

The effect of pregnancy on erythrocyte osmotic fragility

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Erythrocyte osmotic fragility was carried out in 100 pregnant women and 50 non pregnant control subjects. Increased erythrocyte osmotic fragility was observed primarily in the last trimester of pregnancy. Physiological shift in erythrocyte osmotic fragility may create a problem in the diagnosis of hereditary spherocytosis during the last trimester of pregnancy. Twenty eight percent of the pregnant women (28%) showed abnormal results when compared to non pregnant women (p/0.001). The values returned to normal after one week of delivery, indicating that the process is physiological and not pathological. Lack of awareness of this phenomenon could result in an incorrect diagnosis of hereditary spherocytosis.

INTRODUCTION

Pregnancy is accompanied by many physiological changes. The changes in plasma chemistry, cardiovascular changes and haematological changes are of particular interest. The evaluation of haemolytic anaemia during pregnancy may be complicated by the physiological increase in erythrocyte osmotic fragility. Patients suffering from hereditary spherocytosis might show haemolytic anaemia during pregnancy which becomes apparent at the time of pregnancy. Pregnancy thus causes more red cell destruction due to increased splenic blood flow.¹ Various factors affecting the erythrocyte osmotic fragility have been

studied. The thickening of the red cells causes increased osmotic fragility.² Normally disc to sphere transformation takes place in stored blood and in patients who have received blood transfusion. The formation of spherocytes is a phenomenon fundamental to the pathogenesis of haemolytic anaemia. The abnormal erythrocytes change to crenated spherocyte when exposed to a lytic agent invitro and the process is reversible but spherocytic stage is not preceded by crenation and the process is irreversible.³ The present study was conducted to know the effect of different trimesters of pregnancy and one week after delivery, on erythrocyte osmotic fragility.

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MATERIAL AND METHODS

This study was conducted on 100 pregnant women attending the antenatal clinic at Medical College and Hospital, Rohtak. The cases were divided into three categories:

- 20 women of 1st trimester (up to 12 weeks of pregnancy)
- 20 women of 2nd trimester (13 to 28 weeks of pregnancy)
- 60 women of last trimester (29 weeks to full term pregnancy).

The control specimens were taken from 50 non pregnant age-matched healthy women. The cases with known haematological disorders and other diseases that could affect erythrocyte osmotic fragility were excluded from the study.

Erythrocyte osmotic fragility was done after incubating the blood samples at 37° C for 24 hours, by the method described by Dacie and Lewis.⁴ Defibrinated blood was used. Incubated 1 ml volumes of blood in sterile 5 ml screw capped bottles in duplicate. After 24 hours the contents of duplicate bottles were pooled, after thoroughly mixing the sedimented red cells in the overlying serum. In this 0.05 ml of blood were added to 5 ml of range of hypotonic solutions of dilution equivalent to 9.0, 8.5, 8.0, 7.5, 7.0, 6.5, 6.0, 5.5, 5.00, 4.5, 4.0, 3.5, 2.0 and 1.0 gm/litre of NaCl, and immediately mixed by inverting the tubes several times. The tubes were allowed to stand at room temperature for 30 minutes, then remixed and centrifuged for 5 minutes at 1200-1500g. The amount of lysis in each tube was compared with that of 100% lysis tube (1.0 gm/l NaCl) using a photoelectric colorimeter at a wave length of 540 nm. The supernatant from 9.0 gm/l NaCl was used as blank. The highest concentration of saline at which

lysis was just detectable and the highest concentration of the saline at which lysis appeared to be completed were recorded. Fragility curves were drawn on graph paper by plotting the percentage of lysis in each tube against the corresponding concentration of salt solution. Normal almost symmetrical sigmoid curves and curves with long tails due to small proportion of very fragile cells were obtained (Fig. 1). The mean corpuscular fragility (MCF) value i.e. the concentration of saline causing 50% lysis were obtained from the curves.

