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# AN ANALYSIS OF CD4 T CELL RESPONSIVENESS IN DIFFERENT CLINICAL FORMS OF LEPROSY

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**ABSTRACT**  **PURPOSE:** To estimate the CD4 T cell counts in different cases of leprosy and correlate the results with different forms in the leprosy spectrum. **METHOD:** Bacteriological Index of 100 clinically suspected cases of leprosy were estimated. Blood samples were collected to measure the Total Leukocyte Counts, Differential Leukocyte Counts, Absolute T cell counts and CD4 counts. Estimation of Absolute T cell counts was done by E-rossette technique and CD4 counts by flow cytometry. Absolute T cell counts and CD4 counts were then correlated with the Bacteriological Index in different leprosy cases. **RESULTS:** Study group included 20 healthy individuals and 100 leprosy patients. Out of the 100 leprosy patients, 50 patients were paucibacillary and 50 patients were multibacillary cases. The mean Absolute T cell counts in the healthy individuals , paucibacillary and multibacillary leprosy patients were 1260/cu mm, 1351/cu mm and 857/cu mm respectively. The mean CD4 counts in the healthy individuals , paucibacillary and multibacillary leprosy patients were 900 cells/$\mu$l, 893 cells/$\mu$l and 622 cells/$\mu$l respectively. CD4+ counts were lower in multibacillary leprosy patients when compared to that of paucibacillary leprosy patients. **CONCLUSION:** Though expensive, the estimation of CD4 counts in leprosy patients if implemented into routine practice will not only give an idea about the progression of the disease, but also helps in giving appropriate treatment regimens for the paucibacillary and multibacillary cases so as to avoid the risk of relapse and crippling outcomes.

**KEYWORDS :** Leprosy, Absolute T cell counts, CD4 counts

## INTRODUCTION:

Leprosy, caused by *Mycobacterium leprae*, is a chronic infectious disease of low invasive power and pathogenecity. It is manifested in the form of a spectrum with tuberculoid(TT) and lepromatous(LL) forms at either ends and immunologically unstable, borderline forms such as borderline tuberculoid (BT), borderline borderline (BB), and borderline lepromatous (BL) types in between the spectrum. India is home to almost 60% of leprosy cases in the world according to 2018 data released by WHO. The total number of cases in India was 85,302 while 120,334 new cases were detected at the end of 2018 (WHO 2019).[1] On an average, about 100 new cases of leprosy per year are being reported to the Out Patient Department of Dermatology in Government General Hospital, Kakinada, Andhra Pradesh. As it is an obligate intracellular organism, the cell mediated immune response is of main importance in the establishment of pathogenesis and the clinical outcome of the disease. The extremely slow generation time of the bacillus, its inability to grow on artificial media and the non availability of any method for detecting past and present inapparent infection have made the immunological criteria to appear valuable[2].The present study has been undertaken to study the CD4 T cell counts in cases of leprosy by flow cytometry and the results were compared with different forms in the spectrum.

## AIM AND OBJECTIVES:

To perform slit skin smear examination in all suspected cases of leprosy and estimate their bacteriological index after staining the smear with modified Ziehl-Neelsen's technique.To classify the leprosy patients into paucibacillary and multibacillary groups based on their slit skin smear positivity, bacteriological index and number of lesions with which they present. To estimate the Total leukocyte count, Differential leukocyte count and Absolute lymphocyte count. To estimate the Absolute T lymphocyte count by E-rosette technique and CD4 T cell counts by flow cytometry. All the heamatological parameters are estimated in both leprosy patients and healthy individuals.

## MATERIAL AND METHODS

**Ethics statement:** Informed consent for blood and slit skin smears were obtained from the patients following approval of the study by the Institutional Ethical Committee.

**Study design:** The present study was a prospective study, carried out on 100 clinically diagnosed cases of leprosy of all age groups and both sexes attending the Department of Dermatology, Venereology and Leprosy, Government General Hospital, Kakinada.

