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# CYTOKINE RECOGNITION AND PROFILING IN PATIENTS WITH TUBERCULOUS LYMPHADENOPATHY USING ELISA

Immunology

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## ABSTRACT

Tuberculosis (TB) is known to persist as latent infection and it is during this latent phase that the bacilli are able to bypass the host immunity and infect extrapulmonary sites such as lymph nodes. This study aims to find the tentative role of the cytokines released by type-1 and type-2 helper lymphocytes in patients of tuberculous lymphadenitis and understand their role and prevalence during immune response against MTB in these patients. Thirty patients with clinically diagnosed and cytologically proven Tuberculous lymphadenitis constituted the cases. Blood sample of 30 cases and 10 healthy volunteers was collected after obtaining written consent and the separated serum was stored at -80°C. ELISA was carried out using the standard method with the serum of these patients for Th-1 cytokines (IL-2 & interferon-γ) and for Th-2 cytokines (IL-4 and IL-5). The concentrations of the type-1 cytokines, especially INF- γ as well as type-2 cytokines, i.e IL-4 and IL-5 was highly increased in the patients selected. It was also noted that the levels of all the cytokines were increased if the patient had necrosis or AFB positive report on fine needle aspiration as compared to granuloma only report. Patients having tubercular lymphadenopathy show increased INF- γ in the circulation. It was also noted that IL-4 and IL-5 also seem to increase in proportion to the decreased immune status.

## KEYWORDS

ELISA, TB lymphadenitis, IL-4, IFN-gamma

## I. INTRODUCTION

The development of an effective vaccine and new treatment modalities for TB requires a better understanding of the differences in regulation of the immune responses to *Mycobacterium tuberculosis* between individuals who are susceptible or resistant to the infection. (1)

Globally, an estimated 10.0 million people fell ill with TB in 2018, a number that has been relatively stable in recent years. There were an estimated 1.2million TB deaths among HIV-negative people in 2018 (a 27% reduction from 1.7 million in 2000), and an additional 251 000 deaths among HIV positive people (a 60% reduction from 620 000 in 2000).(2)

According to a study, tuberculous lymphadenitis is reported to occur in 25-60% of all EPTB cases. TB involving cervical lymph nodes represents 50% of extrapulmonary TB.(3)

The selection of the samples was done on the basis of the FNA report of the chosen patients which showed Acid fast bacilli or granuloma and necrosis on histopathology.

Serum of the selected patients were used in ELISA against cytokines i.e Th1 cells(IL-2, INF-γ) and Th2 cells(IL-4, IL-5). It coincides with the aim of our study which was analysing the therapeutic statuses of the four cytokines in patients of tubercular lymphadenopathy. A study indicates that Th1 response is known to play a dominant role in the resistance to tuberculosis and that INF-γ is the dominant cytokine in culture positive tuberculosis patients and healthy tuberculosis contact(4). Through this study we tend to find if the same therapeutic profile is repeated in patients of tubercular lymphadenopathy as well.

## II. Aims and Objectives

The aim of the study was to assess the therapeutic status of Th1 and Th2 cytokines in patients of tubercular lymphadenopathy. The objective was to identify confirmed cases of tubercular lymphadenopathy in selected patients and run ELISA for Th1 cytokines i.e IL-2 and INF- γ and Th2 cytokines i.e IL-4 and IL-5.

## III. Materials and Methods

Thirty-six patients were selected from the outpatient department Fine needle aspiration clinic(UCMS and GTB hospital) between May 2013 and July 2013 after taking their informed consent. Out of these, 30 patients were known to have clinically and study relevant tubercular lymphadenopathy. Ten healthy patients were included in the control group of population. Serum was then separated from the blood in separate tubes which was then properly labelled and then stored in a deep freezer at -80ºC. Patient selection was based on the presence of a positive acid fast stain, presence of necrosis or presence of granuloma or epithelioid cells in the histopathology report.

Presence and concentrations of the cytokines (both Th-1 and Th-2) in the serum samples was done by using enzyme linked immunosorbent assay kits for IL-2, INF- γ, IL-4 and IL-5. The readings were assessed using an ELISA reader at 490nm and graphs were obtained.

The data of the concentrations obtained from ELISA was tabulated and the significance of the data between the control groups and the individual cytokines was calculated using the SPSS software. Significance (P value) was also calculated between the test and control subjects of each cytokine(i.e AFB+ve, Necrosis, Granuloma) using the SPSS software. Comparison of the mean concentration of each cytokine was also tabulated in a graphical manner.

