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ORIGINAL ARTICLE

Interleukin-10 and Interferon Gamma as Prognostic Markers in Pulmonary Tuberculosis

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ABSTRACT

Background: *Mycobacterium tuberculosis (Mtb) infection is considered one of the most common causes of human mortality in the world. Many studies had done to explore the role of host defense in controlling the disease but many points are still unclear and need further researches. Many of pro-inflammatory and anti-inflammatory cytokines play a role in the generation and development of tuberculosis (TB) illness. Interferon gamma (IFN-γ) and interleukin-10 (IL-10) have contradictory and conflicting roles in correlation to Mtb infection severity and response to therapy.* ***Objectives:*** *The aim of our study was to estimate the relationship between IFN-γ and IL-10 levels and the different stages of TB and the possible effects of TB therapy on their levels.* ***Methodology:*** *Our study included 40 patients with active pulmonary TB and a control group of 40 healthy individuals. The levels of IFN-γ and IL-10 were measured in the patients at the start of therapy and after 3 and 6 months of TB therapy using enzyme-linked immune-sorbent assay (ELISA).* ***Results:*** *IFN-γ assessment by Quantiferon Gold in Tube test (QFT-GIT), for the pulmonary TB patients was higher than 0.35 IU/ml, so all patients were considered positive at the beginning of therapy. IL-10 was higher in TB patients than the control persons and the difference between the TB patients and the control persons was significant (p value <0.05). After 6 months of treatment, 6 cases not improved with treatment. IFN-γ was still high in those patients and didn't decline after 3 and 6 months of TB therapy as happened in the remaining 34 patients. The mean serum level of IL-10 declined in improved patients after therapy. However, in those 6 resistance patients, IL-10 still high after 6 months of treatment (p value < 0.05). Finally, IFN-γ didn't show difference in correlation with the disease severity, while IL-10 showed higher values with the advanced stage of Mtb (stage 3).* ***Conclusion:*** *IL-10 and IFN-γ could be used as prognostic markers for the success of TB therapy, but only IL-10 shows a correlation with the severity of TB infection.*

INTRODUCTION

Pulmonary tuberculosis (TB) is a major health disease transmitted by aerosol [1]. The World Health Organization (WHO) acts hardly to decrease TB cases. However, the total number of multidrug-resistant TB patients is increasing every year [2].

Tuberculosis (TB) infection is controlled by the immune system mainly the cellular immunity, in which definite cytokines with the aid of T helper 1 (Th1) cells have important roles [3,4,5].

Interferon gamma (IFN-γ) and interleukin 10 (IL-10) are supposed to have controversy roles in the host defense against mycobacterial infection. IFN-γ is considered to be the key cytokine responsible for resistance to TB infection, acts as a powerful macrophage stimulator, increasing the expression of class II histocompatibility complex and the release of nitric oxide [6].

Another important cytokine in the mechanism of TB is interleukin-10 (IL-10). IL-10 decreases the secretion of Th1 cytokines and the expression of MHC class II and costimulatory molecules on macrophages, so, blocking the stimulation of T lymphocytes by inhibiting the expression of MHC class II antigens [7].

IL-10 plays a major role in the negative regulation of IL-12 secretion and co-stimulatory molecule expression, so reducing the secretion of IFN-γ by T-cells. Deficient IL-10 secretion increases the host resistance against TB infection [8]. Also, IL-10 inhibits IL-17 in TB patients[9].

The aim of this study is to enhance pulmonary TB prognosis by correlating the levels of IFN-γ and Il-10 in patients with pulmonary TB with the clinical conditions, laboratory and radiological observations in attempting to monitor the disease progress and severity.

METHODOLOGY

Population selection:

This study is a case control study. Forty patients (27 male and 13 female) with pulmonary TB admitted to Mansoura Chest Hospital and Chest department in the Mansoura University Hospital between June, 2016 and March, 2017 were involved in our study.

The mean age of patients was 36.2 years (SD ± 24.4). All patients were proven to be active pulmonary TB by clinical, radiological and laboratory culture on Lowenstein Jensen media.

Also, 40 healthy persons (29 male and 11 female) were included in our study as a control group. The mean age of the control persons was 29.6 years (SD ± 11.4).Those healthy persons were characterized by; negative TB culture on Lowenstein Jensen media, negative smear by Ziehl-Neelsen (ZN) stain and absent clinical and radiological findings of TB.

Data collection*:*

The following data were collected from both groups (TB patients group and control persons group): age, sex, history of smoking, diabetes mellitus and taking immunosuppressive medications within the last three months. Information regarding radiological findings (site of affection, diameter of lesion, and presence of cavity) were collected from patients at the start of TB therapy.

According to the criteria of Dlugovitzky et al.[10] based on the radiological findings, patients were categorized into one of three stages: ***stage I:*** mild cases, pulmonary TB patients with only one lobe of the lung is affected and without cavities; ***stage II:*** moderate cases, patients with of two or more lobes of the lung are affected, but only on one side and with cavities less than 4 cm; and ***stage III:*** severe cases, patients with both sides are affected and with disseminated cavities.

