SERUM HEAT STABLE ALKALINE PHOSPHATASE—AN INDEX OF PLACENTAL FUNCTION IN PREGNANCY

by

URMILA KAPOOR,* M.D., D.G.O.,

and

H. C. MEHTA,** M.Sc. (Honours),

For many years obstetricians and biochemists have been searching for reliable methods to help in detecting a failing placental function in an effort to reduce perinatal mortality and morbidity. The problem has been tackled in two ways:

(i) Estimation of urinary excretion of hormones and

(ii) Estimation of placental enzymes in serum.

Estimation of hormones in the urine as well as enzymes in the serum, except heat stable alkaline phosphatase (HSAP), have not given consistent results. While various workers have reported a progressive rise in HSAP with the progress of pregnancy, the values of HSAP reported differ from one worker to another. This is due to difference in method, pH used and the temperature at which the serum was heated. Curzen and Morris (1966) assumed the critical temperature of HSAP as 56°C. However, Hunter, a year later, reported a better correlation between the HSAP values and the foetal outcome if the serum was heated at 65°C.

*Asst. Professor, Dept. of Obstetrics and Gynaecology, Medical College and Hospital, Rohtak.

**Assistant Research Officer, Department of Obstetrics and Gynaecology, Medical College and Hospital, Rohtak.

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As the HSAP values differ from week to week and no serial work on the subject under the optimum standard conditions has been done in India so far, the study was undertaken with the following objects:

(i) To standardise the technique for estimation of heat stable alkaline phosphatase; and

(ii) To determine the values of HSAP in different weeks of normal pregnancy in North Indian women, to serve as a reference for placental function.

Material and Methods

The following subjects from the Medical College Hospital, Rohtak constituted the case material for the study:

(1) Thirty women with gestation period of 20-24 weeks.
(2) Twenty women with gestation period of 24-28 weeks.
(3) Thirty women with gestation period of 28-32 weeks.
(4) Thirty women with gestation period of 32-36 weeks.
(5) Twenty women with gestation period of 36-40 weeks.
(6) Fifty women during labour.
(7) Fifty newborn infants.
(8) Fifty women 48 hours after delivery.
(9) Ten males.
(10) Ten non-pregnant females.

The blood was obtained by venepuncture in the case of adults and from the umbilical cord of neonates.

The blood was allowed to clot and serum was diluted with an equal volume of distilled water. It was then heated in a waterbath at 65° for 30 minutes. The serum was cooled and used for HSAP estimation.

HSAP activity was determined by using diphenylphosphate as the substrate and the phenol liberated was estimated by reaction with aminoantipyrine, according to King's method (King 1951); but the pH used was 10.4 and substrate concentration 50mM.

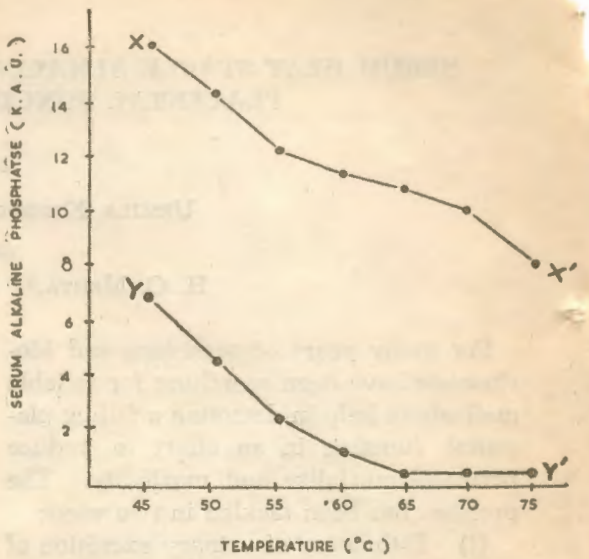

FIG. 1. SERUM ALKALINE PHOSPHATASE ACTIVITY AFTER HEATING AT DIFFERENT TEMPERATURES.

X X' REPRESENTS SAP LEVELS IN PREGNANT FEMALES AT 34 WEEKS GESTATION

Y Y' REPRESENTS SAP LEVELS IN MALES AND NON PREGNANT FEMALES.

Results

Serum alkaline phosphatase activity in samples taken from males and non-pregnant females, heated at different temperatures is given in Table I and graphically depicted in fig. No. I.

TABLE I

Serum Alkaline Phosphatase Activity at Different Heating Temperatures (K.A.U.)

S. No.	Temperature	HSAP in males and n.p. females (Mean of 5 cases)	HSAP in pregnant females (g.p. 34 weeks) (Mean of 5 cases)
1.	45	6.8	16.2
2.	50	4.65	14.4
3.	55	2.42	12.1
4.	60	1.3	11.3
5.	65	0.5	10.8
6.	70	0.4	10.0
7.	75	0.4	7.8

The Table reveals that heat labile alkaline phosphatase disappears at 65°. At 56° however, this fraction is active to some extent and studies of HSAP at this temperature (as carried out by Curzen, 1966) are not of true HSAP. Further, when samples from pregnant females were heated at various temperatures, even HSAP activity decreased beyond 65°. It would, therefore, appear that critical temperature for HSAP is 65° and not 56°. This observation is in accordance with that of Hunter (1969).

HSAP activity estimated at different pH and with 50 mM substrate concentration is depicted in Table II.

