A STUDY OF T AND B LYMPHOCYTES AND DELAYED CUTANEOUS HYPERSENSITIVITY IN NORMAL AND PRE-ECLAMPTIC TOXAEMIC FEMALES

by

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Introduction

One of the most fascinating and challenging phenomena in the field of immunology is the successful grafting of foetus on to the uterus and the maintenance of foetus and placenta throughout gestation. The foetus inherits genetic material from his father and accordingly should be rejected and should be recognised as non-self by the mothers' immunocompetent cells. But rejection does not occur in the majority of the pregnancies. Recent studies have shown that there is a relative decrease in the T lymphocytes in the blood of pregnant women in the first trimester but not in the last trimester. (Howe 1975; Sterlkauskas *et al* 1975).

This study presents the variation in the immune response in different trimesters of normal pregnant females and also the abnormality of immune responsiveness in females with pre-eclamptic toxaemia.

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Material and Methods

The study included 33 normal pregnant females of various trimesters and 32 females with pre-eclampsia.

The control group for normal pregnant females, included 25 non-pregnant females.

The control group for pre-eclamptic toxaemia included 8 normal pregnant females of third trimester.

All the above cases were drawn from the out patient department and the cases admitted in the wards of U.I.S.E. Maternity Hospital of G.S.V.M. Medical College, Kanpur.

Following is an account of the test employed for the assessment of immunological status of the cases under study:

(A) *Lymphocyte Studies*

1. Peripheral lymphocyte count. (P.L.C.) Dacie and Lewis, 1969.

2. T. Lymphocyte (E. Rosettes) % and levels as described by Jondlel *et al* 1972.

3. B. Lymphocyte (E.A.C. Rosettes % and levels as described by Pincus *et al* (1972).

(B) *Skin Test Studies*

Skin test studies were performed by using recall antigens:

(a) P.P.D. antigen (Fundenburg *et al* 1976).

(b) Dermatophytin 'O' (Wanebo *et al* 1975).

A. *Lymphocyte Studies*

1. Peripheral blood absolute lymphocyte count (P.L.C.).

It was obtained by multiplying the percentage of lymphocyte and total leucocyte count taken in hundred.

2. *Lymphocyte Subpopulation Studies*

Seperation of lymphocytes

About 4 ml of blood was collected in vial containing 100 I.U. of heparin. Lymphocytes were separated by layering 3 ml of undiluted blood on 1.5 ml of Ficoll Hypaque mixture (obtained from Neygard & Co. Norway lot No. 70479 as lymphoprep) and centrifuged at 400 g. for 10 mts at 4°C. The condensed layer of lymphocyte at the interface of lymphocyte supernatent plasma was taken in another test tube and cells were washed twice in Hanks balanced salt solution (p.h. 7.2-7.5). The third washing was done with minimum essential medium (p.h. 7.2-7.5) containing A.B. serum in a ratio of 9:1. Viability was tested with 1% trypan blue and count of viable cells was adjusted to 4 x 106 cells/ml.

Assay for T Lymphocytes (E. Rosettes) (Jondel *et al* 1972 with slight modifications).

0.25 ml of peripheral blood lymphocyte was mixed with equal volume of 0.5% washed R.B.C. suspension and centrifuged at 200 g. for 5 mits. at 4°C and kept at 4°c. for 3 hours. The cells were suspended gently and fixed in .6% glutaraldehyde solution. The cells were stained with 0.5% methyle violet. Two hundred lymphocytes were counted and % of E rosettes (lymphocyte with 3 or more erythrocytes attached) was recorded. Total T lymphocyte level was calculated with help of T lymphocyte percentage and absolute lymphocyte count.

Assay for B Lymphocyte (E.A.C. Rosettes) (Pincus *et al* 1972 with slight modifications).

Preparation of E.A.C.

Amboceptor (Anti sheep haemolysin 1:4000 lot No. 2016 c) and lympholysed guinea pig compliment (lot No. 3221 C) along with its solvent (lot No. 3320 K) were obtained from M/s. Behringwereke A.C.W. Germany.

Sheep R.B.C. having a concentration of 5 x 108 cells/ml. were treated with equal volume of diluted amboceptor (1:750 diluted with P.B.S.) incubated at 37°C for 30 mts. Following incubation, the cells (EA) were washed with Hanks balanced salt solution twice and treated with diluted guinea pig compliment (1:1000 diluted with P.B.S.) and incubated at 37°C for 30 mts., the cells (E.A.C.) were washed twice with Hanks balanced salt solution and final solution having sheep R.B.C. 5 x 108 cells/ml was obtained in M.E.M.

E.A.C. Rosettes

Equal volume of E.A.C. (0.5 ml) suspension was mixed with equal volume of lymphocyte suspension (4 x 10 cells/ml) and incubated at 37°C for 5 mts then centrifuged at 300 g. for 5 mts. The cells were throughly mixed and examined in same way as E rosettes. The B cell percentage was obtained and later B cell level was calculated.

