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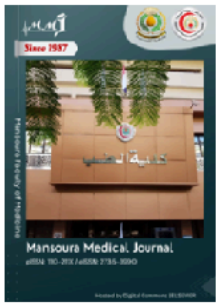

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STUDY OF INTERFERON GAMMA AND INTERLUKIN-12 IN CHILDHOOD TUBERCULOSIS

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STUDY OF INTERFERON GAMMA AND INTERLUKIN-12 IN CHILDHOOD TUBERCULOSIS

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ABSTRACT

This work was planned to evaluate the role of interferon gamma (IFNγ) and IL-12 in tuberculous children and their relation to hypersensitivity and disease susceptibility. For this purpose we studied 19 children with newly diagnosed tuberculosis; 12 with pulmonary TB and 7 with tuberculous lymphadenopathy; in addition to 12 asymptomatic contacts with positive tuberculin test and 13 healthy tuberculin negative children with matched age and sex. Both serum and in vitro production of IFNγ and IL-12 by PBMCs were estimated in all subjects by ELISA technique. Our results revealed that tuberculous patients had significantly higher serum IFNγ and IL-12 than both tuberculin positive and negative control (P<0.001). Similarly, tuberculin positive contacts had significantly higher serum levels of both cytokines compared with healthy negative group. However, in vitro production of IFNγ and IL-12 were significantly higher in patients and tuberculin positive contacts than healthy tuberculin negative subjects (P<0.001) and did not differ signifcantly between patients and tuberculin positive contacts (P = 0.056, 0.86). Significant positive correlation was found between tuberculin diameter and the studied cytokines. Conclusion: serum levels of IFNγ and IL-12 are elevated in tuberculous children than control with correlation to tuberculin reaction denoting a protective role for these cytokines. The relatively depressed in vitro production of IL-12-IFNγ axis in tuberculous patients may predispose to disease progression. Recommendations: adjuvant cytokine therapy using IFNγ and IL-12 especially in advanced and resistant tuber-

culosis is recommended in future studies.

INTRODUCTION

Mycobacterium tuberculosis (MTB) is the etiologic agent of human tuberculosis and is estimated to infect one third of the world's population[1].

Most people who become infected with MTB mount an effective protective immune response but 5-10% develop disease [2] . It has not been fully elucidated which of the components of the immune response against MTB is indicative of resistance or susceptibility [3] .

Many cytokines have been implicated in the protective immunity, pathophysiology and development of tuberculosis[2]. Recent studies have indicated that IL-12/interferon gamma axis is important in mycobacterial infection susceptibility[4]. However, the distinction between changes in cytokine profile attributable to M tuberculosis infection and those associated with active disease is unclear [5,3].

The aim of this work is to study the pattern of serum and in vitro production of interferon gamma (IFNγ) and IL-12 by peripheral blood mononuclear cells (PBMCs) in different forms of tuberculosis infection in children in order to evaluate the role of these cytokines in tuberculosis and to clarify their relation to disease susceptibility and state of hypersensitivity.

SUBJECTS AND METHODS

Subjects :

This work was carried out in the Unit of Infectious Diseases and Malnutrition, Mansoura University Children's Hospital lasting from January, 2002 to March, 2003. The study included 12 patients with newly diagnosed pulmonary tuberculosis (2 males and 10 females) witth mean age of 9.66 years ± 4.84 SD and 7 patients with tuberculous lymphadenopathy (5 males and 2 females) with mean age of 10.2 years ± 4.32 SD. In addition, 12 asymptomatic contact children with positive tuberculin test and 13 healthy tuberculin-negative children with matched age and sex were enrolled in the study as control (table 1,2).

Tuberculin test was done by Mantoux method using 5 PPD units and the diameter of induration was measured 72 hours later. The test was considered positive when the diameter of induration was ≥ 10 mm [6].

Diagnosis of TB patients was confirmed by positive smear, Bactec culture (system 460) or biopsy (table 3).

We excluded from the study patients with chronic debilitating illnesses, malignancy, diabetes or those receiving immunosuppressive therapy.