TABLE II

HSAP Values at Different pH (In women with g.p. 38 weeks)

S. No.	pH	Mean value of HSAP (K.A.U.) (5 cases)	Significance compared to values at pH 10.4 (By 'T' method)
1.	10.0	12.4	Non-significant (P >.05)
2.	10.2	12.9	Non-significant (P >.05)
3.	10.4	13.9	—
4.	10.6	12.8	Non-significant (P >.05)
5.	10.8	11.9	Significant (P >.05)
6.	11.0	10.85	Significant (P >.05)

It can be noted from the table that maximum HSAP activity is realised at pH 10.4. Though the difference in values obtained at pH 10.0, 10.2 and 10.6 were not of statistical significance, the values obtained at pH 10.8 and 11.0 were statistically significantly different from those obtained at pH 10.4.

The values of HSAP with the use of various substrate concentrations and pH 10.4 are given in Table III.

The data indicate the maximum values of HSAP when a substrate concentration of 50 mM or more was used. The difference of values obtained by using 10 mM substrate concentration was statistically significant, whereas that of the value at 25 mM was not.

HSAP values obtained in different groups of normal pregnant women are tabulated in Table IV and have been graphically depicted in fig. II.

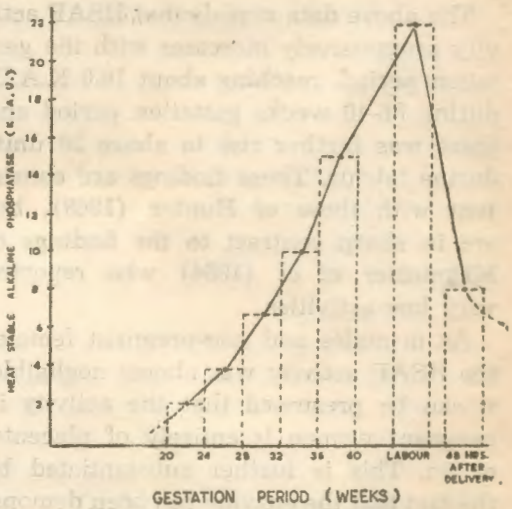

FIG. 2 HASP ACTIVITY IN NORMAL PREGNANT FEMALES AT DIFFERENT STAGES OF GESTATION.

TABLE III

HSAP Values with Different Substrate Concentration (In women with g.p. 28 weeks)

S. No.	Substrate concentration (mM)	Mean value of HSAP (K.A.U.) 5 cases)	Significance as compared to values with 50 mM substrate concentration (By 'T' method)
1.	10	3.6	Significant (P <.05)
2.	25	4.1	Non-significant (P >.05)
3.	50	4.8	—
4.	75	4.8	Nil
5.	100	4.75	Nil

TABLE IV

HSAP Values (K.A.U.) in Various Groups of Patients

S. No.	Group	Range	Mean	S.D.
1.	Normal females (g.p. 20-24 weeks)	1.2 to 4.4	2.25	0.84
2.	Normal females (g.p. 24-28 weeks)	2.1 to 6.0	3.96	1.2
3.	Normal females (g.p. 28-32 weeks)	2.8 to 12.0	6.75	2.5
4.	Normal females (g.p. 32-36 weeks)	6.1 to 22.7	10.53	4.2
5.	Normal females (g.p. 36-40 weeks)	8.5 to 24.8	15.70	4.2
6.	Normal females during labour	13.0 to 34.0	21.40	5.3
7.	Normal females, 48 hrs. after delivery	2.3 to 16.0	8.3	2.9
8.	Newborns (Cord blood)	0.7 to 4.0	1.82	0.64
9.	Males	0.5 to 0.8	0.66	0.08
10.	Non-pregnant females	0.5 to 0.9	0.70	0.09

Discussion

The above data reveals that HSAP activity progressively increases with the gestation period, reaching about 16.0 K.A.U during 36-40 weeks gestation period and there was further rise to above 20 units during labour. These findings are consistent with those of Hunter (1969), but are in sharp contrast to the findings of Mckmaster *et al* (1964) who reported very low activities.

As in males and non-pregnant females the HSAP activity was almost negligible, it can be presumed that the activity in pregnant women is entirely of placental origin. This is further substantiated by the fact that the enzyme has been demonstrated in the placental tissue itself by Mckmaster *et al* (1969)

It was also found that HSAP activity decreased after labour and 48 hours after delivery it was considerably low. In the newborns, however, HSAP activity was insignificant as compared to mothers. It can thus be concluded that HSAP activity increases with the placental function and vice versa. The almost absence of HSAP in newborns is explainable on the basis of histochemical studies. These studies suggest that HSAP is present mainly in the microvilli of the syncytiotrophoblast (Lobel *et al*, 1962), which are in close proximity to the maternal blood, but are separated from the foetal circulation by a number of structures (Wisloki & Padyakula, 1961). It is possible that the material derived from the microvilli goes to the maternal rather than foetal circulation. Another cause of the low HSAP activity in newborns may be the increased breakdown of the HSAP by the foetus resulting in lower concentrations in foetal blood.

Preliminary work shows that levels of HSAP correlate well with abnormal placental function. The clinical implications of this are under study.

Summary

Serum heat stable alkaline phosphatase (HSAP) levels were determined to serve as an index of placental function in normal pregnant women of North India. One hundred and twenty-five women at various stages of gestation were studied for HSAP activity. A progressive rise in HSAP (with advancement of pregnancy) was noted. In males and non-pregnant females HSAP activity was negligible, revealing thereby that HSAP was of placental origin. Still higher values of HSAP during labour but very low values of

HSAP in the newborns, offer a further proof of its placental origin. In some abnormal cases HSAP levels were found to vary considerably from those in normals, depending upon the state of placenta and its function.

The critical temperature for the enzyme (HSAP) was found to be 65°C and the optimum pH and substrate concentration noted were 10.4 and 50 mm respectively.

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