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**ORIGINAL ARTICLE**

## Circulatory level of IL-6 and INF- $\gamma$ in Sudanese leprosy patients

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**ABSTRACT**

**Background:** Leprosy is an infectious disease that affects the skin, peripheral nerves and mucosa caused by Mycobacterium leprae. It is considered as a public health problem for many countries. This study aimed to measure the level of IL-6 and INF- $\gamma$  in the serum of leprosy patients to determine their role in the pathogenesis of the disease.

**Material and methods:** This observational hospital-based case-control cross-sectional study includes 170 individuals, 110 were diagnosed with leprosy (78 male and 32 female), and 60 healthy control (44 male and 16 female). Blood samples were collected and IL-6 and INF- $\gamma$  were measured by Sandwich ELISA using commercial kits.

**Results:** Results revealed a significant difference ( $p < 0.001$ ) in the concentration of serum levels of IL-6, INF- $\gamma$ , and GLO among the studied groups, except for TP ( $p < 0.006$ ). IL-6 shows a very highly statistically significant correlation with INF- $\gamma$  in leprosy patients. INF- $\gamma$  shows the highest concentration in no reaction group, then in type1 reaction group, type2 reaction group, and control group. IL-6 showed significant differences in lepers groups. The highest concentration was found in PB type lepers, followed by MB type.

**Conclusion:** IL-6 and INF- $\gamma$  levels were markedly increased in leprosy patients and their highest concentration obtained in (no reaction) group. Also levels of serum total proteins and globulins were significantly higher in leprosy patients. Serum cytokines estimation may aid in classifying various leprosy forms and reactions types

**Keywords:** Interlukin-6 (IL-6); Interferon- $\gamma$  (INF-  $\gamma$ ); Pauci bacillary (PB); Multi bacillary (MB)

### INTRODUCTION

Leprosy is an infectious disease that affects the skin, peripheral nerves and mucosa caused by Mycobacterium leprae (*M. leprae*) [1]. It is considered as a public health problem for many countries. It is not necessary all who

are exposed to the bacillus will develop the disease, like many immunological and genetic factors having a role [2, 3]. Many trials for leprosy classification, finally in 1996 World Health Organization (WHO) modified a new classification for treatment optimization. Pauci

bacillary (PB), include all patients having less than five skin lesions and multi bacillary (MB) for all others [4,5]. Delay in diagnosis in most regions became due to the stigma [6] that faced leprosy patients in different forms, such as reduced self-esteem, rejection, divorce, loss of a job, and many other types of discrimination [7]. The consequence of that increase in disabilities and deformities grade-2 that results from immune response mediated type 1 or type 2 reaction [8].

Interleukin-6 (IL-6) is a pleiotropic multifunctional immunoregulatory glycoprotein composed of four antiparallel  $\alpha$  helices 6. It has an important physiological role in many functions such as T cell differentiation, homeostasis, nerve cells, and embryonic development [9,10]. The IL-6 is produced by nearly all cells, like monocytes, neutrophils, macrophages, T, and B cells beside the hepatocytes as the main expression site [9]. The gene of IL-6 in humans is located on chromosome 7p15-p21 containing four introns and five exons, with a molecular mass of 21 to 28 kDa and 212 amino acids in length [11]. IL-6 is an interleukin that shows a proinflammatory role, but inhibits IL-1 and TNF- $\alpha$  and activates IL-10 so it gained an anti-inflammatory effect. It is a resolution factor in balancing pro- and anti-inflammatory outcome of the immune response, this discusses the switching from innate to acquired immunity [12].

Interferon-gamma- $\gamma$  (INF- $\gamma$ ) is a homodimeric cytokine produced by natural killers (NK), CD4TH1 cells and CD8 cytotoxic cells. It is located on chromosome 12q15 and secreted as a 143 amino acid polypeptide, resulting in a pyroglutamate amino terminus, the addition of carbohydrate, and a heterogeneous carboxyl terminus after a post-translation modification. It has a potent immunomodulatory, antiproliferative and antiviral activities. After 18 to 24 hours from stimulation, it reaches the maximum level [13, 14]. INF- $\gamma$  is an important factor that controls B cell differentiation, by increasing or decreasing B cell's immune response [15]. Many other functions facilitated by it such as inhibition of

virus adhesion and penetration by changes on the cell membrane, increasing of HLA I and II on the cell membrane, and increasing immunoglobulin secretion in the late stages [15,16].

This study aims to measure the level of IL-6 and INF- $\gamma$  in Sudanese leprotic patients to investigate their role in the pathogenesis of leprosy. Besides their variation of levels in the different reaction types and leprosy types.

