Serum cytokine profile in leprosy and its correlation with clinico-histopathological profile

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Summary
Objectives: To analyse the serum levels of cytokines in leprosy patients, to correlate them with clinico-histopathological profile, and to study the effect of standard multidrug therapy on serum cytokine levels.

Design: Serum immunoassays of TNF-α, IFN-γ, IL-1β and IL-10 were performed by ELISA in 61 newly diagnosed cases of leprosy before starting therapy and during reactional episodes. Of these, cytokine assays could be performed in 17 cases after completion of therapy.

Results: Levels of all the studied cytokines were significantly raised in cases compared to controls (P < 0.05). Levels of TNF-α and IFN-γ were significantly raised in paucibacillary cases whereas all the studied cytokines were raised in multibacillary cases with rise in IL-1β and IL-10 being statistically significant (P < 0.001). No significant difference was however noted between TT and BT type; and BB, BL and LL types. All the studied cytokines were raised in reactional cases as compared to non-reactional cases. Comparing Type 2 reaction (T2R) and Type 1 reaction (T1R) cases, levels of IFN-γ, IL-1β and IL-10 were higher in T2R cases but only IL-10 was found to be statistically significant (P = 0.05) while TNF-α was higher in T1R cases. Post therapy serum levels of all the studied cytokines were significantly lower than pretherapy levels (P < 0.05) and were comparable to controls. Among the paucibacillary cases, levels of all the cytokines were seen to decrease after 6 months of standard multidrug therapy. In the multibacillary cases, mean levels of the cytokines were found to decrease after 1 year of therapy except IFN-γ.

Conclusion: Serum cytokine estimation may have a significant role in classifying various forms of leprosy and can be used to monitor therapy.

Introduction
Leprosy is a chronic infectious granulomatous disease caused by M. leprae which is an obligate intracellular pathogen.1 Cases of leprosy are highly concentrated in...
10 countries—India, Brazil, Myanmar, Nepal, Mozambique, Madagascar, Angola, Central African Republic, Democratic Republic of Congo and United Republic of Tanzania. India alone represents 64% of the prevalence and 78% of new case detection worldwide.\(^2\)

Leprosy is classified according to the degree and type of host immune response. Patients with tuberculoid leprosy have a strong cell mediated immune (CMI) response to \textit{M. leprae}, manifested by positive skin tests (Mitsuda reaction) and marked lymphocyte proliferative responses to \textit{M. leprae in vitro}. In contrast, patients with lepromatous leprosy have a depressed CMI response to \textit{M. leprae}, characterised by negative skin tests and diminished or absent lymphocyte proliferation.\(^3\) The polar groups [Tuberculoid (TT) and Lepromatous (LL)] are stable, but within the central groups [Borderline tuberculoid (BT), Borderline borderline (BB), Borderline lepromatous (BL)], downgrading towards the lepromatous pole, and upgrading (Reversal reactions) towards the tuberculoid pole may occur. The reactional episodes are due to an increase in the CMI and are characterised clinically by the leprosy lesions becoming inflamed and erythematous, with swelling and destruction of the peripheral nerves.\(^4,5\)

Cytokines are low molecular weight glycoproteins produced by immune as well as non-immune cells which act as molecular signals for communication between cells of the immune system.\(^6\) Defects in cytokine production or their expression at the target tissue sites are associated with pathologic states, therefore, delivery of an exogenous cytokine can lead to clinical benefit.

Even though there have been exciting new developments in the field of immunology, there are still many queries related to the immunopathology of leprosy. There is paucity of documented studies on the assessment of serum cytokine levels in leprosy patients by ELISA which is easily available especially in the developing world and has a fair sensitivity and specificity. Literature reveals controversial results on the levels of serum cytokines in various forms of leprosy. Moreover, the effect of multidrug therapy on the cytokine levels has not been substantiated well in the past. With this background in mind, the aim of the present study was to analyse serum cytokine profiles of untreated leprosy patients, compare them with healthy controls, co-relate the patterns with the clinico-histopathological picture and to see the effect of Multidrug Therapy (MDT) on the levels of serum cytokines.

