EVALUATION OF LYMPHOBLAST TRANSFORMATION RATE IN TOXAEMIA OF PREGNANCY

by

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Introduction

Recent advances in the field of immunology have shown that pre-eclampsia and eclampsia may have some immunological aetiological basis. The hypothesis that there is decreased immune responsiveness in the mother during pregnancy has been extensively developed (Howie *et al* 1971) but there are very few reports available on the role of immunological mechanisms in toxaemia of pregnancy. Jenkins *et al* (1973) believed the occurrence of immunological disparity in toxaemia patients and proved by higher transformation index of lymphocyte in mixed lymphocytic culture.

The present study was undertaken to postulate the abnormality of immune responsiveness in toxaemic patients. Mixed lymphocytic culture which is a in vitro test to investigate immunological responsiveness has been done for the evaluation of immune responsiveness in the toxaemic patients.

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Material and Methods

The subject of the present study were 32 toxaemic cases, out of which, 20 had pre-eclamptic toxaemia and 12 had toxaemia. The cases of pre-eclamptic toxaemia were further categorised into mild (9 cases), moderate (7 cases) and severe (4 cases). The finding were compared with 8 females from third trimester of matching socio-economic status and parity without previous history of pre-eclampsia or eclampsia (Table I).

TABLE I
Distribution of Cases

S. No.	Groups	No. of cases
1.	CONTROL (IIIrd Trimester Normal pregnant females)	8
2.	TEST	32
	Pre-eclampsia	20
	Mild	9
	Moderate	7
	Severe	4
	Eclampsia	12

Apart from the mixed lymphocytic culture, few haematological and biochemical investigations were also performed. Haematological investigations included hae-

moglobin estimation, total leucocyte count, differential leucocyte count. Peripheral blood smear was examined for Burr cells. Biochemical investigations included total serum proteins and serum albumin estimation, Plasma fibrinogen levels, blood urea and sero-mucoid protein estimations.

Mixed Lymphocytic Culture was done accorrding to the technique described by Bain *et al* (1963) with slight modifications. A perfect sterile technique was used throughout the procedure.

10 ml of venous blood was collected under all possible aseptic precautions in a sterile container with 250 I.U. of preservative free heparin from both mother and newly born baby within 5 hours of delivery. Mother's and baby's blood was allowed to sediment separately at 37°C for 2 hours in a closed sterile chamber. Plasma from both were taken in 20 ml corning screw-capped sterile bottle for 1 hour so that polymorphonuclear leukocytes become attached to the glass.

The supernatant fluid rich in lymphocytes was drawn from both and washed with Minimum Essential Medium (Micro-Lab. Bombay) P.H. 7.2 twice. One ml of M.E.M. was added to both and the number of lymphocytes were adjusted to 1 x 106 cells/ml. Viability of cells was also done by using 1% Trypan blue exclusion test 95% cells found to be viable.

Culture Medium: 20% fetal, calf serum (Micro Lab. Bombay) in M.E.M. (containing streptomycin 100 units/ml and crystalline penicillin 100 μgm/ml) i.e. 36 ml MEM + 9 ml Fetal Calf serum.

Control Culture

(a) 1 ml of mother's plasma was mixed with 5 ml of culture medium to see the spontaneous transformation of lymphocytes into blast cells.

(b) 1 ml of baby's plasma was similarly mixed with 5 ml. of culture medium.

Test Culture

1 ml of mothers plasma was mixed with 1 ml of baby's plasma in ratio of 1:1 in 5 ml of culture medium.

All the cultures were done in triplicate and were incubated at 37°C for 6 days.

Fixation: Cultures were centrifuged at 800 G for 10 minutes. Supernatant was thrown out and the sediment washed with 1% sodium citrate solution.

The sediment was fixed with ethanol and glacial acetic acid in a ratio of 3:1 for half hour at 37°C, after which it was centrifuged at 800 G for 5 minutes.

The supernatant was drawn out and the sediment was used for making slides. After the slides were dried they were stained with Giemsa's stain and examined under oil immersion.