Clinical samples:

Three blood samples were taken from each pulmonary TB patient. The first sample was taken at the start of TB therapy and then after 3 and 6 months of TB therapy. While, only one blood sample was taken from each control person.

All blood samples in our study were assessed for the levels of IFN-γ and IL-10.

The levels of IFN-γ were measured after stimulation with *Mycobacterium tuberculosis* (Mtb) specific antigens, which are early secretory antigenic target 6 (ESAT-6), and culture filtrate protein 10 (CFP-10) using Quantiferon Gold in Tube test (QFT-GIT) assay (***Cellestis, Carnegie, Australia***). Also, the levels of IL-10 were measured by ELISA kit ***(eBioscience, USA).***

Quantiferon Gold in Tube test assay

QFT-GIT test was done according to the manufacturer's guidelines. The QFT-GIT result was considered negative if the value of IFN-γ in the positive control (after reduction of the value in the nil tube) was ≥ 0.5 IU/ml and the value of IFN-γ in the antigen tube was < 0.35 IU/ml (after reduction of the value in the nil tube). It was considered positive if the level of IFN-γ in the antigen tube was ≥ 0.35 IU/ml (after reduction of the value in the nil tube), whatever the value of the positive-control tube. Finally, It was considered indefinite if IFN-γ level in the antigen tube was < 0.35 IU/ml and in the positive-control tube < 0.5 IU/ ml.

Statistical analysis:

Mean, standard deviation and p value were used to describe data in this study. P value was considered significant if less than 0.05 and highly significant, if less than 0.001. Statistical analysis was done using the Statistical Package for Social scientists (SPSS) for windows 15 *(SPSS Inc., Chicago, IL, USA).*

Ethical issue:

This study was approved by Institutional Research board (IRP) and a written informed consent was obtained from each participant.

RESULTS

Patients in our study were classified according to the radiological findings into three stages using the criteria of *Dlugovitzky:* stage I, mild cases (n = 9), stage II, moderate cases (n = 24), and stage III, sever cases (n = 7).

Table 1: Background characteristics of both pulmonary TB patients and control persons

Group	*TB patients (40)*	*Control persons (40)*
Age (years)	Mean ± SD: 36.2 ± 24.4	Mean ± SD: 29.6 ±11.4
Male/Female	27/13	29/11
Smoking	9 (22.5%)	12 (30%)
Diabetes mellitus	4 (10%)	3 (7.5%)
History of immunosuppressive drugs within three months	0 (0%)	1 (2.5%)
BCG vaccine scar	14 (35%)	19 (47.5%)

This table shows no significant differences between pulmonary TB patients and control persons as regard smoking, diabetes mellitus, history of immunosuppressive drugs and BCG vaccine.

Table 2: IFN-γ and IL-10 in both pulmonary TB patients and control persons

		IFN-γ (IU/ml)	*IL-10 (pg/ml)*
TB patients	At the beginning of treatment	1.4	2.8
	After 3 months of treatment	1.3	2.4
	After 6 months of treatment	0.7	2.0
Control persons		0.17	0.7

This table shows higher IL-10 in TB patients at the beginning of TB therapy than the control persons with a significant p value <0.05. Also, the level of IFN-γ between patients at the beginning of TB therapy and control person is significant (p value < 0.05).

Table 3: IFN-γ and IL-10 in correlation to the stages of TB patients at the start of treatment

	IFN-γ	*IL-10*
Stage 1	1.5	1.6
Stage 2	1.1	2.4
Stage 3	1.3	5.1

This table shows that IFN-γ didn't differ in correlation with the disease severity, while IL-10 show higher values with the advanced stage of TB (stage 3) than the other stages.

Table 4: QFT-GIT and IL-10 results after 3 and 6 months of treatment in TB patients as regard to improvement

	Cytokines Level	*Improved group (N:34)*	*Non improved group (N:6)*
At the start of treatment	**IFN-γ**	1.3	1.8
	IL-10	2.8	2.5
After 3 months of treatment	**IFN-γ**	1.2	1.9
	IL-10	2.1	3.8
After 6 months of treatment	**IFN-γ**	0.4	1.9
	IL-10	1.5	5.1

This table shows 6 cases not respond to treatment after 6 months of treatment. IFN-γ was still high in those patients and didn't decline after 3 or 6 months of therapy as happened in the remaining 34 patients. The mean serum level of IL-10 declined in improved patients after therapy (p value: 0.02). However, in non-improved patients, IL-10 increased after 6 months of treatment (p value < 0.05).

DISCUSSION

Tuberculosis is a growing international health issue. Even with the use of a live attenuated vaccine and new antibiotics, TB is increased worldwide as a major health problem in the community [11].