**Materials and Methods**

A total of 61 untreated cases of leprosy attending the Dermatology Out Patient Department (OPD) of Smt. Sucheta Kriplani Hospital were enrolled for this study. After a written consent, a detailed history was obtained and a thorough physical examination was done. The patients were classified according to Ridley-Jopling’s five subgroups (TT, BT, BB, BL, LL)\(^7\) and a sixth subgroup Pure Neuritic (PN) which was described by the Consensus Classification of Indian Association of Leprologists (1982).\(^8\) Slit smear examination for lepra bacilli and skin biopsies were performed in all cases except the Pure neuritic ones.

The cases comprised 38 paucibacillary (5 TT, 26 BT, 7 PN) and 13 multibacillary (1 BB, 6 BL, 6 LL); 10 patients presented with reactions (six of Type 1 and four of Type 2) and 2 LL patients developed Type 2 reaction during the course of our study.

All patients received the standard WHO-MDT regimen for leprosy [Rifampicin and Dapsone for 6 months in paucibacillary; Rifampicin, Dapsone and Clofazimine for 12 months
(BI \leq 4)/24 months (BI \geq 5) in multibacillary] and patients were followed-up to look for onset of any lepra reaction and to monitor the effect of therapy.

Patients who were already on multidrug therapy or steroids or having any other systemic illness were excluded from the study. Thirty age and sex matched healthy controls were also included. Blood was collected in a plain vial, centrifuged immediately, and sera were stored at \(-70^\circ\)C prior to assay. At least one cytokine belonging to each of the Th1, Th2 and macrophage cytokine subsets were chosen for reasons of feasibility. Cytokine assay for TNF-\(\alpha\), IFN-\(\gamma\), IL-1\(\beta\) and IL-10 was done by ELISA kit method (Diaclone, France). Post-therapy cytokine assays were performed in 17 cases within 1 week of completion of therapy [11 PB (after 6 months) and 6 MB (after 12 months)]. Out of 17 follow up cases, skin biopsies could be performed at the completion of therapy in 10 cases (all PB).

The concentration of individual cytokines in pg/ml in each patient was used for data analysis. Student’s \(t\)-test and chi square test were employed for comparison between two groups. \(P\) values \#0·05 were considered statistically significant. The correlation coefficient \((r)\) was applied between the serum cytokine levels.

The study was approved by the institution’s Ethics Committee and the university’s Review Board and the funding for cytokine kits was provided by the Department of Pathology, Lady Hardinge Medical College.

**Results**

The majority of cases (52·45%) were in the age group of 20–40 years with male to female ratio of 3:1. Mean age of affliction was 30 years. BT was the predominant form of leprosy comprising 26 (42·6%) cases. Nerve involvement in the form of thickened nerves/neuritis was found in 58 (95·1%) cases at the time of sera collection. Ten patients (14·4%) had abnormal facies (madarosis, infiltration or lagophthalmos) out of which two patients (both LL) had leonine facies. Four patients (6·6%) had deformities (claw hand, thenar atrophy or foot drop).

**Clinico-histopathological correlation**

The maximum cases were of Borderline Tuberculoid Leprosy (42·6%). Only two cases out of 61 (i.e. 3·3%) showed discordance in clinical and histopathological findings; 100% of the paucibacillary patients had a BI between 0 and 2+ and 70% of the multibacillary patients

<table>
<thead>
<tr>
<th>CYTOKINES</th>
<th>CASES (N = 61)</th>
<th>CONTROLS (N = 30)</th>
<th>(P) value (Student’s (t)-test)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>MEAN (pg/ml)</td>
<td>STD DEV (pg/ml)</td>
<td>MEAN (pg/ml)</td>
</tr>
<tr>
<td>TNF-(\alpha)</td>
<td>70·17</td>
<td>50·84</td>
<td>19·46</td>
</tr>
<tr>
<td>IFN-(\gamma)</td>
<td>100·01</td>
<td>107·45</td>
<td>25·79</td>
</tr>
<tr>
<td>IL-1(\beta)</td>
<td>93·97</td>
<td>80·98</td>
<td>15·76</td>
</tr>
<tr>
<td>IL-10</td>
<td>63·08</td>
<td>59·48</td>
<td>15·90</td>
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</table>
had a BI between 3+ and 6+. The rest (30%) of the patients had a BI of 0–2 but displayed more than six skin lesions and were placed in the multibacillary group.