Results

Mixed lymphocytic culture was done in 32 toxaemic females and 8 normal third trimester pregnant females which served as control cases. In each group spontaneous and mixed lymphocyte blast transformation rate was assessed.

In cases of pre-eclampsia and eclampsia there was increase in spontaneous as well as mixed lymphocytic transformation as compared to normal third trimester pregnancy but the change was statistically insignificant ($P > .05$) (Table II).

TABLE II

Lymphoblast Transformation Rate After 6 Days of Incubation in Control and Test Cases

Group of cases	No. of cases	Spontaneous Lymphocytic Transformation	Mixed Lymphocytic Transformation
		Mean Blast Cells Per 500 Mononuclear cells	Mean Blast Cells Per 500 Mononuclear cells
CONTROL (Normal IIIrd Trimester)	8	Mean 1.75 S.D. ± 0.91	Mean 1.25 S.D. ± 0.24
Pre-eclamptic toxaemia	20	Mean 2.30 S.D. ± 0.80 Z 0.53 P value > .05	Mean 3.11 S.D. ± 0.71 Z 0.25 P value > .05
Eclampsia	12	Mean 2.51 S.D. ± 0.95 Z 0.59 P value > .05	Mean 3.16 S.D. ± 0.90 Z 0.38 P value > .05

TABLE III

Spontaneous Lymphocytic Transformation Vs Mixed Lymphocytic Transformation

Group of cases	No. of cases	Spontaneous Lymphocytic Transformation	Mixed Lymphocytic Transformation
		Blast Cells Per 500 Mononuclear cells	Blast Cells per 500 Mononuclear cells
Control (IIIrd Trimester Normal Pregnancy	8	Mean 1.75	Mean 1.25 Z 1.06 P value > .05
Pre-eclamptic Toxaemia	20	Mean 2.30	MBean 3.11 Z 2.23 P value > .05
Eclampsia	12	Mean 2.51	Mean 3.16 Z 2.27 P value > .05

When mixed lymphocytic transformation rate was compared with spontaneous transformation rate it was evident that mixed transformation rate was found to be lower than the spontaneous blast transformation rate in third trimester pregnant females, but the change was not significant ($P > .05$). There was a significant increase in mixed lymphocytic transformation rate as compared to spontaneous lymphocytic transformation rate in pre-eclampsia and eclampsia ($P < .05$).

Discussion

The role of immune response in normal pregnant females has been extensively studied, whereas its role in toxaemic females is yet to be studied extensively.

In the present study, the immunological

compitence of lymphocytes was tested in vitro by their response to foreign lymphocytes. When maternal and fetal lymphocytes were mixed together in a suitable culture medium, the number of lymphocytes which transformed into blast cells were increased in cases of pre-eclampsia and eclampsia as compared to normal third trimester pregnancy.

On going through literature, our findings were confirmed by those of Jenkin's *et al* (1973) and Das Gupta (1975) who found a higher transformation index of lymphocytes in mixed lymphocytic culture indicating increased cellular immunity in toxaemia after washing away the serum of toxaemia patient which contained higher levels of seromucoid. These authors believed that the immunological disparity in toxaemia as proved by the higher transformation index and the raised level of seromucoid was a failure of normal protective mechanism.

The increase in blast transformation was found to be more in eclampsia as compared to pre-eclampsia, indicating that greater the severity of disease greater is the number of blast transformed. Our findings were in accordance with those of Stevenson *et al* (1971) and Platt *et al* (1972).

In the present study mixed lymphocyte transformation rate was also compared with spontaneous lymphocytic transformation rate. The spontaneous lymphocytic transformation is believed to occur due to to the presence of DNA synthesis stimulating factor present in the culture medium (Kasakura and Lavenstein, 1965). We found significant increase in mixed lymphocytic transformation rate as compared to spontaneous transformation in both pre-eclampsia and eclampsia, further indicating enhancement of cell mediated immunity in these patients.

Toxaemia of pregnancy appears to be a syndrome of multifactorial aetiology and an extensive study will be fruitful to arrive at a definite conclusion.

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