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Study of serum interleukin-4 in north Indian leprosy patients

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Abstract
Introduction: Leprosy is a chronic infectious granulomatous disease caused by *M. leprae*, which affects mainly the skin and peripheral nervous system. For treatment purposes leprosy is classified as paucibacillary (PB) and multibacillary (MB). Interleukin-4 is a typical Th2 cytokine and mediator of the Th1/Th2 balance.
Aims: Estimation of serum interleukin-4 (IL-4) levels in leprosy patients and compare & correlate with the values of controls.
Materials and methods: The present study was conducted in Department of Biochemistry and Dermatology & Venerology, SGT Medical College, Hospital & Research Institute, Budhera, Gurugram, Haryana and Civil Hospital Gurugram, Haryana. Serum IL-4 was estimated in 50 patients and 50 control by using enzyme linked immunosorbent assay (ELISA).
Result: Serum IL-4 was significantly higher in cases (35.20 ± 4.91 pg/mL) compared to controls (5.52 ± 1.27 pg/mL, p<0.000). Serum IL-4 level was also higher in multibacillary patients (37.40 ± 2.29 pg/mL) compared to paucibacillary patients (26.4 ± 0.90 pg/mL).
Conclusion: The increase in serum IL-4 levels indicates the association of disease progression with Th2 activation.

Keywords: leprosy, interleukin-4 (IL-4), multibacillary, paucibacillary, type-1 & 2 reaction

Introduction

Leprosy is a chronic infectious granulomatous disease. It is caused by *M. leprae* which is an obligate intracellular pathogen [1]. *M. leprae* effects mainly on the skin and peripheral nervous system [2]. In India, prevalence rate of leprosy was 0.68 per 10,000 populations with 86,000 cases on record as on 1 April 2014 [3]. As per WHO, leprosy is classified into paucibacillary (PB) and multibacillary (MB) for treatment purposes [4]. But based on clinically and histopathological features leprosy is classified into tuberculoid (TT), borderline tuberculoid (BT), midborderline (BB), borderline lepromatous (BL) and lepromatous leprosy (LL) [5].
Cytokines are glycoproteins produced by immune and non-immune cells. It acts as molecular signals for communication between cells of the immune system [6]. The defects in cytokine production at the target tissue sites are associated with pathologic states [7]. Thus there has been a surge of research concerning the immune patho-mechanisms with the hope that predictive diagnostic and prognostic parameters will emerge [8]. Th1 cells play role in secreting interleukins 2 (IL-2) and interferon-γ (INF- γ) and resulting in macrophage activation. But Th2 cells have role in secreting IL-4, IL-5, and IL-13, which enhances the production of antibodies and inhibits macrophage activation. Th17 cells produces IL-17 and IL-22 which is involved in inflammation and auto immunity [9].
Yang *et al*. 2011 attributed that cell-mediated immune response and humoral immune response play major roles in leprosy infection. IL-4 is a typical Th2 cytokine and mediator of the Th1/Th2 balance [10]. The regulated expression of CD209 on Schwann cells by the local cytokine can facilitate infection of *M. leprae*, which causes subsequent tissue injury. IL-4 induces CD209 on macrophages and Schwann cells which can bind and take up mycobacteria [11].
Thus considering the above background, we have carried out this study to estimate serum IL-4 levels in leprosy patients to know the role of cytokines in the immune-pathogenesis of leprosy.

Materials and methods

This study was conducted in Department of Biochemistry and Dermatology & Venereology, SGT Medical College, Hospital & Research Institute, FMHS, SGT University, Budhera, Gurugram and Civil Hospital, Gurugram Haryana. The study was approved by Institutional Ethical Committee (IEC), SGT University, Gurugram, and Haryana, India.
50 new and clinically diagnosed cases of leprosy were included in the study who was visiting outpatient department of SGT Hospital and Civil Hospital, Gurugram, Haryana. Patients on immunosuppressive therapy, hormonal therapy, concomitant inflammatory or autoimmune disease and acute or any other chronic infections and who failed to give consent to participate in the study were excluded. 50 age and sex

matched healthy controls were included in this study. After taking written consent from leprosy patients and healthy control, 5ml venous blood was collected in plain tubes. The blood sample was centrifuged and the serum was separated. The serum IL-4 level was estimated by ELISA Assay Kit.

Statistical analysis

Statistical Analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 24.0, for windows (SPSS, Inc., Chicago). The data were expressed as mean ± standard deviation (SD). Independent Student's t-test was used to compare the values (Leprosy *vs.* Controls) and Pearson's correlation coefficient was used to elucidate the association between the variables. $p<0.05$ was considered statistically significant.