#### **METHODOLOGY:**

This observational hospital-based case-control cross-sectional study included 110 leprosy patients (78 Male 70.9% and 32 Female 29.1%), they were admitted to the Abu Rouf leprosy clinic and Khartoum Dermatology Teaching Hospital. And 60 healthy controls (44 Male 73.3% and 16 Female 26.7%) from Khartoum in period of August 2016 to October 2018.

The patients were classified to type 1, type 2 and no reaction. While classified pauci bacillary (PB) and multi bacillary (MB) according to the type of disease. Five ml of venous blood were collected and the serum was used to measure the level of interleukin-6, interferon- $\gamma$ , Total protein (TP), albumin (ALB) and globulin (GLO). The IL-6 and INF- $\gamma$  were measured using Sandwich ELISA kits produced by Sunlong Biotech Company. The serum protein was measured by spectrophotometric kits produced by Biosystem Company.

#### **Statistical analysis:**

The results of this study were statistically analyzed using (IBM SPSS Statistics program – version 21, 64 bits for windows 10). Significant differences between groups were assessed by one-way ANOVA, student t. test. And correlation matrix was done and the r values were obtained with the level of significance.

#### **Ethics approval:**

All patients were assured that all their obtained information will be handled in a confidential atmosphere and it will not affect their life after taking verbal and written consent. All the human studies were carried out according to the guidelines of the Animal and Human Ethical Committee of University of Bahri.

## RESULTS

The mean age of leprosy patients was  $46.3 \pm 16.6$  years, while it was  $42.5 \pm 17.1$  for healthy control group. The leprosy attack duration was  $8.2 \pm 1.2$  years with a range of 1-55 years.

The serum levels of IL-6, INF- $\gamma$ , TP and GLO were revealed a significant difference  $p < 0.001$  between patients and healthy control. The levels were found to be ( $250.2 \pm 11.8$  ng/L), ( $211.8 \pm 105.0$  pg/ml), ( $7.4 \pm 0.6$  g/dl) and ( $3.2 \pm 0.3$  g/dl) respectively. But the ALB level ( $4.1 \pm 0.6$  g/dl) was insignificantly different  $p < 0.057$  between the patient and control groups. All the mentioned parameters were found to be higher in patients group, except ALB is the only higher in control group (table 1).

IL-6 showed a very highly statistically significant correlation with INF- $\gamma$ , the r. value 0.435;  $p < 0.0001$  in leprosy patients. Also, it showed a very highly significant negative correlation with TP in leprosy patients r. value -0.0296;  $p < 0.002$ . While it showed a non-significant correlation with Age in the patient's group, the r. value 0.038;  $p < 0.693$ . Similarly, it showed a non-significant negative correlation with disease duration r. value -0.027;  $p < 0.783$ . On the other hand, INF- $\gamma$  was non-significantly negatively correlated with TP in patients group r. value -0.016;  $p < 0.872$ . Whereas it was

statistically very highly significantly correlated with Age r. value 0.283;  $p < 0.003$ . And highly significant with disease duration in patients group r. value 0.210;  $p < 0.029$  (table 2).

The levels of IL-6, INF- $\gamma$  and GLO were showed significant differences  $p < 0.0001$  among reaction types and control group. For TP the difference was also statistically different  $p < 0.022$ . But for ALB it was insignificant  $p < 0.057$ .

IL-6 ( $28.3 \pm 15.9$  ng/L) and INF- $\gamma$  ( $228.1 \pm 114.8$  pg/ml) were revealed the highest concentration in no reaction group. While the GLO ( $3.4 \pm 0.4$  g/dl) was showed the highest concentration in the type 1 reaction group. But in TP ( $7.5 \pm 0.6$  g/dl) and ALB ( $4.4 \pm 0.7$  g/dl), the highest concentration was determined in the type 2 reaction group (Table 3).

The IL-6, INF- $\gamma$ , TP and GLO showed significant statistical difference in the types of leprosy and control group with  $p < 0.0001$ , except for TP  $p < 0.022$ . While ALB showed no significant difference  $p < 0.057$ .

The highest concentration of IL-6 ( $38.9 \pm 21.2$  ng/L), INF- $\gamma$  ( $279.7 \pm 148.0$  pg/ml), TP ( $7.5 \pm 0.9$  g/dl) and ALB ( $4.4 \pm 0.6$ g/dl) showed in PB type. The GLO highest concentration ( $3.2 \pm 0.3$  g/dl) was obtained in MB type (Table 4).

