

RESULTS

HLA phenotype and gene frequencies :

The phenotype frequencies of HLA class-I and class-II antigens in case of normal and unsuccessful pregnancies are summarized in tables 1,2,3 and 4.

The frequencies of the antigens Cw1, Cw2 of class-I and the antigens DQw1, DQw2 and DQw3 of class-II were significantly higher but the antigens A1, B5, B15, Cw3, Cw5 of class I and the antigens DR1, DR3 of class-II were significantly lower in case of normal pregnancy. The frequencies of A1, B5, B15, Cw1, Cw3, Cw5 of class-I and the antigens DR1, DR3, DQw1 were significantly higher but the antigens Cw1, Cw2 of class-I and the antigens DQw2, DQw3 of class-II were significantly lower in case of unsuccessful pregnancy.

Haplotype, Delta value and Chi-square (χ^2) :

Haplotype (obs.), Haplotype (exp.), Delta value and Chi-square in normal pregnancies have been presented in table 5 for the loci A-B, table 6 for the loci A-C, table 7 for the loci

A-D, table 8 for the loci B-C, table 9 for the loci B-D, table 10 for the loci C-D and in case of unsuccessful pregnancies, table 11 for the loci A-B, table 12 for the loci A-C, table 13 for the loci A-D, table 14 for the loci B-C, table 15 for the loci B-D and table 16 for the loci C-D are summarized.

The delta value of the haplotypes A3-B44 (12), A10-B13, A24(9)-B49(21), A25(10)-B7, A26(10)-B49(21), A28-B5, Aw68(28)-Cw7, Aw68(28)-DRw11(5), B39(16)-Cw6, Bw62(15)-Cw4, were significantly higher in case of normal pregnancy. The delta value of the haplotypes A23(9)-B38(16), A28-B7, B17-Cw6, B5-DR5, B17-DR4, B38(16)-DRw10, B45(12)-DR2, Cw7-DR4 were significantly higher in case of unsuccessful pregnancy.

The most common haplotypes with significant positive linkage disequilibria are given in table 17 for normal and unsuccessful pregnant women. The delta value of the haplotypes A10-B13, Aw68(28)-DRw11(5), Bw62(15)-Cw4, B17-DR4 and the delta value of the haplotypes A23(9)-B39(16), A28-B7, B5-DR5, B38(16)-DRw10, Cw7-DR4 were significantly higher in case of normal and unsuccessful pregnant women respectively.

Incidence of HLA alloantibodies against class-I and class-II antigens :

The incidence of HLA alloantibodies have been

summarized in tables 18, 19, 20 and 21.

Table 18 shows the incidence of 28.08% alloantibodies in case of normal primigravidae and 39.70% in case of multigravidae against the HLA class-I antigens. The presence of HLA alloantibodies were higher in lower age groups both in primigravidae (36.76% upto 20 years of age) and multigravidae (42.85% upto 20 years of age) compared to that of the higher age groups (17.64% in case of primigravidae and 32.5% in case of multigravidae between 26 to 30 years of age).

Table 19 shows the incidence of 5.55% alloantibodies in case of unsuccessful primigravidae and 24.32% in case of multigravidae against the HLA class-I antigens. Lower incidence of HLA-alloantibodies in early age is evidenced only in case of multigravidae (16.66% upto 20 years and 50% in case of 26 to 30 years).

Figure 1 shows the total percentage of positive sera having antibodies against class-I antigens in case of both normal and unsuccessful pregnancies. The levels of the formation of HLA-alloantibodies were higher both in primigravidae (28.08%) and multigravidae (39.70%) in normal pregnancies compared to that of the unsuccessful pregnancies (5.55% in case of primigravidae and 24.32% in case of multigravidae).

Table 20 shows the incidence of 15.75% alloantibodies

in case of normal primigravidae and 16.17% in case of multigravidae against the HLA class-II antigens. The formation of HLA-alloantibodies were observed maximum at the age group of 21 to 25 years both in primigravidae (18.18%) and multigravidae (22.24%).

Table 21 shows the incidence of 16.66% alloantibodies in case of unsuccessful primigravidae and 43.24% in case of multigravidae against the HLA class-II antigens.

Figure 2 shows the total percentage of positive anti-HLA antibodies against class-II antigens in case of both normal and unsuccessful pregnancies. The HLA-alloantibodies against the class-II antigens were higher in unsuccessful pregnancies (16.66% in case of primi and 43.24% in case of multigravidae) compared to that the normal pregnancies (15.75% in case of primi and 16.17% in case of multigravidae).

Quantitative estimation of IgM and IgG by column chromatography :

The highest concentration of IgM was 0.115 mg/ml in case of normal and 0.237 mg/ml in case of unsuccessful pregnancy as has been presented in figure 3. The mean concentration of IgM was 0.350 mg/ml in case of normal and 0.206 mg/ml in case

of unsuccessful pregnancy. The concentration of IgM was lower in normal pregnant women than the unsuccessful pregnant women.

The highest concentration of IgG was 1.15 mg/ml in case of normal and 0.612 mg/ml in case of unsuccessful pregnancy as has been presented in figure 4. The mean concentration of IgG was 0.577 mg/ml in case of normal and 0.70 mg/ml in case of unsuccessful pregnancy. The concentration of IgG level was found higher in normal than the unsuccessful pregnancy.

