AMNIOTIC FLUID ALKALINE PHOSPHATASES IN NORMAL PREGNANCY AND PRE-ECLAMPTIC TOXAEMIA

by

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The non-specific phosphomonoesterase (alkaline phosphatase-orthophosphomonoester phosphohydrolase-E.C. No. 3.1.3.1) of amniotic fluid could be detected as early as twelve weeks of pregnancy (Nadler and Gerbie, 1969). However, amnion cells cultivated in vitro do not show any detectable alkaline phosphatase activity (Kellen *et al.*, 1970). Nevertheless elevated levels of alkaline phosphatase in amniotic fluid are claimed to be of diagnostic value—as an indication of foetal distress (Kellen *et al.*, 1970, Geyer and Schmeider, 1970) and a possible index for the assessment of foetal maturity (Sutcliff *et al.*, 1972). A statistically significant correlation between maternal and amniotic fluid heat stable alkaline phosphatase (HSAP) has been observed by Singh and Matadial (1972). They have reported a statistically significant elevation of HSAP in the amniotic fluid of pre-eclamptic toxaemic patients as compared to normal pregnant women.

The present investigation was, therefore, undertaken to study, the total heat stable and heat labile alkaline phosphatase in amniotic fluid as compared to maternal and foetal status in normal pregnancy and pre-eclampsia complicating pregnancy.

Material and Methods

Thirty cases each of normal pregnancy and pre-eclamptic toxaemia were selected at the time of admission for labour. Diagnosis of pre-eclampsia was made when two out of three signs i.e., hypertension, oedema or proteinuria were present. All these cases had been having treatment in the form of rest, salt restricted diet, sedatives, diuretics and hypotensive drugs as indicated for varying periods prior to labour. Further the patients with pre-eclampsia were divided into two groups, one group of 15 cases having mild pre-eclampsia with blood pressure above 140/90 mm Hg. and oedema and a second group of 15 cases having in addition proteinuria labelled as severe pre-eclampsia. Blood samples were collected during labour from the mother and cord blood from the umbilical vein. The placenta and amniotic fluid free of meconium were collected under sterile conditions. Sera were separated from blood samples and preserved at 4°C till used. The placentae were washed thoroughly with ice-cold normal saline till free of blood and ex-

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—Abbreviations used—TAP—Total alkaline phosphatase; HSAP Heat stable alkaline phosphatase; HLAP Heat labile alkline phosphatase.
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traneous materials and kept frozen at—2°C.A 10% aqueous placental homogenate was used for the enzyme studies. The enzyme activities were determined using 0.01 M phenyl phosphate as substrate in 0.1 M carbonate—bicarbonate buffer at pH 10 and temperature 37° according to the method of King and King (1954) as described in an earlier communication (Tiwana *et al.*, 1971). The heat stable enzyme was prepared by heating at 56°C instead of 65°C (Hunter, 1969) in order that comparison with previous work reported could be made (Tiwana *et al.*, 1971; Sandhoo and Amma, 1972). The enzyme activities are expressed as King Armstrong units (KAU) as milligram phenol liberated in 15 minutes at 37°C by 100 ml. of sera or amniotic fluid or by one gram of wet placental tissue under standard experimental conditions.

Results

The foetal-placental weight in mild and severe pre-eclampsia are shown in Table I.

The mean weights of placenta as well as the newborn were significantly reduced ($P<0.001$) in severe pre-eclampsia as compared to mild pre-eclampsia.

The total and heat stable fractions in the amniotic fluid in severe pre-eclampsia showed a significant increase ($P<0.01$ and $P<0.005$) when compared to the other two groups. No significant differences were observed in the heat labile fractions between the two groups.

The mean total alkaline phosphatase in maternal serum showed significant decrease in both mild and severe pre-eclampsia when compared to normal pregnant women ($P<0.01$). The heat stable fraction showed a decrease in mild

TABLE I

Placental-Foetal Weight in Pre-eclampsia

		Mild pre-eclampsia (15 cases)	Severe pre-eclampsia	Remarks
Placenta (wt. in gms.)	Range	350–400	250–450	$p<0.001$
	Mean	377.3	350	
	S.D.	23.05	53.18	
Newborn (wt. in gms.)	Range	2200–3500	2000–3500	$p<0.001$
	Mean	2960	2539	
	S.D.	468.7	508.9	

TABLE II

Mean Total, Heat Stable and Heat Labile Phosphatase in Amniotic Fluid in Normal Pregnancy and Pre-eclampsia

Status of subjects	No.	Total A.P.	HSAP	HLAP
Normal pregnancy	18	7.78 ±2.10	2.86 ±1.60	4.97 ±1.50
Mild pre-eclampsia	10	7.83 ±2.69	2.91 ±2.26	4.92 ±2.02
Severe pre-eclampsia	10	9.67	3.93 ±1.18	5.74 ±2.60

TABLE III

Mean Total, Heat Stable and Heat Labile Alkaline Phosphatase in Maternal Serum During Labour

Status of subjects	No.	Total A.P.	HSAP	HLAP
Normal pregnancy	30	22.36 ±10.54	12.27 ± 5.76	10.90 ± 6.09
Mild pre-eclampsia	15	15.31 ± 3.43	9.91 ± 3.54	5.40 ± 1.29
Severe pre-eclampsia	15	19.84 ± 5.35	12.61 ± 3.54	7.23 ± 3.54

pre-eclampsia (<0.05) but not in severe pre-eclampsia. Hence the decrease in the total A.P. activity in mild and severe pre-eclampsia is accounted for by a pronounced decrease in the heat labile fraction ($P<0.001$). In severe pre-eclampsia, the values for total, heat stable as well as the heat labile fractions were higher than in mind pre-eclampsia ($P<0.001$).