**Table 1: Comparing mean levels of IL-6, INF-  $\gamma$ , TP, ALB and GLO in leprosy patients and healthy control subjects.**

Blood parameter	Mean $\pm$ SD		P. Value
	Patients	Control	
IL-6	$250.2 \pm 11.8$	$5.5 \pm 3.3$	0.0001
INF- $\gamma$	$211.8 \pm 105.0$	$150.0 \pm 31.4$	0.0001
TP	$7.4 \pm 0.6$	$7.1 \pm 0.6$	0.006
ALB	$4.1 \pm 0.6$	$4.3 \pm 0.6$	0.057
GLO	$3.2 \pm 0.3$	$2.7 \pm 0.2$	0.0001

**Table 2: Correlation of IL-6 with INF- $\gamma$ , TP, age and disease duration.**

Parameters	IL-6		INF- $\gamma$	
	r. value	p. value	r. value	p. value
TP	-0.0296**	0.002	-0.016	0.872
AGE	0.038	0.693	0.283**	0.003
Duration	-0.027	0.783	0.210*	0.029
INF- $\gamma$	0.435**	0.0001	-	-

**Table 3: Comparing mean levels of IL-6, INF- $\gamma$ , TP, ALB and GLO with type of leprosy reaction.**

Parameters	Type1 reaction Mean $\pm$ SD	Type2 reaction Mean $\pm$ SD	No reaction Mean $\pm$ SD	Control Mean $\pm$ SD	P. Value
IL-6	22.6 $\pm$ 8.4	25.1 $\pm$ 10.3	28.3 $\pm$ 15.9	5.5 $\pm$ 3.4	0.0001
INF	208.6 $\pm$ 118.9	202.6 $\pm$ 85.6	228.1 $\pm$ 114.8	150.1 $\pm$ 31.4	0.0001
TP	7.4 $\pm$ 0.7	7.5 $\pm$ 0.6	7.3 $\pm$ 0.7	7.1 $\pm$ 0.6	0.022
ALB	4 $\pm$ 0.9	4.3 $\pm$ 0.6	4.2 $\pm$ 0.5	4.4 $\pm$ 0.7	0.057
GLO	3.4 $\pm$ 0.4	3.3 $\pm$ 0.4	3.2 $\pm$ 0.4	2.8 $\pm$ 0.2	0.0001

**Table 4: Comparing mean levels of IL-6, INF- $\gamma$ , TP, ALB and GLO with type of leprosy.**

PARAMETERS	CONTROL MEAN $\pm$ SD	PB TYPE MEAN $\pm$ SD	MB TYPE MEAN $\pm$ SD	P. Value
IL-6	5.5 $\pm$ 3.3	38.9 $\pm$ 21.2	23.5 $\pm$ 8.9	0.0001
INF- $\gamma$	150.0 $\pm$ 31.4	279.7 $\pm$ 148.0	203.5 $\pm$ 96.2	0.0001
TP	7.1 $\pm$ 0.6	7.5 $\pm$ 0.9	7.4 $\pm$ 0.6	0.018
ALB	4.3 $\pm$ 0.6	4.4 $\pm$ 0.6	4.1 $\pm$ 0.6	0.065
GLO	2.7 $\pm$ 0.2	3.1 $\pm$ 0.5	3.2 $\pm$ 0.3	0.0001

## DISCUSSION

This study was conducted in Sudan to study the correlation of the cytokines IL-6 and INF- $\gamma$  levels in leprosy patients with types of immune reactions and the types of leprosy. Furthermore, to study the plasma protein constituents as a part of the immune response in shifting of the immune response from cellular to the humeral stage.

Our results showed that the concentrations of IL-6 in leprosy patients increased more than forty-five folds than that in the control group, these results were in agreement with Belgaumkar, Stefani, Gabay and Ochoa [17-20], in which they found a significant difference between the levels of IL-6 in patients and control subjects. The IL-6 is the most important inflammatory mediator and stimulates the production of acute-phase proteins [20]. This indicates immune

stimulation by *M. leprae* antigens and they are responsible for neutrophil recruitment in the initial phase and trafficking to the site of infection [17]. So its higher increase in patients proposed a high immune response.

The INF- $\gamma$  concentration in leprosy patients was significantly higher than in the control group. This result agrees with Nada, Saini, and Madan [21-23]. It was moderately increased by more than one and a half folds. The immunopathogenesis of leprosy is primarily due to interaction among subsets of T cells, antigen-presenting cells (APCs), and *M. leprae* antigens as a result of enhanced cell-mediated immunity with a predominant T-helper (Th1) immune response characterized by increased release of interferon [24].