Bacteriological Index (Ridley 1976) was estimated in 100 leprosy patients by obtaining slit skin smears by Slit and Scrape method of Wade[3] and staining them with modified Zeihl Neelsen's method(Figure 1). Leprosy patients were then classified into Paucibacillary and Multibacillary groups. EDTA blood samples were collected from both leprosy patients and healthy individuals. Total leukocyte counts were estimated using haemocytometer, Differential leukocyte by Leishman's staining and Absolute Lymphocyte Counts were calculated. Absolute T cell counts were estimated with the help of E- rosette technique, which consists of separation of lymphocytes from the peripheral blood [4][5], using centrifugation techniques by Boyum[6] in which defibrinated blood is layered on an isoosmotic low viscosity medium (HiSep™ LSM 1077). Mononuclear cells(lymphocytes and monocytes) and platelets are contained in the banded plasma-LSM interphase due to their density(Figure 2). Lymphocytes are then separated by centrifugation and washing procedures using isotonic phosphate buffered saline. Viability of the cells is determined by trypan blue dye exclusion staining. T lymphocytes having surface receptors for sheep red blood cells(SRBC) spontaneously bind them to the surface and form " E" rosettes(Figure 3). The absolute T cell counts were estimated by their E- binding ability of T cells. CD4 counts were estimated by flow cytometry.(Betson Dickinson flow cytometry based method)
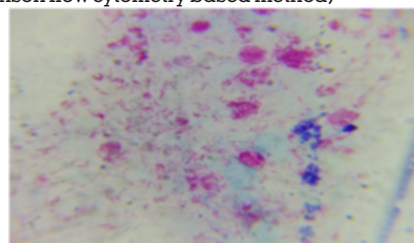


**Figure 1: Clumps of acid fast M.leprae arranged in the form of globi in slit skin smears in a multibacillary patient**
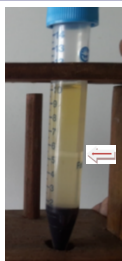
Figure 2: A clear band of interface containing mononuclear cells formed below the supernatant fluid of plasma and platelets
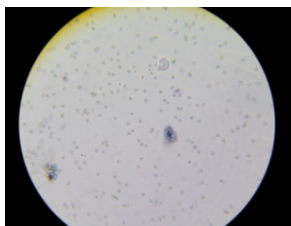


Figure 3: E-rosettes under low power

## RESULTS

Table–i : Number Of Patients Studied In Relation To Their Bacteriological Index [n = 100]

| BACTERIOLOGICAL INDEX | NUMBER OF PATIENTS |
|---|---|
| 0 | 50 |
| 2+ | 24 |
| 3+ | 13 |
| 4+ | 13 |

Table ii : Distribution Of Cases Based On Both Clinical Findings And Skin Smear Results

| STUDY GROUP | CLINICAL PRESENTATION | NUMBER OF CASES |
|---|---|---|
| PAUCIBACILLARY CASES (BI - 0) | Maculo- anaesthetic patches | 20 |
| | Nerve lesions | 8 |
| | Combined (Both patches and nerve lesions) | 22 |
| MULTIBACILLARY CASES (BI – 2+ to 4+) | Nodular lesions | 20 |
| | Facial deformities | 11 |
| | Nerve lesions | 8 |
| | Trophic ulcers | 5 |
| | Combined (Maculo-anaesthetic patches, nerve lesions and nodular lesions) | 6 |
| TOTAL | | 100 |

Table- iii : Mean Findings In The Healthy Individuals In Comparison With Paucibacillary And Multibacillary Groups Of Leprosy

| PARAMETERS | MEAN VALUES | | |
|---|---|---|---|
| | HEALTHY INDIVIDUALS | PAUCIBACILLARY GROUP | MULTIBACILLARY GROUP |
| AGE | 32 | 36 | 41 |
| TOTAL LEUKOCYTE COUNT | 8158 | 8648 | 7768 |
| LYMPHOCYTE % | 30 | 33 | 31 |
| ABSOLUTE LYMPHOCYTE COUNT | 2412 | 2830 | 2378 |
| T CELL % | 55 | 49 | 37 |
| ABSOLUTE T CELL COUNT | 1260 | 1351 | 857 |
| CD4 COUNT | 900 | 893 | 622 |

