DISCUSSION
It is a fact that although leprosy is an infectious disease, a large majority of population goes through a stage of sub-clinical infection. Most of these people are able to mount resistance towards *Mycobacterium leprae* infection. A minor group of population, due to unknown mechanism, is unable to mount a proper immune response against *Mycobacterium leprae* invasion.

The present study was carried out to study cell-mediated immunity in different types of leprosy and control cases. The total number of cases were 90 which included 60 cases of different types of leprosy and 30 cases of control. The leprosy cases were classified histopathologically into tuberculous type-2, borderline tuberculous type-4A, borderline type-4B, borderline lepromatous type-4C and lepromatous type-4D.

T-cell count, B-cell count, lymphoblast transformation percentage, skin tests using lepromin and candid antigen, and nasal, slit-skin smear, and skin biopsies for acid fast bacilli were studied. Uniformity of the study has been maintained by studying the leprosy cases and age and sex matched control cases. The majority of cases were within the third to fifth decade with mean age (±SD) of 36.67 (±10.68). The control cases were almost of similar age group with mean (±SD) of 39.5 (±9.99).
In lepromatous leprosy male to female ratio is generally reported as being around 2:1 and in tuberculoid no sex differentiation has been found (Anderson and Kissane, 1977). Similar incidence has also observed in present study as 5 were male out of 9 cases of tuberculoid type while in other types male preponderance was noted.

Variation in the presence of Myco. leprae in nasal smear, slit-skin smear and skin biopsy:

Myco. leprae has special affinity for skin, nerve tissues and nasal mucosa. It may be demonstrated in skin-slit smear, nasal smear and skin biopsy. In present study, 2 cases were positive for Myco. leprae in skin-slit smear out of 16 and 17, IL and LI cases respectively while positive nasal smears in 50, IL and IL were 1, 3 and 4 out of 8, 10 and 9 respectively. The skin biopsies were positive in all types of leprosy except tuberculoid.

The number of positive cases in skin biopsy for Myco. leprae in 50, 50, IL and IL were 5, 7, 6 and 13, out of 16, 18, 10 and 10 cases respectively.

It is evident from above findings that the number of positive cases were less in nasal and slit-skin smear as compared to skin biopsy. This may be explained as patient were under treatment with DDS for longer duration and bacilli may have cleared from skin and invaded the internal organs.
T-CELL VARIATION IN CONTROL AND DIFFERENT TYPES OF
LAPROXY CASES:

Control Cases:

In the present study, 35 age and sex matched control cases have been studied. T-cell percentage ranged from 52 to 77 with mean (± SD) of 66.80 (± 5.11). Absolute T-cell count ranged from 1239 to 2973 with mean (± SD) of 1866 (± 510.47).

Similar were the observations of Ren et al (1976) who had reported mean (± SD) of T-cell percentage 68.6 (± 7.7). Other workers also have reported nearly similar mean T-cell percentage which ranged from 40.8 to 77 percent (Dwyer et al, 1973; Lim et al, 1974; Ghagie et al, 1977; Sharma et al, 1979).

There are various factors which affect T-cell percentage in normal human peripheral blood. Mendes et al (1973) had studied the conditions in E and EAC rosette formation in great detail for T and B-cells respectively. They had found that E and EAC rosette obtained at Duke University were 55.4 percent and 18.3 percent and at Faculdade Paulista de Medicina were 20.4 and 26.8 percent respectively. Rosette formation between T-cells and sheep red blood cells was found to be temperature dependent with maximum values between 10°C to 20°C and no rosette formation at 37°C. They had also mentioned that variation in rosette percentage was also observed
when some normal donors was tested on different days and this is also affected by storage of blood and/or lymphocytes.

Therefore to minimize these effects, in present study the fresh samples were used and tests were carried out at 4 to 6°C. From the above mentioned facts, it is clear every laboratory should have its own control value for comparison with study cases. The minor differences in the present study and other studies may be due to above mentioned facts.