Interleukin-6 is a cytokine which is secreted by immune and nonimmune cells

and shows functional pleiotropy and redundancy. IL-6 plays an important role in the differentiation of several cell types. IL-6 has a negative regulation of CD4 Th1 cell differentiation. While IL-6-directed Th2 differentiation is mediated by IL-4, inhibition of Th1 differentiation by IL-6 is independent of IL-4. IL-6 upregulates suppressor of cytokine signaling 1 (SOCS1) expression in activated CD4 T cells, thereby interfering with signal transducer and activator of transcription 1 (STAT1) phosphorylation induced by IFN- $\gamma$ . Inhibition of IFN- $\gamma$  receptor-mediated signals by IL-6 prevents autoregulation of IFN- $\gamma$  gene expression by IFN- $\gamma$  during CD4 T cell activation, thereby preventing Th1 differentiation. Thus, IL-6 promotes CD4 Th2 differentiation and inhibits Th1 differentiation [25,26]. The moderate increase in INF- $\gamma$  can be explained for this reason.

Serum total protein and globulin showed higher concentration levels in patients with a highly significant statistical difference when compared to the control group. In immune response of cell-mediated immunity, the macrophage and other immune T cells secrete acute-phase proteins. Moreover, IL-6 is known to enhance B cell responses and augment antibody formation diverting to humoral immunity, thereby potentiating immune complex formation. The secretion of antibodies (gamma globulins) increases the globulin fraction, consequently the concentration of the total serum protein [27]. On the other hand, serum albumin was non-significantly higher in the control group than in patients. Albumin is related to nutritional status rather than other effectors, besides its irrelevance to infection directly. It is exclusively produced by the liver and not by T cells that is why not increased due to inflammation or infection and may be decreased if the liver is negatively affected [28].

In lepra, the result of IL-6 showed a significant difference in comparison of patients groups with the control group. This is in concordance with results of Ochoa, Saini, Moraes, Stefani and Belgaumkar [17,18,20,22,29]. But when compared with the patient's subtypes, the mean differences were not statistically significant except between typ1 and no reaction groups which is agreed only with Moraes [29]. Our study displayed that Il-6 in type 2 reaction group was higher than that in type1 reaction group. This agrees with many previous studies like Ochoa and Saini [20,22] and disagrees with Moraes and Stefani [18,29]. In our result the highest concentration was found in no reaction followed by type 2 reaction then type1 reaction group. This is agreed by Belgaumkar [17] and disagreed by Stefani [15] while the other studies compared just the reactive types.

The concentration of INF- $\gamma$  in our study was higher in patients groups than in the control group. The highest level was for (no reaction) group followed by the type 1 reaction and type 2 reaction. This agreed with Belgaumkar [17] results. In our study, there is no statistical difference between the subtypes except between type 2 and no reaction group and the vice versa was in Belgaumkar [17] study. The level in type 1 is higher than in type 2 with a significant difference in Saini result, and no statistical difference between type 2 and no reaction group. This is contrasting our results. In Moraes' study type2 showed a higher level than type1 [29].

Type 1 reactions or reversal reactions that occur in patients with borderline forms of the disease are caused by spontaneous increases in T cell reactivity to mycobacterial antigens. They are associated with the infiltration of INF- $\gamma$  CD4 lymphocytes. The elevated levels of INF- $\gamma$  in type1 reaction indicate an exaggerated cell-mediated immune response which culminates

in the clearing of bacilli and concomitant tissue damage [17].

Type 2 reaction is a systemic inflammatory response to the deposition of immune complexes which occurs in BL and LL leprosy. Type 2 reactional patients in our study had higher levels of IL6 compared to type 1 patients. IL6 is known to enhance B cell responses and augment antibody formation, thereby potentiating immune complex formation [17].

According to the types of leprosy, the serum IL-6 concentration showed a highly significant difference between leprosy types and the control group. It was higher in the patients of PB leprosy type than in MB type. Our result agrees with Nada and Saini [21,22]. Also INF- $\gamma$  was higher in PB than in MB which is concordant with the results of De Almeida-Neto, Nada, Saini, and Madan [21,23,30].

The pauci bacillary (PB) types of infection are portrayed by the power of a Th1-type immune response, the nearness of all around shaped granulomas at the site of the injury because of fiery CMI, and control of mycobacterial replication. In contrast, in the multi bacillary (MB) forms no granuloma is seen for inefficient CMI, and high bacterial load and antibody levels are detected. Cytokines play a critical role in triggering host-pathogen interactions [31].

### CONCLUSION

Leprosy is distributed all over the country and can implicate all jobs, vocations and ethnic groups. IL-6 in leprosy patients increased more than forty five folds than that in the control group. The Level of INF- $\gamma$  was higher in patients groups than in control group. Serum IL-6 and INF- $\gamma$  concentrations were higher in the patients of PB leprosy type than in MB type. Levels of serum total protein and globulin were significantly higher in leprosy patients when compared to control group.

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