Table: iv - Comparison Of Absolute T Cell Counts And Cd4 Counts In Healthy Individuals, Paucibacillary And Multibacillary Groups

| GROUP | ABSOLUTE T CELL COUNTS | | CD 4 COUNTS | |
|---|---|---|---|---|
| | RANGE | MEAN | RANGE | MEAN |
| HEALTHY INDIVIDUALS | 1030 - 1635 | 2060± 171.15 | 704 - 1223 | 899.6±142.23 |
| PAUCIBACILLARY LEPROSY | 1972- 4221 | 1351.32± 404.58 | 642-1325 | 893.28±177.79 |
| MULTIBACILLARY LEPROSY | 540-1619 | 856.7± 236.31 | 401-895 | 622.02±105.92 |

[P value < 0.05]

## DISCUSSION

A total number of 120 individuals were investigated in the study, of which 100 were leprosy patients and 20 were healthy individuals. The sex prevalence of leprosy patients revealed a male dominance with male is to female ratio being 2.3:1. This can be justified by the increased risk of exposure in adult males due to their work outside the household. Traditional beliefs, low status, limited mobility, illiteracy and poor knowledge of leprosy may be suggested as important socio-cultural factors responsible for under reporting of cases of women with leprosy. The age of the patients ranged from 11years to 66 years with a mean of 38.11 years. Majority of the patient in this study presented with maculo-anaesthetic patches(48%) followed by nerve involvement(44%), nodular lesions(26%), facial deformities(11%) and trophic ulcers(5%). The patients were grouped into paucibacillary and multibacillary groups by assessing their bacteriological index ranging from bacteriologically negative to 2+, 3+ and 4+ index. 50% of the smear negative cases presenting with less than five lesions i.e., either pure maculo-anaesthetic patches(20%) or pure nerve lesions(8%) or combined lesions(22%) were grouped under paucibacillary group. Rest of the 50% smear positive cases presented clinically with more than five lesions including pure nodular lesions(20%), facial deformities(11%), nerve lesions(8%), trophic ulcers(5%) and combined lesions (6%) and were grouped under multibacillary group(Table II). The cases were grouped according to the National guidelines and technical manual on leprosy, 2005[7]. The heamatological parameters estimated were the total leukocyte count, differential leukocyte counts, absolute lymphocyte counts, absolute T cell counts and CD4 counts and their mean values were compared as shown in the Tables III. The mean total leukocyte counts were found to be 8158/cumm, 8648/ cu mm and 7768/cu mm in healthy individuals, paucibacillary patients and multibacillary patients respectively. The mean absolute lymphocyte counts were found to be 2412/cumm, 2830/ cumm and 2378/ cumm in healthy individuals, paucibacillary patients and multibacillary patients respectively. The mean values of total leukocyte counts and the absolute lymphocyte counts were found to be high in paucibacillary cases when compared to that of healthy individuals and multibacillary patients. This increase in values could be because of chronic antigenic stimulation and active lymphocyte proliferation in a host with good immunological reserve in paucibacillary cases. Cell mediated immunological status of an individual, is best depicted by enumerating the absolute T cell counts. In this study the mean absolute T cell count was 1351.32 ± 404.58/cu mm and mean CD4 count was 893.28 ± 177.79/cells/$\mu$l in paucibacillary leprosy and the mean absolute T cell count was 856.7 ± 236.31 /cu mm and mean CD4 count was 622.02 ± 105.92 cells/$\mu$l in multibacillary leprosy(Table IV). In this study, highly significant reduction was observed in the absolute T cell counts of multibacillary leprosy patients when compared to that of healthy individuals. This finding is in agreement with other workers such as Behra et al.(1982) [8],K.R.L.S.Kirani(1985) [2], and Aziz et al (1996) [9]. The factors responsible for immunological disturbances in leprosy are poorly understood[10]. Different reasons were given by different