CYTOKINE ANALYSIS

Levels of all the studied cytokines were significantly raised in cases rather than controls ($P < 0.05$) (Table 1).

In the patient group (including all subtypes), mean serum levels of TNF-α, IFN-γ, IL-1β and IL-10 were $70.17 \pm 50.84 \text{ pg/ml}$, $100.01 \pm 107.45 \text{ pg/ml}$, $93.97 \pm 80.98 \text{ pg/ml}$ and $63.08 \pm 59.48 \text{ pg/ml}$ respectively.

Between the paucibacillary (PB) and multibacillary (MB) cases, higher levels of TNF-α and IFN-γ were found in PB patients whereas higher levels of IL-1β and IL-10 were found in MB cases ($P < 0.05$) (Figure 1).

No significant difference was noted among the cytokine levels between cases of TT and BT leprosy; and between BL and LL cases.

All the studied cytokines were raised in reactional cases as compared to non-reactional cases (Figure 2).

When cases with T1R were compared with non-reactional PB cases, it was found that levels of TNF-α and IL-1β were significantly higher in T1R than in PB cases ($P < 0.001$). IFN-γ was raised in T1R cases as compared to PB cases however was not statistically significant (Table 2).
When cases with T2R were compared to non-reactional multibacillary cases, it was found that all of the studied cytokines were raised in T2R than in non-reactional multibacillary cases but only IFN-γ levels were statistically significant ($P < 0.05$) (Table 3).

Between T2R and T1R patients, levels of IFN-γ, IL-1β and IL-10 were higher in T2R cases than T1R cases but only IL-10 was found to be statistically significant ($P = 0.05$) while TNF-α was higher in T1R cases (Table 4).

The bacillary index (BI) showed a positive correlation with levels of TNF-α and IFN-γ and a negative correlation with levels of IL-1β and IL-10 (Figure 3).

Positive correlation was found between TNF-α and IFN-γ levels and between IL-1β and IL-10 levels (Figure 4A and 4B).

**EFFECT OF THERAPY ON CYTOKINE LEVELS**

Post-therapy serum levels of all the studied cytokines in the paucibacillary group were significantly lower than the pre-therapy levels ($P < 0.05$) and were comparable to controls (6 months post-therapy) (Figure 5).

The difference in the mean levels of TNF-α and IFN-γ, before and after MDT was found to be significant ($P < 0.01$).
In the multibacillary cases, mean levels of TNF-α, IL-1β and IL-10 were found to decrease after 1 year of treatment but did not come down to the level of healthy controls. However, two multibacillary cases showed increase in IFN-γ levels (these two cases went into T2R during the study period).

**Histopathology Post Therapy**

Post therapy skin biopsies of the 10 PB cases, taken within a month of completion of therapy, were found to be normal with the disappearance of the inflammatory granulomas.

**Discussion**

Leprosy provides an ideal model to address the role of T-cell subsets in human diseases. Cytokines play important roles in both protection and immunopathology of leprosy and are considered important components of lepra reactions. *M. leprae* is not toxic to its host cells, and the pathologic process reflects the immune response of the patient to the organisms. At the tuberculoid pole, patients exhibit a strong CMI response to *M. leprae* leading to control of bacillary replication. At the lepromatous pole, patients exhibit a defective CMI response to *M. leprae* thereby causing a high bacillary load.

In the present study, serum levels of TNF-α, IFN-γ, IL-1β and IL-10 were raised in all leprosy cases, both paucibacillary and multibacillary. TNF-α and IFN-γ were raised in paucibacillary leprosy, whereas all the studied cytokines were raised in multibacillary leprosy cases with rise in IL-1β and IL-10 being statistically significant ($P < 0.05$).

