

**DIAMINE OXIDASE LEVELS IN HUMAN BLOOD AND THEIR
POSSIBLE RELATIONSHIP TO COMPLICATIONS OF EARLY
PREGNANCY**

by

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Abortion is a major problem for the obstetrician and an increasingly common cause of maternal mortality and morbidity. When a patient presents with signs and symptoms of threatened abortion, it is uncertain whether the ovum is already dead or still viable. If the ovum is viable, then the treatment to ameliorate the symptoms is justifiable but, in cases where the ovum is already dead, such treatment may lead to unnecessary expense and disappointment, besides increasing chances of missed abortion. According to Eastman (1961) the ovum is already nonviable for six weeks or more before the signs and symptoms of threatened abortion appear, especially if it is in the first three months. Thus, in a threatened abortion, any investigation that could give some clue regarding the viability and well-being of the foetus in utero would be of great significance in obstetrics.

It has been shown by Kahlson (1958) that the growing foetus produces large amounts of histamine. In order to protect the mother from the deleterious

effects of this amine in the maternal circulation, there has to be some mechanism for its dextoxication. Marcou (1938) found a high histaminolytic activity in the maternal blood during pregnancy and Zeller (1938) called this substance diamine oxidase. This histaminolytic activity is found to be such a typical and definite feature of human pregnancy that it is of importance to study this enzyme in various abnormalities of early gestation

Studies on vaginal cytology and cervical mucus fern test in prognosticating the outcome of pregnancy in cases of threatened abortion have been carried out by different workers with varying results. (Schuman 1944; Randall 1953; Kishore and Agarwal 1957; Menon *et al*, 1959; Bhinde *et al*, 1963; Abrams and Abrams 1964; Jacobson 1965). Progestational agents have been used in these cases with widely conflicting reports Hertig and Sheldon (1943) found a high incidence of foetal abnormalities in the abortuses from spontaneous abortions.

Thus the following study was undertaken in the Irwin Hospital, New Delhi, in order to (1) estimate the levels of plasma diamine oxidase in normal and abnormal pregnancies and to assess the value of variations, if any (2) to correlate

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diamine oxidase levels with the cervical mucus fern test and vaginal cytology in prognosticating the outcome of pregnancy, (3) to study the effect of progestational compounds (Proluton or Gestanin) in threatened abortion, (4) to assess the effect of these agents on enzyme levels, (5) to study the morphology of the conceptus whenever the material was available.

Material and Methods

The clinical material was collected from the outpatient and antenatal clinics and the maternity wards of the department of Obstetrics and Gynaecology, Irwin Hospital, New Delhi. Cases were divided into three groups consisting of, (1) 15 non-pregnant normal women (2) 45 normal pregnant women at various stages of gestation (3) 85 cases of abnormal pregnancy who were further divided into 5 groups;

(a) 6 cases of threatened abortion, (b) 7 habitual aborters, (c) 7 cases of doubtful pregnancy, (d) 4 cases of missed abortion, (e) 7 cases of incomplete abortion.

Plasma histaminase levels were determined in all cases. In addition, vaginal cytology was done in 17 normal and 25 cases of threatened abortion; cervical mucus fern test was carried out in 17 normal and 41 cases of threatened abortion; Histopathologic examination of the conceptus was done in 24 cases.

Serial enzyme estimations, repeated cytology and fern tests were carried out at fortnightly intervals until either the patient reached the period of viability or aborted.

A detailed history was taken. Physical examination was conducted and case records maintained. In the history, special care was taken to exclude cases

of bronchial asthma, allergy and skin diseases, i.e. conditions which are known to raise the diamine oxidase levels.

All patients were given symptomatic treatment. In addition, progestational therapy was given to such patients who had previous history of repeated abortions or who were elderly. Ten of them received Proluton 250 mg I/M biweekly and the rest were given 5 gm. of Gestanin thrice daily until the patient reached 28th week of gestation.

Methods

Estimation of diamine oxidase was carried out by the volumetric method of Kapeller-Adler (1952). The principle of the test depends on the decolorization of Indigo Carmine by the hydrogen peroxide formed during the enzymatic destruction of histamine by the histaminase in the blood.

Vaginal smears were taken from the lateral vaginal wall opposite the tip of the cervix and fixed and stained by Papanicolaou method.

Cervical fern test was carried out in the normal manner and graded as; negative, +, ++, and +++.

Abortuses were classified according to Carnegie classification of Mall and Meyers (1921).

Results

(1) The maximum number of abortions occurred in patients aged between 21 years and 25 years (68.1%) and the rate of abortion was the least in patients below the age of 20 years. (46.1%).

(2) Many of the patients were paras 3 and 4. Foetal wastage was the highest in those who had more than 4 children (77.7%).