## IV. RESULTS

For the study 36 patients-16 male and 20 female patients were taken ranging from age 8 to 70 years. 32 patients presented with cervical lymphadenitis, 2 patients presented with inguinal lymphadenitis while 2 presented with submandibular lymphadenitis. We noted 14 cases with a positive AFB stain, 18 cases showing necrosis and 11 cases with granuloma on histopathology.

The data chart of the mean concentration of each cytokine of the test and control cases is given in table-1. IL-2 levels were found to be extremely low(in traces), so IL-2 has not been mentioned.

**Table 1: mean of concentrations of each cytokine**

| Column1 | Mean of Concentrations (pg) | Column2 | Column3 |
|---|---|---|---|
| | INF-γ | IL-4 | IL-5 |
| Test | 54.49 | 5.05 | 2.57 |
| Control | 6.3 | 0.7 | 1.54 |

Interferon-gamma levels were consistently high. It is to be noted that levels of IL-4 and IL-5 were considerably increased as depicted in figure-1.

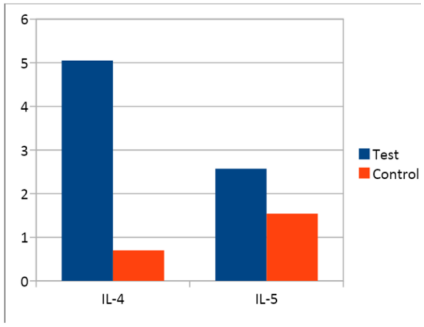


**figure-1 : IL-4 and IL-5 Levels (in pg)**

For the three morphological indices (AFB+ve, Necrosis and Granuloma) observed, the mean of concentration (in pg) of each cytokine is given in table-2. While representing mean data given in table-2 for each cytokine, it was observed that patients having granulomas in their cytology report had decreased levels of each cytokine as compared to those patients who had AFB+ve report.

| Conc (pg) | AFB+ve | Necrosis | Granuloma |
|---|---|---|---|
| INF-γ | 59.56 | 57.92 | 38.82 |
| IL-4 | 5.05 | 4.79 | 3.14 |
| IL-5 | 2.56 | 2.5 | 1.98 |

**Table 2: Concentration of each cytokine observed in different morphological indices.**

## V. DISCUSSION

It was observed that levels of INF- γ, IL-4 and IL-5 were increased in test subjects as compared to the controls. Increase in IL-4 can be associated with the decreased immune response of the patients and it points towards disease progression. High INF- γ levels can be assessed as showing resistance to the infection as seen in a study which showed that resistant families had increased INF- γ levels while increased IL-4 levels pointed towards families with disease progressors.(5)

Th-1 response is essential for protective immunity against tuberculosis(5). But we also observed an increase in IL-4 and IL-5 levels,which is th-2 cell response. Studies indicate that in patients of Tuberculous lymphadenitis, increase in INF- γ is associated with enhanced production of IL-10 and IL-4(6). If IL-4 levels are decreased in-vitro, say by giving anti-IL-4 factors, it increases potential chances of passive immunoprophylaxis(7). Increased Th-2 cell cytokines (i.e. IL-4 and IL-5) with suppression of the Th-1 cell cytokines is suggestive of an immunosuppressive response(8). Studies show that this trend was also followed by IL-5 which is inversely associated with low INF- γ levels(9) and hence a low th-1 response, correlating with IL-5 levels in AFB+ve patients in our study.

Patients who had a AFB+ve report on cytology showed increased levels of IFN-gamma, IL-4 and IL-5 as compared to the reports with granuloma. Hence having granulomas in the cytology is directly related to a good immune response and hence reduced th-2 response.

Our research can open newer areas of analysis in Anti-tubercular drugs regimens and it caters to questions as to how cytokine profile of each patient can be correlated to their FNA reports and how can we administer pro-th-1-cytokines and anti-th-2-cytokines for treating multiple drug resistant cases, e.g MDR-TB or XDR-TB.

## VI. CONCLUSIONS

1. It can be concluded that in patients of tuberculous lymphadenitis, a considerable and persistent rise in Interferon-gamma is observed along with an increase in the th-2 cytokines- IL-4 and IL-5. However IL-2 does not play much role in the immune response.
2. The rise in IL-4 and IL-5 can be directly related to the immune status of the patient, the prediction of a possible high risk status and hence disease progression.
3. The FNA report is an important marker for estimating the th-1 v/s th-2 cytokine profile in patients of tubercular lymphadenitis---- Acid fast stain and necrosis showed a shifting immune response to th-2 cells, hence poor prognosis. Whereas findings of granuloma indicated protective th-1 response and a good prognosis.
4. Further research is needed as to how newer drugs can be obtained in achieving treatment against resistant strains of the tubercle bacilli using immunology as its treatment basis.

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