SIGNIFICANCE OF CHANGES IN LEUCOCYTE ALKALINE PHOSPHATASE IN ABORTION

by

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Increased leucocyte alkaline phosphatase (LAP) activity during pregnancy was reported by Valentine and Beck (1957) with the use of a quantitative chemical technique. Their findings were confirmed by Pritchard (1957) in a large series, who also demonstrated that there is a consistent rise in LAP activity throughout pregnancy and a return to the non-pregnant level within 6 to 7 weeks postpartum.

The study of Quigley *et al* (1960) by the semi-quantitative cytochemical technique of Kaplow (1955) in 138 women during various stages of pregnancy further substantiated Pritchard's findings, showed an elevated level of LAP activity as early as 18th, 24th and 30th days of gestation.

Harper and Quigley (1961) then explored the possibility of applying LAP test to the diagnosis of early pregnancy and found 100 per cent correlation in 70 women who were tested in less than 70 days from the first day of the last menstrual period. The study of Climie *et al* (1962) also showed 100 per cent correlation between elevated LAP activity and pregnancy in 63 women with normal intrauterine pregnancies who were tested after 7 weeks of gestation.

The present study was carried out to evaluate the role of leucocyte alkaline phosphatase (LAP) to measure the placental function and to determine the prognosis of abortion.

Material and Methods

LAP activity estimation was done in 40 normal women at various phases of gestation and 113 cases of different types of abortion which include, (a) threatened, (b) missed, (c) incomplete and (d) inevitable. The patients were taken from the antenatal, out-patients' department, labour room and wards of the Department of Obstetrics and Gynaecology, Patna Medical College & Hospital, Patna.

Normal pregnant women were studied during the period of gestation from 8 to 40 weeks and they served as control. For the sake of convenience these cases were further sub-divided into the following groups:-

Group I—8 to 15 weeks, Group II—16 to 23 weeks, Group III—24 to 31 weeks, and Group IV—32 to 40 weeks.

Cytochemical Demonstration of Leucocyte Alkaline Phosphatase

It is believed that alkaline phosphatase participate in both protein and carbohydrate metabolisms within the cell. The cytochemical demonstration of alkaline phosphatase depends upon the formation of an insoluble, coloured precipitate at

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the site in which a substrate has been hydrolysed. The site of hydrolysis shows the site of enzyme activity and hydrolysis takes place when the tissue is incubated with an organic phosphate ester at pH 9 or higher. The method adopted was a modification of original Takamutsu histochemical method with Azodye coupling technique.

Collection of Blood: Thin smears were made on dry, clean glass slides and dried in the air.

Reagents: (a) Fixative solution—10 ml. of 37% formaldehyde was added to 90 ml. of absolute methanol and kept in the refrigerator between uses (b) stock —0.2M Propanediol solution—10.5 gm. of 2-amino-2 methyl 1, 3 propanediol was added to 500 ml. of distilled water and stored in refrigerator (c) working 0.05m propanediol buffer pH 9.75 to 25 ml of stock solution (d) 5 ml. of 0.1 HCl was added and diluted to 100 ml. with distilled water. This was also stored in the refrigerator, (e) substrate mixture—35 mg. of sodium alphanaphthyl acid phosphate and 35 mg. of fast blue RR, were added to 35 ml. of working buffer, filtered and used at once. The pH was 9.5 to 9.6, and (f) lastly the slides were counterstained with Mayer's Aqueous haemotoxylin.

Procedure: Blood smeared slides were immersed in the fixative solution at ice-box temperature for 30 seconds. Then these slides were incubated in the substrate mixture for 10 minutes at room temperature, washed in running tap-water for 10 seconds and dried. After that the slides were counter-stained with Mayer's haemotoxylin stain. Under oil immersion, 100 successive polymorphonuclear leucocytes were counted with grading as follows:

Zero — Colourless cytoplasm (Microphotograph No. I)

One — Diffuse pale brown cytoplasm, no granules (Microphotograph No. II)

Two — Brown with or without occasional clumps of brownish black precipitate (Microphotograph No. III)