Methods :

Serum samples were withdrawn from all patients before treatment and control groups and preserved at $-20^{o}C$ till cytokine assay. Simultaneously, 15 ml of whole heparinized blood were also withdrawn from all subjects for in vitro culture. Peripheral blood mononuclear cells (PBMCs) were obtained by sedimentation over Ficoll (Biotest AG. Landsteinestrasse 5.D-63303 Dreieich. Germany). PBMCs (2×10^{6}/ml) were suspended in PRMI 1460 (Hyclone 1725 south hyclone road Logan. Utah 84321) containing 10 % heat inactivated fetal calf serum and were cultured with heat killed atypical mycobateria with ratio of 10:1 MTB to target cells for 48 hours. Supernatent fluids were then collected and preserved at $-20^{o}C$ till assay for in vitro cytokine production [7].

Interferon gamma assay was done by sandwich enzyme-linked immunosorbant assay (ELISA) according to instructions of manufacturers (Immuntech). IL-12 assay was also done by the same technique (IL-12+ P40 EASIA, BIOSOURCE, Europe S.A.).

Statistical analysis :

Statistical analysis was done using SPSS (statistical package for social science program version10,1999). Data were parametric. Student T test was used for comparison between groups. Spearman correlation coefficient test was used to study the relation between variables. Significance is considered if $P < 0.05$.

RESULTS

The results of this study revealed that tuberculous patients and tuberculin positive contacts had significantly higher serum and in vitro IFNγ production compared with healthy tuberculin negative group ($P< 0.001$). Serum IFNγ was significantly higher in patients than tuberculin positive contacts ($P=0.007$) without significant difference between them regarding in vitro IFNγ (table 4). There were no significant differences between pulmonary or extra-pulmonary patients as re-

gards IFNγ levels (table 6).

Similarly, serum and in vitro IL-12 was significantly higher in both tuberculous patients and tuberculin positive contacts compared with healthy tuberculin negative subjects and only serum IL-12 was significantly higher in patients compared with tuberculin positive contacts (table 5). Also there were no significant differences between pulmonary or extrapulmonary regarding IL-12 levels (table 6).

There were significant positive correlation between both IFNg gamma, IL-12 and tuberculin diameter. Also, both cytokines correlate positively with each other (table 7).

Table (1) Age of studied group (ANOVA test)

Group	*Mean ± sn*	*F*	*Significance*
Group1 (pulm.) (N=12)	9.66 ± 4.84	1.82	P=0.158
Group2 (extrapulm.) (N=7)	10.2 ± 4.32		
Contact tuberculin Reactor (N=12)	10.44 ± 5.08		
Healthy non reactor (N=13)	10.61 ± 4.64		

Table (2) sex distribution of studied groups (chi – square test)

Sex	*Pulmonary N=12*	*Lymphadenopathy N=7*	*Tuberculin reactors N=12*	*Healthy non reactors N=13*	*P*
Male No (percent)	2 (16.7%)	5 (71.4 %)	4 (33.3%)	5 (38.5%)	0.50
Female No(Percent)	10 (83.3%)	2 (28.6%)	8 (66.7%)	8 (61.5%)	

Table (3) Results of Diagnostic tests in tuberculous patients

Group	Skin test		Positive smear	Bactect	Biopsy
	Positive	Negative			
Pulmonary T.B (N=12)	8	4	9	3	-
Extrapulmonary (lymphadenpathy)(N=7)	6	1	1	-	6

Table (4) Inter comparison of tuberculous patients, asymptomatic tuberculin reactors and healthy non-reactors regarding serum and in vitro I FN production (T-test)

Parameter	T.B patients N=19	Tuberculin reactor N=12	Healthy tuberculin non-reactors.	Significance
Serum IFN (pg/ml) Mean ± SD	3.94 ± 1.13	2.90 ±1.00	1.43 ±0.67	P1 =0.007* P2< 0.001 * P3 <0.001*
In vitro IFN (pg/ml) Mean ± SD	4.85 ± 2.07	3.64 ± 1.75	1.09 ±0.43	P1=0.056 P2<0.001* P3<0.001*