Skin Test Studies

Dermatophytin 'O' Skin Test: under asceptic precautions 1:100 diluted antigen was prepared in P.B.S. (P.H. 7.2). After sterility testing, 0.02 ml was injected I/D on the flexor aspect of left arm. Reading

was made after 48 hours and area of induration measuring more than 5 mm in diameter was taken as positive (Wanebo 1975). *P.P.D. skin test*: (Feudenburg *et al* 1976) 0.1 ml of 1:1000 diluted old tuberculin was injected intra-dermally and reaction was measured after 48 hours. Area of induration measuring 10 mm or more was taken as positive.

Observations

The study included 33 normal pregnant females and 32 females with pre-eclamptic toxamia.

Study Group

Out of 33 normal pregnant females, 13 were from first trimester, 12 from second trimester, and 8 from third trimester of pregnancy. The cases from third trimester served as control for pre-eclamptic cases. Their age ranged from 19-40 years (mean 27.8, S.D. ± 5.67).

Control Group for Normal Pregnant Females

Out of 25 non-pregnant females, 12 were primigravida and 13 were multigravida. Their age ranged from 17-52 years (mean 30.60 S.D. — 10.98).

Study of Lymphocytes in Females of Normal Pregnancy in Various Trimesters

The average, range and S.D. of T & B lymphocytes are shown in Table I and II. It is evident that there is no significant change in P.L.C. in control vs first, second and third trimesters ($P > .05$). T cell % and levels are markedly decreased when compared with non-pregnant control females, ($P < .001$) in first and second trimesters. At the same time, there is re-

TABLE I

T-lymphocyte Studies in Control and Normal Pregnant Females in Various Trimesters

Type of cases	No.	PLC/cu mm	T-Cell %	T-Level/cu. mm.
CONTROL	25			
Range		1512-3800	62-76	1058-3118
Mean		2498.1	69.4	1784.4
S.D.		± 892.6	± 3.7	± 511.5
FIRST TRIMESTER	13			
Range		1520-3120	22-38	456-904
Mean		2456.1	27.4	665.1
S.D.		± 431.7	± 4.3	± 121.6
		P>.05	P <.001	P <.001
SECOND TRIMESTER	12			
Range		1782-3120	26-66	570.2142
Mean		2609.2	44.4	1182.7
S.D.		± 532.9	± 13.6	± 500.7
		P >.05	P <.001	P <.001
THIRD TRIMESTER	8			
Range		2750-3104	65-72	1900-2203
Mean		2901.8	69.4	2012.3
S.D.		± 170.6	± 2.3	± 114.4
		P >.05	P <.001	P <.001

TABLE II

B-Lymphocyte Studies in Control and Normal Pregnant Females in Various Trimesters

Types of cases	B-Cell %	B-cell level/cu mm.
CONTROL		
Range	11-18	254-447
Mean	14.43	349
S.D.	± 2.096	± 56.47
FIRST TRIMESTER		
Range	31-39	674-1072
Mean	35.83	911
S.D.	± 2.88	± 130.58
	P<.001	P<.001
SECOND TRIMESTER		
Range	20-28	518-750
Mean	23.86	638-21
S.D.	± 2.85	± 92.46
	P<.001	P<.001
THIRD TRIMESTER		
Range	9-17	226-470
Mean	13.05	322.65
S.D.	± 2.33	± 63.90
	P>.05	P>.05

ciprocal rise in B cell % and levels which statistically significant ($P < .001$) in same trimesters as compared to normal non-pregnant females. In the third trimester, there is no change in T cell % and levels, B cell % and levels when compared with control ($P > .05$). This indicates depression in the cellular immunity and elevation of humoral immunity in early pregnancy and reversion towards normal in late pregnancy.

Study of Lymphocytes in Females with Pre-Eclamptic Toxaemia

The range average and variation of P.L.C./cu mm, T cell % and levels, B cell % and levels in control and test cases are shown in Table III and IV. It is evident that in cases of pre-eclampsia there is no significant change in P.L.C., B. Cell % and levels ($P > .05$) but T cell % is significantly raised when compared with normal third trimester pregnant females ($P < .001$).

Skin Test Studies in the Cases under Study

The result to various recall antigens in various trimesters are shown in Table V. There is significant depression of response to P.P.D. in first trimester (positive 38.5%) as compared to control cases (positive 84%). Similar results were obtained by Dermatophytin '0' (positive 15.4 vs 68%-positivity in controls) in first trimester. In second trimester percentage of positivity increased but was below normal. In the third trimester, there was further increase in the positivity to these antigens but still below normal.