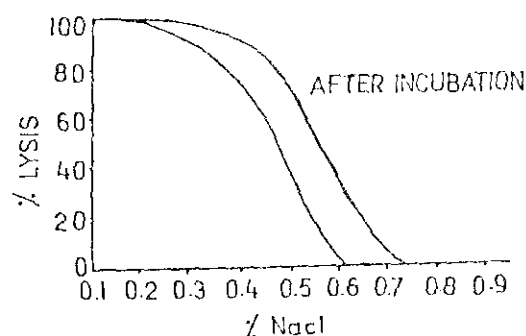


Fig. 1: Showing normal erythrocyte osmotic fragility curves.

RESULTS

Mean corpuscular fragility (after incubation) in 50 control cases varied from 0.482 to 0.592 with a mean of 0.554 ± 0.011 , while mean corpuscular fragility in 100 pregnant women varied from 0.482 to 0.724 with a mean of 0.588 ± 0.057 . Mean corpuscular fragility (after incubation) in pregnant women according to trimester is given in Table 1.

Statistical analysis showed that it was significant in 11nd and highly significant in

Table 1: Showing MCF (after incubation) in control and pregnant women of different trimesters.

	Control	Ist trimester	11nd trimester	Last trimester
Range	0.482-0.592	0.482-0.592	0.490-0.625	0.484-0.724
Mean + SD	0.554 ± 0.011	0.567 ± 0.035	0.572 ± 0.040	0.600 ± 0.067

Table 2: Statistical analysis of MCF (after incubation) of pregnant women of different trimesters Vs control.

	Difference of means	Standard error	t-test	p value	Remarks
Pregnant women Vs control	0.024	0.0059	5.762	<0.001	Highly significant
1st trimester Vs control	0.013	0.0079	1.630	>0.05	Not significant
2nd trimester Vs control	0.018	0.0090	2.000	<0.05	Significant
3rd trimester Vs control	0.046	0.0088	5.227	<0.001	Highly significant

last trimester of pregnancy as shown in Table 2.

Twenty eight women (28%) out of 100 pregnant women showed increased mean corpuscular fragility after incubation and these were abnormal values. Out of these 28 pregnant women, 27 women were in last trimester of pregnancy and one in the second trimester of pregnancy. Mean corpuscular fragility in pregnant women showing abnormal values was in the range of 0.596 to 0.724 with a mean of 0.628 ± 0.023 . Statistical analysis showed that when results of pregnant women showing abnormal values were compared with control group, it was very highly significant ($p/0.001$) Table 3. After one week of delivery mean corpuscular fragility returned to normal and values were from 0.493 to 0.587 with a mean of 0.556 ± 0.160 . Statistical analysis of the results when compared with control was not significant ($p/0.05$) Table 3.

survival is a controversial issue.^{5,6,7} A common but unappreciated haematological effect of pregnancy is an increase in erythrocyte osmotic fragility.^{8,9,10} This may be encountered as abnormally increased osmotic fragility during the evaluation of mildly anaemic but otherwise normal pregnant women. The mechanisms underlying increased osmotic fragility in pregnancy are not yet understood. They may include hormonal effect of gestation¹¹ or lowered plasma osmotic pressure. From increased erythrocyte osmotic fragility in late pregnancy, one may make an erroneous diagnosis of hereditary spherocytosis. In the present study 28% pregnant women showed abnormal values of MCF, while previously abnormal values have been observed in 22% pregnant women. Maximum women showing abnormal values, 27 out of 28 were in the last trimester of pregnancy and our findings are in

Table 3: Statistical analysis of cases showing abnormal values during pregnancy and in cases one week after delivery Vs control.

	Difference of means	Standard error	t-test	p value	Remarks
Pregnant women	0.074	0.0046	16.087	<0.001	Highly significant
Women one weeks after delivery	0.002	0.030	0.0666	>0.05	Not significant

DISCUSSION

Pregnancy is accompanied with changes in major haematological values and indices but whether erythrocytes have normal

accordance with the findings of other workers.¹² Returning of MCF to normal one week after delivery showed that the process is physiological and not pathological.¹³

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