**Leprosy Cases:**

The absolute lymphocyte count was almost equal in all types of leprosy. The mean ($\pm$SD) of absolute lymphocyte count in TT, MB, BB, BL and LL was 3629.77 ($\pm$777.47), 3688.71 ($\pm$828.96), 2777.60 ($\pm$615.99), 3274.4 ($\pm$804.66) and 2828.82 ($\pm$585.66) respectively. It indicates that there is no correlation in absolute lymphocyte count among different groups.

The mean $\gamma$-cell percentage ($\pm$SD) in control was 66.88 ($\pm$2.11) which was decreased to 45.28 ($\pm$4.62) in LL as shown in Table IX. Similarly there was decrease in absolute $\gamma$-cell count towards lepromatous pole. The mean $\gamma$-cell percent was decreased in all types of leprosy cases. The fall in mean $\gamma$-cell percentage was maximum in LL and minimum in TT as compared to control cases. 3B cases at intermediate pole while MM was closer to TT and BL was closer to LL.
The difference in mean T-cell percentage between control and total leprosy cases was statistically highly significant \( (P < 0.005) \) and was significant also when control cases were compared with different type of leprosy cases separately. The difference in TT and BR was only significant \( (P < 0.05) \) but it was highly significant with BB, BL and LL \( (P < 0.005) \).

Other workers had also reported the similar findings that there were decrease in mean T-cell percent from control to LL \( (\text{deep et al, 1973; Lim et al, 1974;}
\text{ Glogle et al, 1977; Hakiemanis et al, 1977}) \).

Recently Shrama et al \( (1979) \) had studied a complete spectrum of leprosy. They had combined the TT and BR group and found that mean \( (\pm SD) \) of T-cell percentage in control, TT + BR, BB, BL and LL cases which were 55.07 \( (\pm 7.29) \), 41.37 \( (\pm 10.25) \), 40.76 \( (\pm 9.48) \), 39.28 \( (\pm 7.3) \) and 37.00 \( (\pm 11) \). It indicates that there was gradual decrease in the mean T-cell percent from control to LL which is similar to present study with minor differences which may be due to factors mentioned earlier.

Contrary to the findings of present study, Nea et al \( (1976) \) had observed that there was no difference in mean T-cell percent in control and LL. They had found mean \( (\pm SD) \) of T-cell percent which were 70.4 \( (\pm 6.3) \) and 68.9 \( (\pm 7.7) \) respectively.
The present findings were again in accordance with the findings of Mendes et al (1974) who had found that significant decrease in the proportion of T-cell in peripheral blood as well as the depletion of lymphocytes in paraaortal area of involved lymphnodes. Similar finding has been reported by Turk and Waters (1971).

Methin et al (1980) had reported severe depletion of lymphocyte in white pulp of spleen which were replaced by large number of macrophage and plasma cells. A reduction in T-cell count in lepromatous patients is due to extensive involvement of lymphatic system including all superficial groups of lymphnode and spleen. T-cell replacement in spleen seems to be effect of disease rather than cause. The spleen filters large quantity of blood every hour and there is constant exchange of cells between organ and blood. A large number of circulating macrophage may finally find their way in to spleen where they settle down and replace the small lymphocytes. Fragments of bacilli and even acid staining organisms had been detected in spleen although skin smear were negative. They had assumed that bacilli and product of bacilli continued to persist in internal organs especially in reticuloendothelial system even after the skin smear were negative. The presence of large amount of antigen prevents the restoration of cell mediated immunity even after many years of apparent disease arrest.
The nonspecific depletion of cell-mediated immunity can be due to an absolute reduction of T-cells. The specific defect of lymphocytes in lepromatous patients producing tolerance to Mycobacterium continues to be present even after all demonstrable bacilli are eliminated.

Beyer et al (1979) has suggested the mechanism for alteration in T, B ratio. First was that the para-cortical, area which was normally heavily populated with T-cell, were often extensively invaded by macrophage index bacteria. Second was presence of antigen within granulomas of these disease for a longer period of any induces sustained production of immune-suppressive factors resulting in anergy.

Khalimov et al (1977) had suggested that in bacteriologically negative BL cases, the mean value for circulating lymphocytes were not significantly different from those of normal control. Thus T-lymphocyte deficiency did not appear to be genetic because number of T-cells returned to normal in bacteriologically negative BL patients. T-cell deficiency which may be the reason for diminished cell mediated immune response, is further supported by prolonged survival of skin allografts in lepromy patients (Hsu et al, 1977).