Skin test studies in pre-eclamptic toxaemia are shown in Table V indicating an increased in skin test positivity with recall

TABLE III

Study of T Lymphocytes in Control and Pre-eclampic Toxaemia Females

Types of cases	No. of cases	Parity P.	Parity M.	PLC/cu mm Range Mean ± SD	T. Cell % Range Mean ± SD	T cell level cu.mm Range Mean ± SD
1. 3rd Trimester Normal Pregnancy	8	3	5	(2750-3104) M 2902.80 SD ± 170.63	(65-72) M 69.37 SD ± 2.33	(1900-2203) M 2012.25 SD ± 114.40
2. Pre-Eclamptic Toxaemia	32	16	16	(2200-3612) M 2982.19 SD ± 475.01 P >.05	(68-84) M 76.38 SD ± 1.50 P <001	(1650-2889) M 2272.03 SD ± 361.88 P >.05

TABLE IV

Study of B Lymphocytes in Control and Pre-eclamptic Toxaemia

Types of cases	No. of cases	B Cell % Range Mean ± S.D.	B. Cell level/cumm. Range Mean ± S.D.
Third Trimester Normal Pregnancy	8	9-17 13.05 ± 2.33	226-470 322.65 ± 63.90
Pre-Eclamptic Toxaemia	32	19-30 20-81 ± 1.57 P >.05	518-750 638-21 ± 92.46 P >.05

TABLE V

Results of Skin Testing

Types of cases	No.	P.P.D. Positive	P.P.D. Negative	Candida Positive	Candida Negative
Control	25	84%	16%	68%	32%
1st Trimester	13	38.5%	61.5%	15.4%	84.6%
2nd trimester	12	41.7%	58.3%	25%	75%
3rd trimester	8	62.5%	37.5%	37.5%	62.5%
Pre-Eclamptic Toxaemia	32	66.70%	31.30%	81.25%	18.75%

antigens as compared to normal IIIrd trimester pregnant females.

Discussion and Conclusion

In the present study, in females of first trimester, there was a highly significant fall in the percentage and levels of T cells ($P < .001$) and a relative rise in B cell percentage and levels ($P < .001$). In the second trimester T cell % and levels con-

tinued to show a highly significant fall ($P < .001$) and B- cells showed reciprocal rise. Similar findings were observed by Purtilo *et al* (1972), Lewis *et al* (1966) and Finn *et al* (1972) who found a depressed cell mediated immunity as reflected by decreased T cells levels in first trimester as well as in the second trimester of normal pregnancy. This depressed cellular immunity may help to implant and protect the foetus from rejection by the mother's immunocompitent cells. This inversion of T cells may represent a physiological depletion of T lymphocytes (Waldman *et al* 1974).

The relative increase in B cell level in first and second trimesters may reflect an assistance in allograft acceptance through the production of blocking antibodies or factors (Helstrom and Helstrom, 1970).

During the third trimester, the fall in T cell was not observed and they reverted back to normal. Similarly, B cell counts also reverted back to normal. By this time the foetal allograft may have already overcome the potential danger that might have existed for the rejection by a T cell mediated reaction.

The absolute lymphocyte count did not show any significant change ($P > .05$) in any trimester. Our findings as regards this are in conformity with the observation of Knobloch *et al* (1975), Sterlkauskas *et al* (1875) and Brain *et al* (1972) who could not find any appriciable change in absolute lymphocyte count in their series of pregnant females. On the other hand, Purtilo *et al* (1972) observed a marked supression of lymphocytes in one way mixed lymphocytic culture but they have not specified the exact period of pregnancy when this supression was noted.

Skin test studies with recall antigens revealed a depression response of both antigens (P.P.D. and Dermatophytin 'O'). This depression was most marked in the first trimester. Positivity increased in second and third trimesters, but still it was below normal controls. This cutaneous response was depressed as a whole during normal pregnancy. A depressed tuberculin response was shown by Finn *et al* (1972), Walker *et al* (1972).

In cases of pre-eclamptic toxaemia, appreciable increase in T cell percentage and increased skin reactivity to skin antigens were observed which indicated enhancement of cell mediated immunity. Similar findings were observed by Jenkins *et al* (1973) and Das Gupta (1975) who demonstrated higher transformation index of lymphocytes in pre-eclamptic toxaemia.

This increase in cellular immunity may be due to immunological disparity. Further studies shall be usefull in arriving at definite conclusion regarding the immunological aspect of etiology of pre-eclamptic toxaemia which is still a subject of debate.

Conclusions

Following conclusions were drawn from the present study:

First and Second trimester normal pregnancy

— Significant fall in T-lymphocyte levels ($P < .001$).

— Significant relative increase in B-lymphocyte levels ($P < .001$).

— Depression in cutaneous reactivity to recall antigens.

Third trimester normal pregnancy

— T and B lymphocyte levels reverted back to normal.

— Skin test positivity to recall antigens increased but was still below normal.

Pre-Eclamptic Toxaemia

— Significant increase in T lymphocyte

percentage as well as increased skin reactivity to recall antigens indicating enhancement of the cell mediated immunity.

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See Fig. on Art Paper I