Results

In this present study, out of 50 patients 37 (74%) were male and 13 (26%) were female of mean age 31.92±11.26 years. On the basis of clinical features, patients were subdivided into multibacillary (MB) and paucibacillary (PB). In this study 40 (80%) patients have multibacillary and only 10 (20%) patients have paucibacillary.

Among the 50 leprosy cases, 8 (16%) patients were type-1 reaction, 4 (8%) patients were type 2 reaction and 38(76%) were without reaction. In this study, 50 healthy controls were included, among them 40 (80%) male and 10(20%) female of age group 32.18±10.83 years.

Serum IL-4 was increased in cases (35.20±4.91 pg/mL) compared to controls (5.52±1.27 pg/mL) with p< 0.000. The level of IL-4 was raised in multibacillary patients (37.40±2.pg/mL) compared to paucibacillary patients (26.40±0.90pg/mL). The level of IL-4 was higher in type-2 reaction (37.37±3.85 pg/mL) compared to type-1 reaction (35.05±5.43pg/mL) and without reaction (35.0±4.95pg/mL).

Table 1: Comparison between case & control

Parameters	Case (n=50)	Control (n=50)	t-value	*p*-value
IL-4	35.20 ± 4.91	5.52 ± 1.27	41.35	0.00***

p <0.05 Significant**; *p* <0.01Highly significant***; *p*>0.05Not significant*

Table 2: Comparison between multibacillary & paucibacillary case

Parameters	Multibacillary (n=40)	Paucibacillary (n=10)	t-value	*p*-value
IL-4 (pg/mL)	37.40 ± 2.29	26.40 ± 0.90	23.78	0.00***

p <0.05 Significant**; *p* <0.01Highly significant***; *p*>0.05Not significant*

Table 3: Comparison between multibacillary, paucibacillary case & control

Parameters	Multibacillary (n=40)	Paucibacillary (n=10	Control(n=50)	t-value	p-value
IL-4 (pg/mL)	37.40 ± 2.29	-	5.52 ± 1.27	78.65	0.00***
	-	26.40 ± 0.90		49.01	0.00***

p <0.05 Significant**; p <0.01Highly significant***; p>0.05Not significant*

Table 4: Comparison between type-1 reaction, type-2 reaction & without reaction

Parameters	Type-1 reaction (n=8)	Type-2 reaction (n=4)	Without reaction (n=38)	t-value	*p*-value
IL-4 (pg/mL)	35.05 ± 5.43	-	35.0 ± 4.95	0.02	0.93*
	-	37.37 ± 3.85		0.92	0.36*