### Table 2. Cytokine levels in paucibacillary cases compared to cases with type 1 reaction

<table>
<thead>
<tr>
<th>CYTOKINES</th>
<th>PB (N = 31)</th>
<th>PB with T1R (N = 6)</th>
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<tr>
<td></td>
<td>MEAN (pg/ml)</td>
<td>STD DEV (pg/ml)</td>
</tr>
<tr>
<td>TNF-α</td>
<td>61.66</td>
<td>28.55</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>77.07</td>
<td>60.90</td>
</tr>
<tr>
<td>IL-1β</td>
<td>24.71</td>
<td>19.68</td>
</tr>
<tr>
<td>IL-10</td>
<td>19.09</td>
<td>14.87</td>
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### Table 3. Cytokine levels in multibacillary cases compared to cases with type 2 reaction

<table>
<thead>
<tr>
<th>CYTOKINES</th>
<th>MB (N = 13)</th>
<th>MB with T2R (N = 4)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>MEAN (pg/ml)</td>
<td>STD DEV (pg/ml)</td>
</tr>
<tr>
<td>TNF-α</td>
<td>55.32</td>
<td>76.78</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>55.17</td>
<td>62.42</td>
</tr>
<tr>
<td>IL-1β</td>
<td>131.95</td>
<td>98.73</td>
</tr>
<tr>
<td>IL-10</td>
<td>60.30</td>
<td>29.96</td>
</tr>
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It has been hypothesised that IFN-γ and TNF-α play a role in immunoprotection and immunopathology, whereas IL-10 and IL-1β are immunosuppressive. Our results are consistent with this hypothesis. IFN-γ activates antimicrobial mechanisms in macrophages by inducing Nitric oxide synthase, leading to the production of Nitric oxide, a powerful microbicidal molecule. TNF-α is necessary for granuloma formation containing bactericidal macrophages that play an essential role in preventing the extension and dissemination of mycobacterial infection. IL-10 is a potent inhibitor of IFN-γ production and to some extent of TNF-α. It suppresses the macrophage mediated

<table>
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<tr>
<th>CYTOKINES</th>
<th>T1R (N = 6)</th>
<th>T2R (N = 6)</th>
<th>P value (Student’s t-test)</th>
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<tr>
<td>TNF-α</td>
<td>118·23</td>
<td>105·45</td>
<td>0·7</td>
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<tr>
<td>IFN-γ</td>
<td>166·61</td>
<td>352·31</td>
<td>0·07</td>
</tr>
<tr>
<td>IL-1β</td>
<td>78·49</td>
<td>135·78</td>
<td>0·09</td>
</tr>
<tr>
<td>IL-10</td>
<td>21·96</td>
<td>157·26</td>
<td>0·05</td>
</tr>
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</table>

**Figure 3.** Scatter plots showing correlation between serum cytokines and bacillary index.
Figure 4. (A) Scatter plots showing correlation between serum cytokines. (B) Scatter plots showing correlation between serum cytokines.
destruction of intracellular pathogens, leading to increased bacillary load.\textsuperscript{19} Raised levels of IL-1\textbeta in LL patients can be explained in view of enhanced B-cell responses and antibody production in LL patients.\textsuperscript{20}

The results of present study were similar to those shown by Moubasher \textit{et al.}\textsuperscript{12} Belgaumkar \textit{et al.}\textsuperscript{21} showed increased levels of IFN-\gamma in tuberculoid leprosy, while Sarno \textit{et al.}\textsuperscript{22} demonstrated increased levels of TNF-\alpha. On the contrary, Parida \textit{et al.}\textsuperscript{23} showed that lepromatous leprosy is associated with a rise in TNF-\alpha and IL-1\textbeta whereas tuberculoid leprosy is associated with low serum TNF-\alpha and IL-1\textbeta. Similarly, an increase in TNF-\alpha was seen in lepromatous leprosy by Pisa \textit{et al.}\textsuperscript{24} Watson \textit{et al.}\textsuperscript{25} and Jayapal \textit{et al.}\textsuperscript{26} observed that the quantity of IL-1\textbeta produced by LL/BL patients before therapy was significantly less when compared to healthy controls.