(3) 41.7% of the patients had previous history of abortions. The salvage rate in

random aborters was slightly better than in those with the history of previous abortions (36%). The outcome of pregnancy with threatened abortion among those who had had 3 or more previous abortions was a 100% failure.

(4) Fifty-eight out of 60 patients with threatened abortion presented with a history of vaginal bleeding and 30 of them had abdominal pain in addition. Two patients gave a history of premature rupture of membranes without any vaginal bleeding or abdominal pain.

(5) Thirty-five out of 60 cases of threatened abortion aborted giving an incidence of 58.3% in the study group. 65.7% of the abortions occurred at the gestational age of 9 to 16 weeks. Foetal wastage was highest (70%) from 6 to 12 weeks of pregnancy. After the 24th week, there was no case who threatened to abort indicating that the pregnancy is well established after the 24th week.

(6) 15% of the cases sought medical advice within 12 hours of the onset of symptoms and 11.1% aborted. The prognosis was poor in cases who came to the hospital long after the onset of symptoms.

(7) Haemoglobin estimation of these cases showed that 53.3% of them had a haemoglobin level of 10,11 gms%. The percentage of abortions was the highest in those who had Hb 12 gms% or more, the next being in the group 10-11 gms% indicating that anaemia did not contribute much in the causation of abortion in the present study.

Plasma histaminase levels in 15 normal non-pregnant women were found in the range of 0.0 to 0.4 P.U./ml., with an average of 0.166 P.U./ml. There was no significant difference in the enzyme levels at different phases of the menstrual cycle. The values also did not vary appreciably with age or parity.

The difference in the enzyme titre between non-pregnant and normal pregnant cases was highly significant ($P < 0.001$) as early as the 6th week of gestation. The titres steadily increased from the 6th week and reached a maximum at the 28th week. The only exceptions were at the 18th and 22nd weeks when the titres obtained were higher than those expected (Table I).

TABLE I
Histaminase Levels in Normal Pregnancy at Various Stages of Gestation

Period of gestation in weeks	No. of cases	Range of histaminase in P.U./ml.	Mean histaminase in P.U./ml.
6	4	0.3-0.4	0.32
8	7	0.3-0.6	0.4 ± 0.1
10	10	0.2-0.6	0.38 ± 0.09
12	7	0.3-0.7	0.46 ± 0.15
14	14	0.4-2.0	0.63 ± 0.41
16	15	0.3-2.2	0.7 ± 0.43
18	10	0.4-2.5	0.91 ± 0.59
20	15	0.4-1.3	0.73 ± 0.3
22	9	0.5-2.8	1.13 ± 0.7
24	12	0.5-1.4	0.77 ± 0.35
26	15	0.6-1.4	0.94 ± 0.28
28	19	0.6-1.6	1.0 ± 0.36

When the initial histaminase levels in cases of threatened abortion were compared with those of normal pregnancy at the corresponding gestational age, all were found to be lower (Table II) except in one case at the 24th week. This was a primigravida who came with premature rupture of membranes without any bleeding or pain. The enzyme titre was high at the time of admission (0.9

P.U./ml) and the level continued to be high on follow-up.

Cases of threatened abortion who continued the pregnancy successfully after the initial threat had comparatively higher enzyme levels than those who eventually aborted (Table III).

On follow-up the enzyme titres gradually rose to normal or subnormal levels in those who continued their pregnancy.

TABLE II

Comparison of Mean Histaminase Levels in Threatened Abortion and During Normal Pregnancy

Gestation in weeks	No. of cases	Mean histaminase in threatened abortion	No. of cases	Mean histaminase in normal pregnancy	P. value
6	3	0.26 ± 0.1	4	0.33 ± 0.05	<0.01
8	5	0.14 ± 0.11	7	0.4 ± 0.1	<0.01
10	7	0.3 ± 0.16	10	0.38 ± 0.19	0.2
12	7	0.34 ± 0.12	7	0.46 ± 0.15	0.05
14	12	0.30 ± 0.13	14	0.63 ± 0.41	<0.01
16	10	0.37 ± 0.15	15	0.7 ± 0.43	<0.01
18	7	0.31 ± 0.09	10	0.91 ± 0.49	0.1
20	3	0.43 ± 0.15	15	0.72 ± 0.3	<0.02
22	3	0.4 ± 0.17	9	1.13 ± 0.7	<0.02
24	1	0.9	12	0.77 ± 0.3	
26	—	—	15	0.94 ± 0.28	
28	3	0.45 ±	19	1.0 ± 0.36	<0.001

TABLE III

Comparison of Mean Histaminase Levels in Threatened Abortion Cases Between Those Who Aborted and Those Who Continued Pregnancy

Gestation in weeks	Total No. of cases	Mean histaminase in cases who aborted	P.U./ml. cases who aborted	P. Value
6	3	0.2 (1)	0.3 (2)	
8	5	0.1 (4)	0.3 (1)	
10	7	0.26 (5)	0.4 (2)	0.1
12	7	0.22 (4)	0.43 (3)	<0.01
14	12	0.27 (6)	0.33 (6)	<0.001
16	10	0.32 (6)	0.45 (4)	0.1
18	7	0.28 (4)	0.36 (3)	
20	3	0.3 (1)	0.5 (2)	
24	1	—	0.9 (1)	
26	0	—	—	
28	2	0.48 (2)		

In those who eventually aborted the enzyme levels remained stationary or fell from initial readings.