A number of studies have demonstrated the role of IFN-γ in the control of TB. Experiments in mice deprived from the IFN-γ genes have complained fulminant infection by Mtb [12].

In this study, 80 subjects (40 patients with active pulmonary TB and 40 healthy control subjects) were included. The 40 pulmonary TB patients were diagnosed as pulmonary TB by clinical, radiological and laboratory culture while, the 40 healthy persons included in our study, were negative for acid fast smear by ZN stain, negative for TB culture and with no clinical or radiological findings of TB.

All TB patients in our study were positive by QFT-GIT test. Out of the 40 pulmonary TB patients, 34 (85%) patients were improved clinically and laboratory after 6 months of TB therapy. The mean QFT-GIT at the start of TB therapy was 1.3 IU/ml and after 3 months of therapy declined to 1.2 IU/ml and after 6 months of therapy decreased to 0.4 while, in patients without improvement was 1.8 IU/ml and after 3 months of TB therapy was 1.9 IU/ml and after 6 months of treatment was 1.9 IU/ml. The decline in QFT-GIT values with treatment showed statistically significant p value (<0.05) as regard improvement after 6 months of TB therapy and this means that the decrease of IFN-γ release assessed by QFT-GIT test could be used to as a tool to follow up the response to treatment.

Previous research on the effect of therapy on QFT-GIT results has generated conflicting results. Some research reported that the reactions to TB-specific antigens decline adequately with TB therapy. So, QFT-GIT can be used as a tool to monitor the efficacy of TB therapy, while other research reported that the responses might not decline adequately. So, QFT-GIT cannot be used as a tool to monitor the treatment.

Our findings agree with Katiyar et al. [13] who reported 22.4% of patients showed persistent high IFN-γ response after the initial phase of therapy and that the persistence high IFN-γ concentration is positively associated with the likelihood of remaining culture-positive after 2 months of treatment (p value: 0.007). They reported that the higher IFN-γ after 2 months of TB therapy is positively correlated with the positive sputum culture after the initial phase of treatment.

Also, in Sauzullo et al.[14] study, out of 11 TB patients, 5 patients had persistent high IFN-γ, that did not improved after 6 months of TB therapy, whereas the other 6 patients showed clinical and bacteriological improvement.

On the other side**,** Dyrhol-Riise et al [15] research, reported that IFN-γ release should not be used as a tool to follow up the success of treatment.

The explanation for the persistence positive QFT-GIT after 3 months of TB therapy in patients with improvement is not clear. There are many explanations for that; as the steady present of residents of sensitized T-cells after completing treatment and disappearance of Mtb for years [16] and the role of genetic polymorphisms on the variation of IFN-γ response [17]**.**

In our study, the mean serum level of IL-10 declined in improved patients after therapy. However, in non-improved patients, IL-10 increased after 6 months of TB therapy (p value: < 0.05). IL-10 level remained significantly higher in TB patients at the end of TB therapy compared with control persons. These observations could be used to monitor the success of TB therapy.

This agrees with Almeida study, which showed a relation between higher IL-10 at the end of treatment and the persistence of TB [18]**.**This is explained by that the increase in IL-10 secretion, inhibit the immune system and enhance TB progression [19,20]. This agrees also with Henao et al.,[21] study which reported that mice with defective IL-10 secretion show potent immunity against TB.

Also, Boussiotis et al., [22] reported higher IL-10 release in anergic TB patients, suggesting that the higher IL-10 secretion play a role in inhibiting the immune system and flaring of the disease.

Ndishimye et al., [23] showed similar results to our study. The study showed higher IL-10 levels in TB patients than the control persons with a significant p value (p <0.05). In addition, it showed that the patients group with normal chest X-Ray findings had lower IL-10 concentrations than patients group with abnormal X-Ray findings (p=0.03). Also, showed that the level of IL-10 in patients at the start of TB therapy was higher than after 3 months of TB therapy and after 6 months of TB therapy (p=0.01) and the level of IL-10 in patients at the end of TB therapy remained significantly higher than the control persons.

This agree with Almeida et al., [18] research which shows that IL-10 and other suppressor cytokines of the immune response are found in samples of TB patients, while one month after treatment their level decreases significantly in improved patients.

Also, Feruglio et el. [24] reported that early inhibition of IL-10/TGF-β pathway facilitate Mtb clearance and response to treatment.

This study shows that IFN-γ didn't differ in correlation with the disease severity, while IL-10 show higher values with the advanced stage of TB. Jamil et al.,[25] study reported that IFN-γ /IL10 ratio can be used as a marker to determine disease severity in both pulmonary and extra-pulmonary tuberculosis. This can be explained by the higher IFN-γ in TB patients have no role, while IL-10 value used in the ratio is the responsible marker to determine disease severity.

CONCLUSION

IL-10 and IFN-γ could be used as prognostic markers for the success of TB treatment. Also, IL-10 shows a correlation with the severity of TB infection.

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