Leprosy reactions are major causes of hospitalisation and disability of patients with leprosy. Type 1 reaction or reversal reaction which occurs in patients with borderline forms of disease represents delayed type hypersensitivity reaction with increased cell-mediated immune reactivity to antigens of \textit{M. leprae} that can rapidly produce nerve damage. It is associated with infiltration of cytokine secreting CD4 lymphocytes in skin lesions and nerves resulting in edema and painful inflammation. High levels of cytokines indicate that this reaction represents a state of immune hypersensitivity and exaggerated CMI response that can lead to clearing of bacilli and concomitant tissue damage.\textsuperscript{27}
Type 2 reaction is a systemic inflammatory response to the deposition of immune complexes that are responsible for vasculitis, arthritis, panniculitis and nerve injury. Transient IFN-γ production by Th2 cells is operative in ENL as opposed to a more established production of IFN-γ by Th1 clones in Reversal Reaction. Moreover, TNF-α and IL-1β are proinflammatory cytokines that play a role in chronic inflammatory pathologies. These two cytokines are responsible for the vasculitic lesions characteristic of leprosy reactions.

In our study we found that all the studied cytokines were raised in reactional cases as compared to non-reactional leprosy as was also shown by Moubasher et al. Rise in the levels of IFN-γ and TNF-α has been demonstrated by Iyer et al., Bhattacharya et al., Parida et al., and Esquenazi et al. in reactional leprosy. In the present study, on comparing T2R and T1R, TNF-α was found to be higher in T1R whereas higher levels of IFN-γ, IL-1β and IL-10 were seen in T2R cases and rise in IL-10 was statistically significant. This contrasts with the findings of Sarno et al. and Barnes et al. who reported higher TNF values in T2R cases as compared to T1R cases. During our study period, the two patients who went into T2R were showing initial TNF-α, IL-1β and IL-10 levels > 100 pg/ml. Raised TNF-α in T1R indicates a state of high immunity and plays an important role in granulomas becoming more epithelioid and activated, with concomitant tissue damage. IL-10 plays a role in the pathogenesis of T2R. Its high levels enhance B-cell responses and augment antibody formation potentiating the formation of immune complexes. The results of the present study suggest that a significant rise in TNF-α and IL-10 may predict the occurrence of T1R and T2R respectively, whereas rise in IL-1β and IFN-γ may predict the occurrence of both T1R and T2R. Thus serial estimation of cytokine expression may allow early indication of occurrence and evolution of reactional inflammation in leprosy. Further studies are however required to establish this.

The effect of multidrug therapy of leprosy on the serum cytokine levels has not been widely reported in the past. Most of the researchers have observed the effect of steroid treatment in reactional leprosy only. In this study, we found that serum levels of all the studied cytokines in paucibacillary cases significantly decreased after 6 months of MDT and came down to the level of control subjects. In the multibacillary cases, mean serum levels of all the cytokines were found to decrease after 1 year of MDT except IFN-γ. Two multibacillary cases showed increase in IFN-γ levels after treatment suggesting conversion to a higher immunity state. In the study of Moubasher et al. the serum levels of the studied cytokines were significantly reduced after 1 year of treatment in paucibacillary leprosy patients. After 1 year of MDT (but not 6 months) paucibacillary patients showed a significant reduction in all the studied serum cytokines to levels comparable with those of healthy controls. Multibacillary patients also showed a significant reduction in all studied serum cytokines, but the levels were higher than those of healthy controls after one year of therapy. On the other hand, Jayapal et al. found no change in the quantity of IL-1β produced by LL/BL patients after 6 months of MDT.

Cytokines are produced in response to antigenic stimulation of the immune system. If these antigens are removed, there will be a decrease in the levels of cytokines. Multidrug therapy decreases the bacterial load in leprosy patients and the decrease in antigenic stimulation of immune system possibly causes reduction in serum cytokine levels. Moreover dapsone and clofazimine have anti-inflammatory effects which can explain the reduction of cytokine levels particularly the proinflammatory cytokines like TNF-α and IL-1β.
Conclusion

This study concluded that serum cytokine estimation may have a significant role in classifying various forms of leprosy. It can help in predicting the course of the disease and monitoring therapy. Furthermore, the increased cytokines suggest a potential target for therapeutic modulation. Additional studies may determine the sensitivity and specificity of these cytokines as leprosy disease markers.

Estimation of cytokines like TNF-α, IFN-γ, IL-1β and IL-10 can help predict the development of reactional states in leprosy. More research is needed in this regard as revelation of the cytokine patterns during reactions, might lead to development of specific targeted immunotherapy which can prevent such episodes, thus saving the patient from physical and psychological trauma.

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References