p <0.05 Significant**; *p* <0.01Highly significant***; *p*>0.05Not significant*

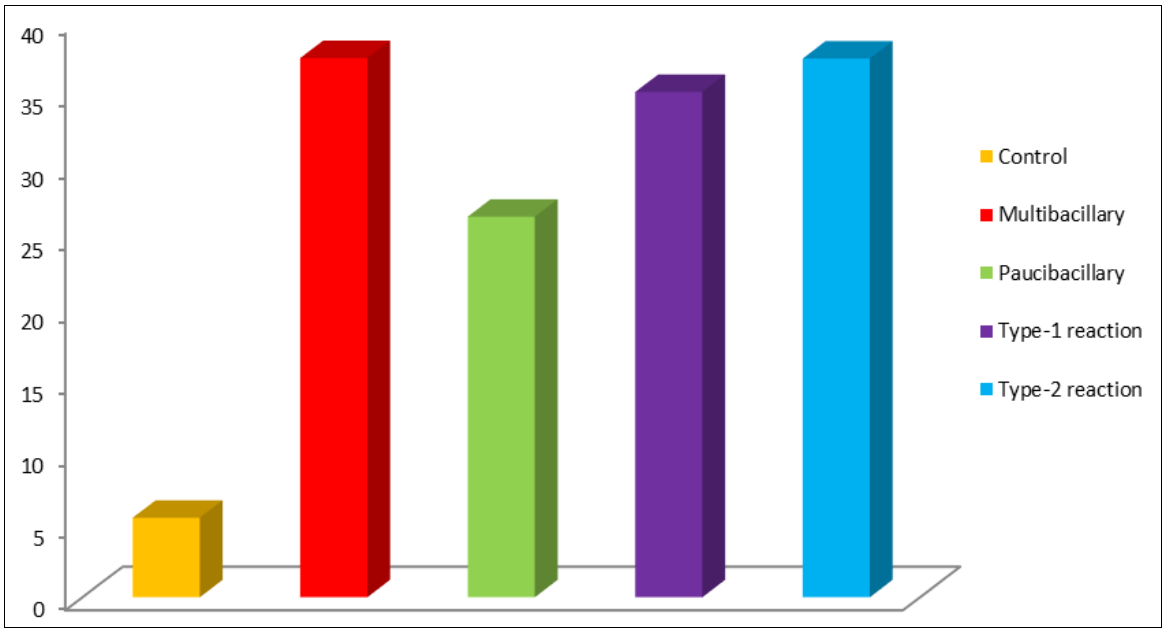

Fig 1: Graphical representation of total no. of cases with types & control

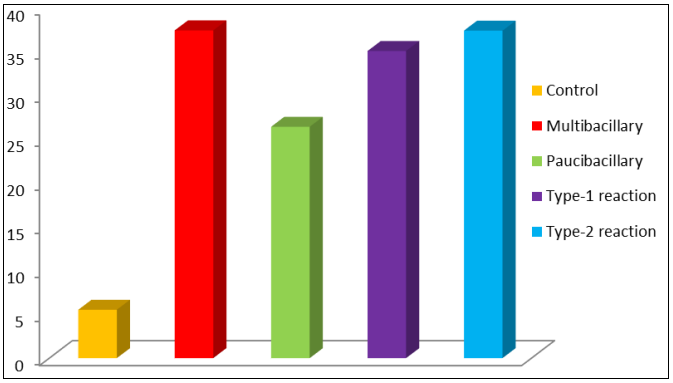

Fig 2: Graph showing level of IL-4 in cases with types & control

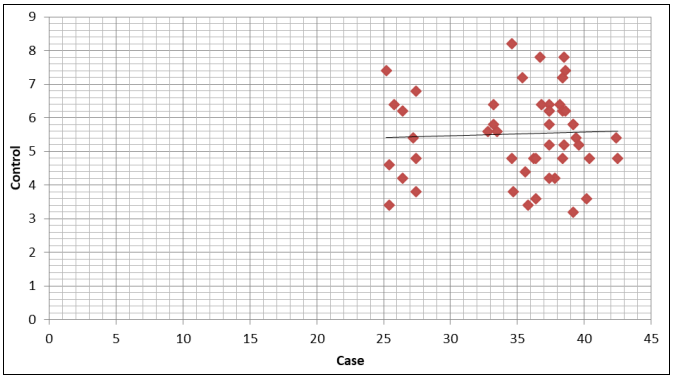

Fig 3: Graph showing positive correlation of IL-4 between case & control.

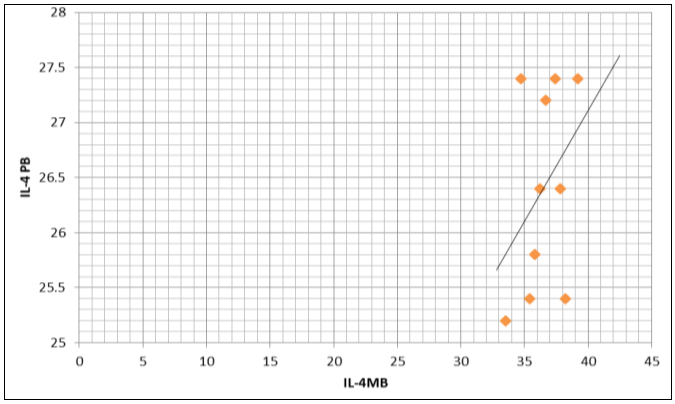

Fig 4: Graph showing positive correlation of IL-4 between MB & PB.

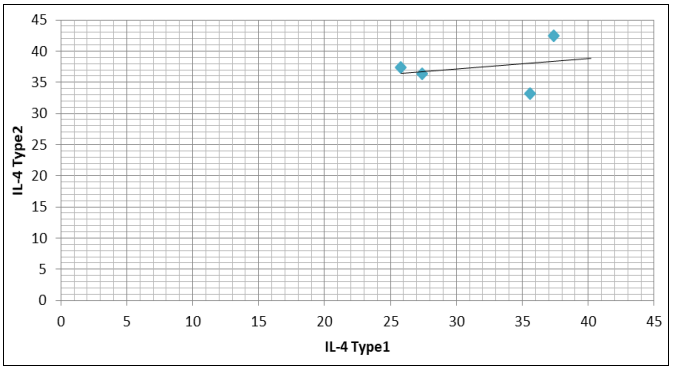

Fig 5: Graph showing positive correlation of IL-4 between type-1 & type-2.