Habitual Abortion

There were 8 cases with a history of 3 or more consecutive abortions, 2 were admitted with vaginal bleeding and the rest prophylactically for rest and observation at the critical time. Two aborted and one of them had the enzyme level of 0.3 P.U./ml. She showed progesterone deficiency in her vaginal smear and cervical mucus. She aborted in spite of progestational therapy. The conceptus was pathologic. The second case in this group was a known case of hypertension and was admitted at the 6th week with vaginal bleeding and B.P. of 160/100 mm. of Hg. The initial enzyme level was 0.4 P.U./ml. The B.P. returned to normal after treatment. Nine days later she left the hospital against medical advice only to return again after 26 days with another bout of bleeding. Now the enzyme level was only 0.2 P.U./ml. She aborted after 2 days and the conceptus showed foetal hydrops and the chorionic villi were avascular. The other six habitual aborters had their histaminase levels within normal limits. One of them developed drug rash after Proluton injection following which she had high histaminase levels on two occasions.

Missed Abortion: All the 4 cases in this group showed persistently low levels of histaminase which did not show any rise with treatment.

Doubtful Pregnancy: There were 9 cases who gave history of amenorrhoea for 6-14 weeks. Their obstetric examination did not give conclusive evidence of pregnancy at the time of admission. One of them had an initial histaminase reading of 0.3 P.U./ml and aborted within few

hours of admission. Another had 0.2 P.U./ml. and aborted the next day. The diagnosis of abortion was established in these patients after examining the abortuses.

The enzyme levels in the other 7 patients ranged from nil to 0.2 P.U./ml. which were within the normal non-pregnant levels. Diagnosis of delayed period was confirmed after causing withdrawal bleeding in them with Orasecron tablets, 1 daily for 3 days.

Incomplete Abortion: All the 7 cases in this group presented with low levels histaminase equivalent to non-pregnant levels.

Vaginal Cytology: The control group consisted of 17 normal pregnant women in whom the C.I. was determined every month till the 28th week. C.I. index below 15% was considered as normal, while above that was taken as abnormal. As seen in Table IV the cornification in-

TABLE IV
Cornification Index in Normal Pregnancy

Gestation is weeks	No. of smears studied	Mean percentage of cornified cells
4-8	4	9.7%
9-12	5	7.2%
13-16	9	6%
17-20	5	5.2%
21-24	5	5.6%
25-28	9	5.44%

dex gradually fell from the 6th till the 28th week as the pregnancy advanced.

Out of 25 cases of threatened abortion in whom vaginal cytology was done, 12, i.e. 48% had normal pregnancy smears and needed no treatment, one of them aborted. On further scrutiny it was found that she had ferning in the cervical mucus but the foetus had cord round the neck twice which might have been the cause of foetal death.

Among the 13 cases with abnormal smears at the time of admission, 10 aborted in spite of progestational therapy. Three cases continued their pregnancy successfully. Their smears reverted to normal within 15 days of treatment and their cervical ferning disappeared.

Amongst 8 habitual aborters, 2 had vaginal bleeding and both had high C.I. and both aborted.

Of the other 6, 3 had cornification index of 15-20 in the smears and all the 3 reverted to normal with 15 days of treatment.

Ferning of Cervical Mucus: Forty-one patients had this test done of which 26 (63.4%) had no ferning and 7 of them aborted. Of the 15 cases with positive ferning, 8 continued to have ferning in spite of progestational therapy and 7 of them aborted. In 7 cases the fern test became negative with treatment. One of them aborted in whom the cause of abortion could not be elicited. It is evident from Table V that the cases with posi-

aborted. One of the remaining 6 had positive fern, but became negative after treatment and continued pregnancy successfully.

Correlation between the Value of Histaminase Test, Vaginal Cytology and Cervical Fern Test in Prognosticating the Outcome of Threatened Abortion.