Three — Brownish black, unevenly distributed granular precipitate (Microphotograph No. IV)

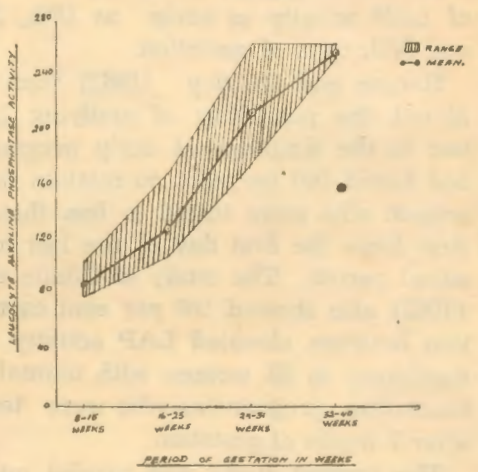
Four — Uniform deep black granular precipitate (Microphotograph No. V).

100 consecutive cells were counted and the sum of scores of the cells was considered the score for that slide.

Graph I gives the LAP activities between 8th to 40th week of gestation.

GRAPH I

SHOWING THE MEAN AND RANGE LEUCOLYTE ALKALINE PHOSPHATASE ACTIVITY OF NORMAL PREGNANCY.



In normal pregnancy LAP activities were estimated from 8th week of pregnancy. Progressive rise of LAP scoring

was accompanied by the advancement of pregnancy. A gradual rise upto 23 weeks followed by a steeper rise upto 30 weeks was observed. From 31 weeks onwards the LAP remained relatively constant at that level.

TABLE I

LAP Activities in Different Groups of Normal Pregnancy in 40 Cases

Groups of normal pregnancy in weeks	L A P activities			
	Range	Average	S.D.	S.E.M.
Group I (8-15)	80-106	89	7.94	2.38
Group II (16-23)	108-170	130	20.70	6.21
Group III (24-31)	178-265	215	29.62	8.89
Group IV (32-40)	255-265	259	3.32	1.00

LAP activity started appearing after 8 weeks of pregnancy followed by progressive rise. Steep rise was marked during 24 to 31 weeks and after that level is continued relatively at a constant level, though the mean was raised.

there is very little diminution in LAP activity. In cases of incomplete abortion the LAP picture is not conclusive. In 50 per cent of the cases there is marked diminution in the LAP range, while in 50 per cent of the cases range of diminution is only 10 to 25%. This has been explained as due to the stilll active placental function. In all the cases of threatened abortion which ended in inevitable abortion there is marked diminution of LAP activity. In cases of

TABLE II

Shows the Distribution of Types of Abortion of Weeks of Pregnancy in 113 Cases

Types of abortion	6-10 weeks	11-15 weeks	16-20 weeks	21-24 weeks
Threatened	14	7	11	10
Incomplete	12	14	Nil	Nil
Threatened ending in inevitable abortion	26	11	Nil	Nil
Missed	Nil	2	3	3
Total	52	34	14	13

Majority of threatened abortion cases were evenly distributed between 6th to 24th weeks of gestation. Most of the inevitable abortion cases occurred between 6th to 15th weeks, while missed abortion cases were found between 16th to 24th weeks of gestation.

Table III gives the LAP activity of different types of abortion and their end results. In all the threatened abortion cases which ended in normal delivery missed abortion the diminution of LAP activity ranged between 50-75 per cent.

Graph II gives the LAP activity in serial estimation in different types of abortion.

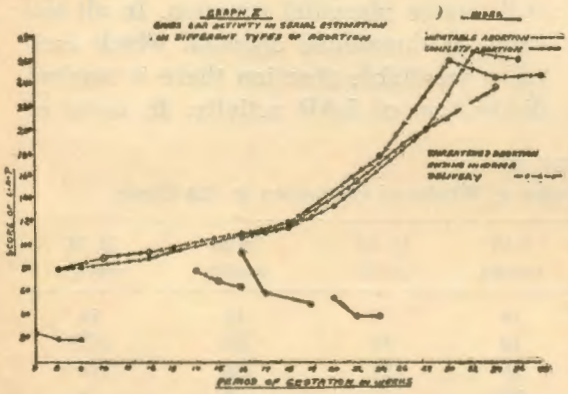
Discussion

Alkaline phosphatase is an enzyme which is present in all the tissues and body fluids. It is found in maternal serum, cord blood, placenta and leuco-