Discussion

In this present study, among 50 newly diagnosed leprosy patients 37 (74%) were male and 13 (26%) female with mean age group 31.92 ± 11.26 years. This is comparable with the studies of Sehgal *et al*. (1982) [12], Rao *et al*. (2006) [13] and Abdullae *et al*. (2013) [14]. As per Rao *et al*. (2006) [13] male to female ratio was 3:2 which showed male majority and it may be due to socio-cultural situation and greater exposure to men [13].

In this present study among cases 40 (80%) were multibacillary and only 10 (20%) were paucibacillary (Figure 1). Moreover, among the cases 8 (16%) were type-1 reaction, 4 (8%) were type-2 reaction and 38 (76%) were without reaction (Figure 1). The cases of this study were compared with 50 healthy controls with mean age group 32.18 ± 10.83 years. Among 50 controls 40 (80%) were male and 10 (20%) female (Figure 1).

In this present study, circulating level of IL-4 was increased in cases (35.20 ± 4.91 pg/mL) compared to controls (5.52 ± 1.27pg/mL) with $p<0.00$ (Table1, Figure 2). The serum level of IL-4 has positive correlation between cases and controls (Figure 3). The circulating level of IL-4 was highly raised in multibacillary (37.49 ± 2.29pg/mL) compared to paucibacillary (26.40 ± 0.90 pg/mL) and control (5.52 ± 1.27pg/mL) with $p<0.00$ (Table 2, 3 & Figure 4). This result is in accordance to Abdullae *et al*. (2013) [14] and by Poovamma AS *et al*. (2017).[15] Both of them have concluded that multibacillary leprosy patients had higher level of IL-4 compared to paucibacillary and controls [14, 15].

As per Reja *et al*. (2013) pathology of leprosy was primarily due to immune interaction between subsets of T-cells and *Mycobacterium leprae* antigens which produce cytokines [16]. These cytokines communicate signals between immune response and tissue damage [17].

In this present study, circulating level of IL-4 was increased in type-2 reaction (37.37 ± 2.29 pg/mL) compared to type-1 reaction (35.0 ± 4.95 pg/mL) (Table 4, Figure 2). The comparison between type-1 reaction, type-2 reaction and without reaction were statistically non-significance with $p>0.05$ (Table 4). But positive relation was established between type1 and type2 reaction (Figure 5). The finding of present study was similar with Abdullae *et al*. (2013) and by Poovamma AS *et al*. (2017) [14, 15]. As per Abdullae *et al*. (2013) level of serum IL-4 was lowest in type-1 reaction and concluded that overproduction of IL-4 in lepromatous leprosy may derive their liability to develop erythema nodosum leprosum (ENL) [14]. Likewise, Poovamma AS *et al*. (2017) reported that serum IL-4 had highest level in type-2 reaction which is similar to the result of present study [15].

In this study, circulating level of IL-4 was raised in case compared to controls with highest in MB and type-2 reaction. This provide the information regarding balance between Th1 and Th2 cells [15]. However, Th1 and Th2 cells can cross regulate one another, secretion of IL-4 is suppressed by IFN-γ and over production of Th1- type cytokines is associated with type 1 lepra reaction [18, 19]. But type 2 reaction occured in patients with poor cell mediated immunity to *M. leprae* [15]. In Erythema nodosum leprosum all cytokines associated with neutrophil chemotaxis and antibody production were elevated [20]. IL-4 enhances humoral immunity and inhibits cell

mediated immunity [21]. IL-4 contributes to high antibody levels and unhindered replication of bacilli in leprosy patients [15]. In this study paucibacillary patients have also increased level of IL-4 compare to control. It may be due to the difference between lesional and circulating cytokine toward tuberculoid pole which is well known for localized neurocutaneous disease rather than systemic [14].

Thus, present study showed that the serum level of IL-4 in different types of leprosy patients play major role in diagnostic and monitoring the leprosy patients to prevent from further damage and deformities.

Conclusion

The current study revealed that, an increased serum IL-4 level could be related to disease progression with Th2 activation in different leprosy patients with reaction. The results of this study would be useful in diagnosing various types of leprosy and further planning for the treatment.

Conflict of Interest: - None declared.

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