**T-cell variation in control and lepromay cases.**

T-cell percentage was gradually increased from control to BL. The mean (±SD) of T-cell percentage in control was 20.86 (±5.40) and gradually increased up to 43.8 (±6.6) in lepromatous leprosy as shown in Table XII.
The difference in B-cell percentage in control and total leprosy cases as well as different type of leprosy cases separately is shown in Table XIII. The difference was highly significant in control and total leprosy cases ($P = \leq 0.005$). It was only significant ($P = \leq 0.05$) in control and TT but was highly significant ($P = \leq 0.005$) in other types. When TT was compared with BT, BB, BL and LL, it was highly significant in all except with BT where it was only significant ($P = \leq 0.05$).

Caul and Forsmark et al. (1979) had reported marked increase in B-cell percentage 60% to 88% of B-cell in peripheral blood. Sharma et al. (1979) had studied B-cell percentage in complete spectrum of leprosy and observed slight increase in B-cell percentages. They observed mean ($\pm SD$) of B-cell percentage in control, TT + BT, BB, BL and LL which was 27.67 ($\pm 5.77$), 32.86 ($\pm 5.71$), 30.83 ($\pm 7.09$), 31.86 ($\pm 7.31$) and 29.97 ($\pm 7.97$) and concluded that there was very minimal increase in B-cell percentage which was statistically insignificant. Dua et al. (1976) had also observed no significant difference in increase of B-cell in lepromatous leprosy.

Dupuj et al. (1978) had studied B-cell percentage only in control and lepromatous groups and found significant increase in B-cell percentage in peripheral blood in lepromatous group. They observed 27 percent in control and 85 percent B-cell in lepromatous group similar to the
observation of present study. Other workers also had shown that patients with lepromatous leprosy had high proportion of circulating lymphocytes possessing membrane bound immunoglobulin (B-cells). It had been proposed that such increase in B-cell numbers might represent over compensation for a deficiency of T-lymphocytes.

The findings of present study were also in accordance with Chegla et al (1977), who studied mean (± SD) of B-cell percentage in control, TT, BB and LL groups and found 27 (± 5.6), 36 (± 8.6), 37 (± 6.6) and 36 (± 10.7) respectively.

The findings of present study were again supported by the study of Verma et al (1971) who had observed significant increase in B-cell percentage, lymphocytes obtained from crushing the lymph node.

As B-lymphocytes are involved in antibody production, Abo et al (1978) had reported anti mycobacterium leprae antibodies with indirect fluorescent technique in both lepromatous, tuberculoid, and indeterminate forms but the proportion of positive sera and titres observed was highest in lepromatous sera.

It had established that human antibodies produced by B-cells could specifically enhance or inhibit the response of T-cells (cited by Chegla et al, 1977).
It had also been suggested that T-cell might be affected by depressive humoral factor (Bullock, 1968). Lymphoblast transformation response to PHA in control cases and leprosy cases.

Blood lymphocytes were found to be stimulated to blastogenesis by mitogens such as PHA and could be a measure of cell mediated immune response (David and David, 1972). In present study, there was gradual decrease in lymphoblast transformation to PHA from control to LL. The mean (+SD) of lymphoblast transformation percentage in control, TT, ET, EE, EL and LL was 38.15 (+3.06), 34.68 (+5.44), 27.57 (+4.23), 22.87 (+3.69), 18.68 (+3.21) and 13.07 (+2.89) respectively as shown in Table XIII.

The difference in lymphoblast transformation response to PHA in control and total leprosy cases as a whole and separately with different type of leprosy cases was found to be highly significant (F = ∞.008) except control and TT where it was only significant (F = ∞.08). The difference between TT and ET, TT and EE, TT and EL and TT and LL was also highly significant (F = ∞.008). It indicates that there was marked decrease in lymphoblast transformation response towards lepromatous pole.
The findings of the present study were almost similar to the usually reported findings of Chai et al (1980) and Dubey et al (1981) who had also observed the diminished blastogenic response to PHA with slight variation. Chai et al (1980) had reported mean (± SD) of lymphoblast transformation percentage in control, TT, ET, BE, EL and LL which were 34.6 (± 8.5), 30.6 (± 10.1), 30.9 (± 6.8), 19.1 (± 13.8) and 16.7 (± 10.3) respectively. Dubey et al (1981) had reported the percentage of lymphoblast transformation which had decreased from 33 to 40 percent in TT to 5 to 20 percent in LL.