TABLE III

Shows the LAP Activities in Different Types of Abortion in 113 *Cases*

No. of cases	Duration of pregnancy in weeks	Expected L.A.P. activity	Observed L.A.P. activity	Diminution of LAP activity in percentage	Remarks
14	6-10	68- 84	68- 84	Nil	Continued with pregnancy
12	6-10	68- 84	28- 68	10-70	Ended in evacuation of uterus
26	6-10	68- 84	27- 29	63-65	Ended in inevitable abortion
7	11-15	90-100	70-100	Nil	Ended in normal delivery
14	11-15	90-100	75- 80	27	Ended in evacuation of uterus
11	11-15	90-100	45- 78	20-50	Ended in inevitable abortion
2	11-15	90-100	43- 45	53-54	Missed abortion
11	16-20	108-135	108-135	Nil	Normal delivery
3	16-20	108-135	20- 30	73-76	Missed abortion
10	21-24	135-178	130-170	Nil	Threatened abortion ending in normal delivery
3	21-24	135-178	60- 67	50-52	Missed abortion



cytes during pregnancy. The leucocyte alkaline phosphatase as well as the serum alkaline phosphatase are raised in pregnancy and are considered to be under the influence of placental hormones.

The foeto-placental complex is affected by the maternal and foetal complications before the microscopic changes take place in the placenta. So, when the functional unit of the placenta is affected, it produces not only the macroscopic changes in it, but also diminishes its functional capacity to produce hormones and enzymes.

Besides the hormonal activities for the study of placental function there are enzymatic activities in blood and other tissues. The LAP activity is influenced by the hormones secreted by the placenta, the insufficiency of which can be detected at a very early stage by the changes in the leucocyte alkaline phosphatase. The present study is under taken to assess the placental function by LAP activities so that suitable treatment can be carried out in gestational bleeding.

In this series of 113 cases, in group of threatened abortion all the cases had serial estimation and were found to have LAP activity within the limit of normal pregnancy. This coroborates the findings of Diamant *et al.*, (1970). In the series of cases of incomplete abortion low LAP score was seen in 50 per cent of the cases, while in 50 per cent there was a small diminution in LAP activity. This type of finding was also seen in Diamant's

series of cases of incomplete abortion and could be explained as due to the presence of still adequate placental function. All the cases of threatened abortion ending in inevitable abortion showed low score. Low score of LAP activity was noted in all the cases of missed abortion.

It can be reasonably concluded that serial estimation of leucocyte alkaline phosphatase will help in the management of different types of abortion.

Summary

LAP activity was estimated in 40 cases of normal pregnancy. It was detected from 8th week of gestation with the score level of 80. It gradually rose to 265 by 40th week of gestation. LAP activity in 113 cases of different types of abortion was estimated. Marked reduction in LAP score was found in inevitable and missed abortions, it remained at normal level in cases of threatened abortion which ultimately ended in term delivery.

References

1. Climie, A. R. W., Heinrichs, W. L. and Foster, I. J.: Tech. Bull., 32: 95, 1962.
2. Diamant, Y. Z., Zuckerman, H., Sadovsky, E. and Polishuk, W. Z.: Amer. J. Obst. & Gynec., 106: 872, 1970.
3. Harper, W. B. and Quigley, H. J.: Obst. & Gynec., 17: 238, 1961.
4. Kaplow, L. S.: Blood, 10: 1023, 1955.
5. Pritchard, J. A.: J. Lab. & Clin. Med., 50: 432, 1957.
6. Quigley, H. J., Dawson, E. A., Hyun, B. H. and Custer, R. P.: Amer. J. Clin. Path., 33: 109, 1960.
7. Valentine, W. N. and Beck, W. S.: J. Lab. & Clin. Med., 38: 39, 1957.

See Figs. on Art Paper III-IV