The total and heat stable fractions ($P<0.001$) but the differences were not related to the severity of the disease. The heat stable fraction was found to be relatively more affected in cord blood sera of severe pre-eclampsia ($P<0.001$). The heat labile fraction was lower in both mild and severe pre-eclampsia ($P<0.001$).

TABLE IV

Mean Total, Heat Stable and Heat Labile Alkaline Phosphatase in Cord Blood Sera

Status of subjects	No.	Total A.P.	HSAP	HLAP
Normal pregnancy	28	17.33 ± 6.07	1.19 ±1.74	16.14 ± 6.24
Mild pre-eclampsia	15	13.82 ± 4.25	0.49 ±0.64	12.53 ± 4.62
Severe pre-eclampsia	15	14.76 ± 5.38	0.14 ±0.24	14.62 ± 5.36

The total alkaline phosphatase activity of cord blood sera was significantly lower in mild and severe pre-eclampsia showed an increase in the placenta from both mild and severe pre-eclampsia ($P<0.001$) though the increase was not

TABLE V

Mean Total, Heat Stable and Heat Labile Alkaline Phosphatase in Cord Blood Sera

Status of subjects	No.	Total A.P.	HSAP	HLAP
Normal pregnancy	30	17.66 ± 6.04	14.11 ± 9.04	3.02 ±0.53
Mild pre-eclampsia	15	26.73 ± 9.28	26.35 ± 9.50	0.34 ±0.26
Severe pre-eclampsia	15	22.25 ± 7.25	22.00 ± 8.54	0.25 ±0.72

related to the severity of the disease. Corresponding decrease was observed in the heat labile fraction ($P<0.001$) though the decrease was not related to the severity of the disease.

Matadial, (1972). Raised levels of HSAP (3.93 ± 18 KAU) were observed in severe pre-eclampsia but not in mild pre-eclampsia (2.91 ± 2.26). The source of the raised HSAP in amniotic fluid in cases

TABLE VI

Mean Total and Heat Stable A.P. in Amniotic Fluid, Maternal Serum and Placenta

	Normal pregnancy (30 cases)	Mild pre-eclampsia (15 cases)	Severe pre-eclampsia (15 cases)
Amniotic fluid			
Total A.P.	7.78	7.83	9.67
HSAP	2.86	2.91	3.93
Cord Blood			
Total A.P.	17.33	13.82	14.76
HSAP	1.19	0.49	0.14
Maternal serum			
Total A.P.	22.36	15.31	19.84
HSAP	12.27	9.91	12.61
Placenta			
Total A.P.	17.66	26.73	22.25
HSAP	14.11	26.35	22.00

The total alkaline phosphatase is lowest in amniotic fluid in all groups of cases. In the cord blood the heat labile fraction is the major component, whereas in the placenta the heat stable fraction is the major component.

Discussion

The alkaline phosphatases of amniotic fluid resembled the maternal sera qualitatively in its thermal stability. The heat stable fraction accounted for 36.7%, 37.2% and 40.6% of the total alkaline phosphatase activity in amniotic fluid obtained from cases of normal pregnancy, mild and severe pre-eclampsia, respectively. Kellen *et al.*, (1970) found 30% HSAP in amniotic fluid from normal pregnant women. The mean value for HSAP in amniotic fluid in normal pregnancy observed (2.86 ± 1.6 KAU per 100 ml) is similar to that reported by Singh and

of severe pre-eclampsia appears to be from the placenta rather than the maternal blood, since significantly higher values for HSAP were seen in placental but not in maternal blood in cases of severe pre-eclampsia studied. A greater retention of HSAP seen in placentae in severe pre-eclampsia with a parallel decrease in the sera of cord blood agrees well with the vascular changes in the placenta associated with this disorder (Amma *et al.*, 1971, 1973).

Although the source of heat labile alkaline phosphatase, both in maternal and cord blood is not well delineated, it showed a parallel change with that of amniotic fluid. The differences in the heat labile enzyme in amniotic fluid in mild and severe forms of pre-eclampsia are not significant. As reported previously from this laboratory, (Sandhoo and Amma, 1972, 1973) both maternal and cord blood showed significant decrease in HSAP

activity in pre-eclampsia as compared to normal pregnant women.

The foregoing data thus indicate that the amniotic fluid heat stable alkaline phosphatase could be of value in assessing the severity of pre-eclamptic toxaemia in addition to the assessment of foetal maturity (Sutcliff *et al.*, 1972). The distribution pattern of HSAP and HLAP in the maternal placental foetal compartments also explains the changes in maternal sera observed in pre-eclampsia (Hunter, 1969; Curzen and Southcombe, 1970). Curzen and Hensel, (1972) suggested that the unequal expulsion of the enzyme protein might be the reason why no correlation was observed between serum HSAP and urinary oestrogens.

Summary

1. The non-specific phosphomonoesterase (Alkaline phosphatases) of amniotic fluid were studied from 15 patients each of mild and severe pre-eclampsia and compared with 30 cases of normal pregnancy.

2. The changes in the total, heat stable and heat labile enzyme in maternal and cord blood sera as well as placental homogenates were studied in the same groups.

3. The placental-foetal weight ratios were observed in the same groups of subjects.

4. In mild pre-eclampsia, the following features were observed, (a) no changes in total or HSAP in amniotic fluid, (b) decrease in total and HSAP in maternal serum, (c) decrease in total HSAP and HLAP in cord blood, (d) increase in total and HSAP of placenta.

5. In severe pre-eclampsia, the features observed were, (a) Significant increase in total and HSAP in the amniotic fluid, (b) decrease in total A.P. and HSAP in maternal serum, (c) decrease in total AP, HSAP and HLAP in cord blood, (d) increase in total and HSAP in placenta.

6. The significance of these changes is discussed.

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