermehren (1939), Speert *et al* (1950) and Young *et al* (1946) reported a gradual fall of alkaline phosphatase level in puerperium. The present study reveals that both abrupt and gradual falls of maternal heat stable alkaline phosphatase activity take place after delivery in lactating as well non-lactating women.

Maternal and cord blood at delivery

The average value of heat stable

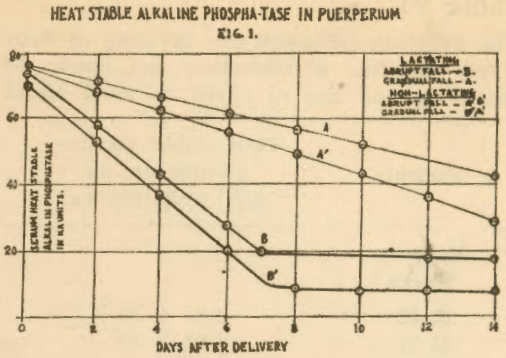

Fig. 1

alkaline phosphatase in cord blood was found to be 0.40 ± 0.1 K.A. units. It is lower than that of maternal blood (7.3 ± 1.25 K.A. units). Similar low level of total alkaline phosphatase in cord blood as compared to that of maternal serum, has been observed by Stearns and Warweg (1933), Meranze *et al* (1937) and Kerleau *et al* (1939). It has been shown by electron microscopy that E. Coli alkaline phosphatase is situated in an intermediate layer of the cell walls just inside the outer membrane. If an analogous situation were to exist in the cases of human placenta, it is reasonable to suppose that phosphatase would gain access to maternal and not to the foetal circulation as such different levels exist in mother and foetus.

Further by comparing the levels of heat stable alkaline phosphatase in maternal (7.3 ± 1.25 K.A. unit) and foetal blood (0.4 ± 0.1 K.A. units) it would seem reasonable to assume that osseous activity in the mother at the end of pregnancy is at a higher level than in the foetus. Hence the higher enzyme levels exists in the mother.

Table VI

The relation between the activity of heat stable alkaline phosphatase and weeks of maturity and sex of child, in cord blood

Variant	Heat stable alkaline phosphatase in K.A. units/100 c.c.
Maturity Weeks	
32-33	0.2 ± .01
36-37	0.37 ± .01
40-41	0.40 ± .01
Sex of child	
Male	0.46 ± 0.1
Female	0.31 ± 0.1

Table VI discloses that there is no significant difference in heat stable alkaline phosphatase levels in cord blood of the cases bearing either a male child or a female child. This shows that sex of a child does not affect the level of heat stable alkaline phosphatase in cord blood. Further it is observed that the level of heat stable alkaline phosphatase in cord blood increases with increased maturity.

Fig 2 is a scattergram with the maternal heat stable alkaline phosphatase as the *abscissa* and corresponding cord/mother ratio as the ordinate. The intersecting lines in the graph are drawn at the mother average enzyme level as the vertical line and the average cord/mother ratio as a horizontal line. The diagram shows that all the points are concenterated in all the quadrants close to the horizontal line. This shows that there is a clear co-relation between the maternal and cord levels.

It seems that earlier workers (Speert *et al* (1950); Lapan *et al*

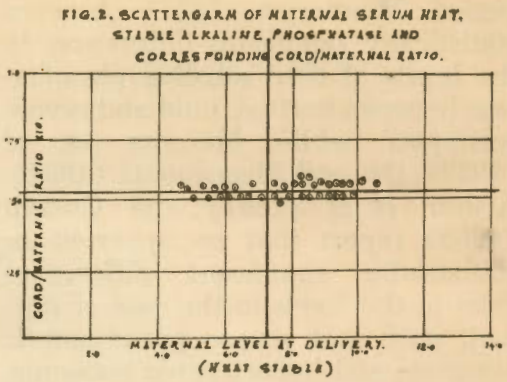

Fig. 2

(1959), Chowdhury *et al* (1965) could not observe such co-relation because they tried to correlate cord blood level with the sum of maternal heat stable and heat labile alkaline phosphatase while the latter does not undergo any change during pregnancy.

Summary and Conclusion:—

(i) Serum heat stable alkaline phosphatase activity was studied in normal males, healthy non-pregnant women and in cases of normal and abnormal pregnancy.

(ii) The range of heat stable alkaline phosphatase for males has been reported as 0.4 to 0.1 K.A. units while it was from 0.3 to 0.9 K.A. units for non-pregnant healthy females.

(iii) During pregnancy, heat stable alkaline phosphatase was found to be increasing while heat labile phosphatase remained more or less constant.

(iv) The average value of heat stable alkaline phosphatase for pregnant women, in the third trimester, was 7.52 ± 1.3 K.A. units.

(v) No relation was found between age and socio-economic status of the

mother and serum heat stable alkaline phosphatase level at term.

(vi) Neither the weight nor the sex of a child was found to affect maternal serum heat stable alkaline phosphatase.

(vii) The degree of gravidity was not related to the level of maternal serum heat stable alkaline phosphatase.

(viii) There was no significant difference in the serum heat stable alkaline phosphatase level in the cases of mild, severe, pre-eclamptic and hypertensive toxaemic pregnancy as compared to the value of normal pregnancy.

(ix) Both gradual and abrupt falls in the level of maternal serum heat stable alkaline phosphatase at postpartum were observed.

(x) The average value of heat stable alkaline phosphatase in cord blood was 0.4 ± 0.1 K.A. units.

(xi) Neither the weight nor the sex of a child was found to affect the activity of heat stable alkaline phosphatase in cord blood.

Acknowledgement

The authors wish to express thanks to Dr. M. L. Gupta, M.D., F.A.M.S., Professor and Head, Department of Physiology and Bio-Chemistry, S.M.S. Medical College, Jaipur for providing laboratory facilities for the present study.

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