Each of these investigations was evaluated poor, equivocal and good. Histaminase level more than 50% below normal pregnancy level for the corresponding gestational age was considered poor, value less than 50% below the normal value was considered equivocal and within normal limits was considered good. In the case of vaginal smear C. I. below 15% was good, 15 to 25% was equivocal and more than 25% was poor. Cervical mucus ferning of Grade II & III were poor, Grade I was equivocal and no ferning was considered good. As seen in table VI when the vaginal smear showed good progestational effect, the incidence of foetal wastage was minimal. Fallacy by this test was the least when compared with the other two tests. In cases showing equivocal effects, accuracy of fern test was more reliable. The outcome as predicted by cytology and cervical mucus was more accurate and reliable in cases who showed "poor" result by all the three methods.

Effect of Treatment: Twenty-one cases of threatened abortion received progestational therapy i.e. 11 Proluton and 10 gestanin.

The salvage rate was not much different from the untreated group (42.8% as compared with 41% untreated) on further analysis, the salvage rate of the Proluton treated group was higher than the group treated with Gestanin (54.6% and 30%).

TABLE V
Effect of Cervical Mucus Ferning on Percentage of Abortion

Cervical mucus	No. of cases	No. aborted	Percent- of abortion
No ferning	26	7	26.9%
Positive ferning	15	9	60%
(a) Grade I	10	4	40%
(b) Grade II	3	3	100%
(c) Grade III	2	2	100%

tive ferning had a worse prognosis than those without ferning. On further analysis, all who had ++ and +++ ferning aborted, showing that treatment had no effect on them.

Amongst 8 habitual aborters, 2 with vaginal bleeding had positive fern and

TABLE VI

Correlation of the Value of Histaminase Test, Vaginal Cytology and Fern Test in Prognosticating the Outcome of Pregnancy in Threatened Abortion

Investigation done	Good			Equivocal			Poor		
	No. of cases	Abort-ed	%age	No. of cases	Abort-ed	%age	No. of cases	Abort-ed	%age
Histaminase	7	2	25.7%	9	4	44.4%	9	5	55%
Fern test	9	2	22.2%	11	4	36.3%	5	5	100%
Vaginal cytology	12	1	8.3%	6	3	50%	7	7	100%

Among 8 habitual abortors, 4 had Gestanin and 4 Proluton. One from each group aborted.

The progestational agents did not seem to have any enhancing effect on histaminase titres. The mean enzyme levels in the treated and untreated groups were similar.

Morphology of the Conceptus

There were 24 abortuses of which only 9 were normal. One had cord round the neck. Thirteen cases showed pathologic embryos. The details are given in Table VII.

TABLE VII

Morphology of the Foetus in 24 Cases

Gross features	No. of cases	Percent-age
Chorionic villi only	2	8.3%
Villi, chorion and amnion.		
No embryo	4	16.6%
Villi and chorion only	—	—
Nodular foetus	2	8.3%
Cylindric foetus	—	—
Stunted foetus	—	—
Macerated foetus	7	29.1%
Normal	9	37.5%

Changes in the Placenta

Histopathologic examination of the placenta was carried out in 29 instances. Chorionic villi were degenerated and

nonviable in 9 instances. Hydatid degeneration was found in 5 specimens. Placental haemorrhages and normal villi were seen in 2 cases. Normal villi were found in 13 patients.

When placental changes were correlated with the morphology of the foetuses, it was found that all the 5 cases who showed hydatid degeneration in the villi were associated with pathologic embryos.

Discussion

The classical work of Kahlson *et al* (1958) had shown that the growing foetuses produce histamine and the maternal plasma levels of histaminase are an indication of the foetus in utero. Rising levels of diamine oxidase were present in normal pregnancy in the present group of patients as early as the 6th week of gestation and a steady rise in the enzyme level was found in all cases of normal pregnancy. It was possible to diagnose pregnancy in doubtful cases with fair accuracy using this test.

As long as the enzyme levels were high or rising, the prognosis for the foetus was good and the symptoms subsided with treatment. When the enzyme levels were low or stationery, patients almost always aborted.

Though the test was fairly accurate in prognosticating the outcome of pregnancy in threatened abortion, the results

obtained by vaginal cytology and cervical mucus fern test proved more accurate. Thus, of the three tests, cervical fern test proved the best, easy to carry out, less time consuming than the other more elaborate procedures.

When a patient threatened to abort, use of progestational agents was found to be as effective as any other symptomatic treatment with placebo, proving that progesterone deficiency was not the cause but the effect of abnormal pregnancy. Progestational compounds produced good results, but there are no control series.

The incidence of pathologic ova was high in the present series indicating that the failure of treatment in these cases was probably due to this reason. Therefore, in these cases as judged by the low levels of diamine oxidase, the patient should be treated only symptomatically lest she goes on to missed abortion.

If diamine oxidase reflects foetal welfare in the uterus, then it is logical to conclude that in threatened abortion the prognosis can be improved by increasing the maternal enzyme levels by administering the enzyme itself. Further studies and experimentation will be needed in

this field to clearly understand the relationship of these changes.

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