These findings were further supported by the findings of other workers who had also studied the lymphoblast transformation response to various other antigens by measuring the DNA synthesis of cells by radioactive thymidine uptake. They had shown diminished response from TT to LL (Nac et al, 1971; Fuji Chiangung et al, 1971; Hehn et al, 1973; Takemura et al, 1975; Harger et al, 1975; Takemura et al, 1975; Harger et al, 1975; and Shams et al, 1979).

However Ulrich et al (1979) found no significant difference in control, tuberculin-positive, borderline, and Lepromatous patients in their responses to PHA and pokeweed mitogen (PWM).
Although these findings were much clear to the findings of other worker with slight variation. This discrepency in the findings of PHA stimulation may be due to the effects of drugs received during the study. Sen Gupta et al (1979) had observed diminished response in healthy volunteers after receiving the DDS orally. Similar were the observations of Beigelman and Fisseni (1974) after studying the effect of DDS in vitro.

It has been suggested that T-cell number is significantly reduced towards lepromatous pole as shown in present as well as in other studies too. Hence, reduced lymphoblast transformation response to PHA may be due to less number of T-cell towards lepromatous end of spectrum. PP and PT patients do not show much reduction in T-cells and thus probably showed slight diminution in lymphoblast transformation response to PHA.

Dulock and Fisseni (1971) had observed the presence of depressor activity to blastogenesis in plasma of leprosy cases who also claimed that depressor activity did not get altered by antileprosy treatment. In contrast to this Eshay et al (1981) had shown that there was significant improvement in their blast transformation percentage in LL patients taking DDS over untreated LL cases.
Nelson et al (1971) had reported that the lepromatous patient as a whole had no evidence of intrinsic defect in the ability of lymphocytes to respond to PTH. Lymphocytes from Indian lepromatous patients with stable disease did show a depressed response when cultured in normal reference sera. In unselected Malay and Indian lepromatous patients a depressed response was clearly apparent only when the cells cultured in autologous sera. In the Chinese lepromatous patients the lymphocyte actually responded better than those of normal Chinese even in autologous sera. Thus there was racial difference in response to PTH induced transformation.

Response of Lepromatous and Canida Antigens in Control and Lepromy Cases:

The findings of the present study showed that lepromin test was positive in TT, MT and MM while ML and LI patients failed to respond to lepromin antigen. Similar results were obtained with canida antigens with the difference that percentage of positive cases were less. The percentage of positive lepromatous cases in control, TT, MT, MM were 66.6, 66.6, 66.6 and 66 respectively while with canida were 28.6, 46.4, 81.4 and 20 respectively.
Buck et al (1963) had only used candida antigen for testing delayed hypersensitivity reaction in their study of control, tuberculoïd and lepromatous cases. They had also observed diminished response in leprosy cases both in tuberculoïd as well as in lepromatous type and reported that positive response against candida were seen in 40.7%, 10.4% and 14.8% in control, tuberculoïd and lepromatous type respectively. The discrepancy in present study and study of Buck et al (1963) who had observed positive cases even in lepromatous type, may be due to the fact that they had divided the cases only in two groups namely tuberculoïd and lepromatous group. So some of borderline type of cases may be included in the lepromatous type who had given the positive response.

From the findings of present study, it is clear that lepromin test is positive upto 85% cases, and this may be a good measure of immunity during the course of therapy or during the reversal reactions, when patient is shifting from one pole to another pole.

Thus it is established from the present study that there is gradual decrease in T-cell percentage, PPD response, and cutaneous delayed hypersensitivity tests from tuberculoïd pole to lepromatous pole. Borderline group cases in between the two polar forms in the Ridley-Jopling scale. It comes close to