workers for the reduction in the T cells in the peripheral blood such as depletion of T cells in the paracortical areas, inherent loss of thymus control, immunological tolerance & immunological deviation, presence of anti lymphocyte antibodies, inadequacy on the part of the lymphocytes to exert their function, suppressor activity of phenolic glycolipid over PBMCs, alteration of T cell kinetics, genetic factors, nutritional factors and enviromental factors. It is observed that there was no significant reduction in CD4 counts in paucibacillary patients when compared to the counts in healthy individuals. But, the CD4 counts in multibacillary patients were significantly reduced when compared with the healthy individuals. The reduction in the CD4 T cell counts in multibacillary patients in the present study correlated well with the study of T.Hussain et al. (2015)[11]. who estimated CD4+ T-lymphocyte counts among leprosy patients and the values were found to be 569±407 cells/μl in paucibacillary patients and 425±345 cells/μl in multibacillary patients. It was reported that the CD4 counts were lower among both paucibacillary and multibacillary patients when compared to healthy controls. The CD4+ cells are the key cells which are infected and destroyed progressively leading towards the poor immune status and approaching towards the risk for opportunistic infections. CD4+ cell counts are monitored to assess immune-suppression and disease progression most commonly in HIV patients. In our study on leprosy, we have observed that CD4+ counts were lower in multibacillary leprosy patients when compared to that of paucibacillary leprosy patients.

## CONCLUSION

Although it has been many years since the existence of a suppressor population in patients with leprosy, there is still controversy over its role in the emergence of the disease and the different clinical presentations. Our study and the other studies suggest that as immunity is severely compromised in leprosy, an estimation of CD4+ cell counts will give an idea about the status of T cell subsets in leprosy patients. Though expensive, the estimation of CD4 counts in leprosy patients if implemented into routine practice will not only give an idea about the progression of the disease, but also help in giving appropriate treatment regimens for the paucibacillary and multibacillary cases so as to avoid the risk of relapse and crippling outcomes.

## REFERENCES

1. Anand V, Kunte R, Jathar S et al(2020). Evaluation of National Leprosy Eradication Programme in Pune city of Maharashtra from 2008 to 2019 –A Record Based Study. Indian J Lepr. 92: 211-219.
2. K.R.L.S.Kirani; 1985;Cell Mediated Immunity in patients with Leprosy; A study of circulating T lymphocytes by 'E' rosette techniques : p 13
3. Wade HW. The histoid variety of lepromatous leprosy. Int J Lepr 1963; 31: 129-42.
4. Jondal M,Holm G and Wigzell H.Surface markers on human B and Tlymphocytes1. A large population of lymphocytes forming non immunerosettes with sheep red blood cells. J.Exp.Med.1972;136(2):207-15.
5. Gradwohl 1980: Gradwohl's Clinical Laboratory Methods and Diagnosis, 8th Ed.,Vol.I, p.794; Vol II, p1237.
6. Bøyum, A. Isolation of mononuclear cells and granulocytes from human blood.Scand. J. Clin. Lab. Invest. 21, Suppl. 97 (Paper IV), 77–89 (1968)
7. M.S. Alam et al.; Demography, clinical presentation and laboratory diagnosis of leprosy by microscopy, histopathology and PCR from Dhaka city in Bangladesh; Lepr Rev (2017) 88, 122–130.
8. Behra R N, Sen P C, Sharma BM and Rajendran P.CMI in tuberculoidleprosy – A study on T lymphocyte population by E rosette technique. J.Ind.Assoc.for Communicable Diseases 1982; 5 : 23 – 27.
9. Aziz et al. Prognostic evaluation of Cell Mediated Immunity in Leprosy and correlation with clinicopathological status of Leprosy patients. Indian.J. Microbiol 1996; 39 (1): 5-12.
10. Dwyer JM,Bullock WE,Field JP.Disturbance of the blood T:B lymphocyte ratio in lepromatous leprosy. N.E.J. M.1973; 288:1036–39.
11. T. Hussain et al. Cd4+, Cd8+, Cd3+ Cell Counts And Cd4+/Cd8+ Ratio Among Patients With Mycobacterial Diseases (Leprosy, Tuberculosis), HIV Infections, And Normal Healthy Adults: A Comparative Analysis Of Studies In Different Regions Of India, Journal of Immunoassay and Immunochemistry Dec 2014 , 36:4, 420-443.