Quantitative estimation of IgM and IgG by immunodiffusion technique :

Figure 5 shows the concentration of IgM and IgG in mg/dl in normal and unsuccessful pregnant women. The concentration of IgM was 190.52 mg/dl in case of normal and 236.95 mg/dl in case of unsuccessful pregnancy and that of the IgG was 128.86 mg/dl in case of normal and 984.11 mg/dl in case of unsuccessful pregnancy. The concentration of IgG level was found higher in normal than the unsuccessful pregnant women and the concentration of IgM was lower in normal pregnant women compared to that of the unsuccessful pregnant women.

T-lymphocyte, B-lymphocyte and their respective blast cells
in peripheral blood :

The total percentage of blast cells in case of normal and unsuccessful pregnant women have been presented in figure 6. 25.33% blast cells have been observed in case of normal whereas in case of unsuccessful pregnant women, the percentage was 35.35. The total percentages of T-cells were 61.18 and 61.53 in case of normal and unsuccessful pregnant women respectively. The percentage of B-cells was higher (42.33%) in case of unsuccessful pregnancy than that of the normal pregnant women (31.31%). The percentages of T-blast cells were 31.3 and 33.47 in case of normal and unsuccessful pregnant women respectively. The percentages of B-blast cells were 25.12 and 30.8 in case of normal and unsuccessful pregnant women respectively. The total percentage of B-blast cells in case of unsuccessful pregnant women was higher than that of the normal pregnant women though the T-cell percentage in both the cases were almost same. Higher percentage of blast cells (T+B cells) has been observed in case of unsuccessful pregnant women.

Percentage and the ratio of CD4⁺ & CD8⁺ T-lymphocytes :

The percentages of CD4⁺ (helper/inducer) and CD8⁺ (cytotoxic/suppressor) cells in case of non pregnant (virgin),

normal pregnant and unsuccessful pregnant women have been presented in table 22.

Percentages of $CD4^+$ T-cells were 45.05, 30.06 and 35.06 in case of non (virgin), normal and unsuccessful pregnant women respectively. The percentages of $CD8^+$ T-cells were 23.25, 17.87 and 17.31 in case of non (virgin), normal and unsuccessful pregnant women respectively. $CD4^+$ T-cells were higher in unsuccessful pregnant women compared to that of the normal pregnant women but it was much lower when compared with the non pregnant (virgin) women ; whereas $CD8^+$ T-cells were slightly increased in normal pregnant women compared to unsuccessful pregnant women. The percentage of $CD8^+$ T-cells was much higher in case of non-pregnant (virgin) women.

The ratio of $CD4^+$ and $CD8^+$ T-cells were 1.93 : 1, 1.68 : 1 and 2.02 : 1 in case of non (virgin), normal and unsuccessful pregnant women respectively. In case of unsuccessful pregnancy, the ratio of $CD4^+$ and $CD8^+$ T-cells was much higher than non (virgin) and normal pregnant women. This ratio was slightly decreased in normal pregnant women than the non-pregnant (virgin) women.

Blastogenic transformation of peripheral blood lymphocytes
in vitro with PHA-P :

To asses the cell mediated immune response (CMI) of

the pregnant women as well as to investigate the reason(s) of the induction of CMI response in case of unsuccessful pregnancy, or in otherwords, the reason(s) of the inability to inhibit the CMI response to avoid foetal rejection upto the period of the delivery of full grown baby by the unsuccessful mothers, several parameters have been studied.

The lymphocytes of the normal and unsuccessful pregnant women were cultured separately by using autologous sera for different hours and observed that in case of unsuccessful pregnancy, the percentage of blast cells were much higher than the normal pregnancy (Fig. 7). 5 μ g dose of PHA-P was used in each case and the lymphocytes were cultured in vitro for 24, 48 and 72 hours.

The lower percentage of blast cells (21.75% in 24 hours, 27% in 48 hrs. and 31% in 72 hrs.) in case of normal pregnancy may be due to some factors those are present in the serum and possibly responsible for the inhibition of cell activation. This was evidenced when the lymphocytes of the unsuccessful pregnant women were cultured with the sera taken from the normal pregnant women having the same blood groups and compared with the degree of blastogenic transformation of the lymphocytes of normal pregnant women, cultured with autologous sera (Fig.8). In both the cases, the percentages of the blast cells were more or less same and lower than the percentage of blast cells of unsuccessful pregnant women, cultured with autologous sera (fig.9).

As this has been indicated from the above results that the CMI response was slightly inhibited during pregnancy especially in case of normal pregnancy, possibly to avoid the rejection of the foetal allograft, it has been investigated whether the degree of the blastogenic transformation of lymphocytes of normal pregnant women, just before delivery and after delivery were comparable with the degree of the blastogenic transformation of lymphocytes of non pregnant (virgin) women. The lymphocytes of the non pregnant (virgin), immediately before delivery and after delivery of normal pregnant women were cultured with autologous sera. Fig. 10 shows that the blastogenic transformation of the lymphocytes of non pregnant (virgin) and delivered women at 24, 48 and 72 hours were very close to each other and higher than the normal pregnant women before delivery. This shows that the pregnant women after delivery possibly start regaining the CMI response to reach upto the normal level. Figures 11, 12 and 13 are showing the comparative account of the degree of blastogenic transformation of lymphocytes at different hours.