**P is significant if < 0.05* *P1: patients versus tuberculin reactors.*
P2: patients versus healthy non-reactors. *P3: contact reactors versus healthy non-reactors.*

Table (5) Inter-comparison of tuberculous patients, contact tuberculin reactors and healthy non-reactors regarding serum and in intro IL-12 production (T- test)

Parameter	T.B patients N=19	Tuberculin reactor N=12	Tuberculin non reactor N=13	Significance
S IL-12 (pg/ml) Mean ± SD	375.26±96.74	200.66 ±64.63	62.46 ±36.18	P1 =0.001* P2< 0.001 * P3 <0.001*
Culture IL-12 (pg/ml) Mean ± SD	88.15 ±46.76	85.83 ± 34.03	33.92 ±24.86	P1=0.86 P2<0.001* P3<0.001*

**P is significant if < 0.05* *P1: patients versus tuberculin reactors.*
P2: patients versus healthy non-reactors. *P3: contact reactors versus healthy non-reactors.*

Table (6) comparison of pulmonary versus extra-pulmonary tuberculous patients regarding serum and in intro IFN and IL-12 (T-test)

Parameter	*Pulmonary T.B N=12*	*TB Lymphadenopathy N=7*	*Significance*
Serum IFN (pg/ml) mean ± SD	3.63 ± 1.02	4.48 ± 1.18	0.06
Culture IFN (pg/ml) mean ± SD	4.94 ± 2.26	4.61 ±1.84	0.64
Serum IL –12 (pg/ml) mean ± SD	367.5 ±87.19	388.57 ±117.53	0.55
Culture IL-12 (pg/ml) mean ± SD	91.25 ± 43.22	82.85 ±55.51	0.64

Table (7) Pearson inter-correlation of serum IFN γ- , sIL-12, culture IFN γ, IL-12, age and tuberculin diameter.

Paramount	*Age*	*Tuberculin diameter*	*ESR*	*sIL – 12*	*Culture IL-12*
IFN	r 0.227 P= 0.138	r 0.37 P=0.013*	r-0.205 P=0.4	r 0.718 P<0.001*	r 0.259 P=0.009
Culture IFN	r – 0.114 P=0.441	r 0.395 P=0.008*	r –0.372 P= 0.117	r 0.683 P <0.001*	r 0.586 P<0.001*
Serum IL –12	r 0.161 P=0.296	r 0.477 P =0.001*	r –0.455 P=0.05		
Culture IL-12	r – 0.113 P = 0.467	r 0.412 P = 0.005*	r –0.173 P=0.479		

** p is Significance if p < 0.05*

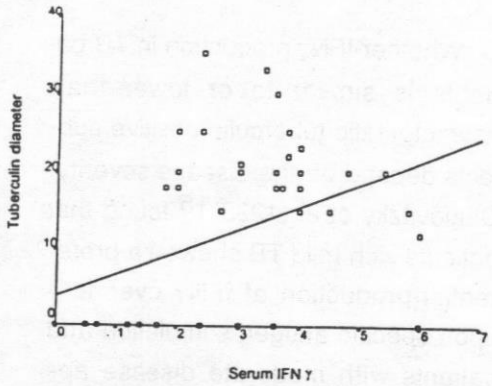

Fig. (1) : Correlation between tuberculin diameter & serum IFNγ.

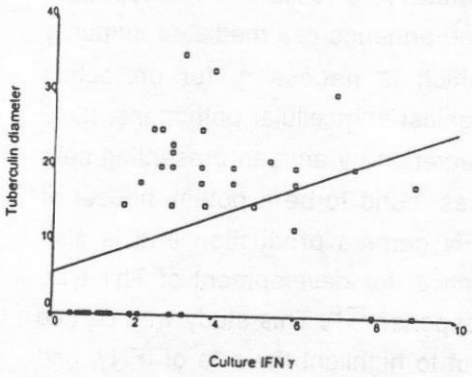

Fig. (2) : Correlation between tuberculin diameter & culuture IFNγ.

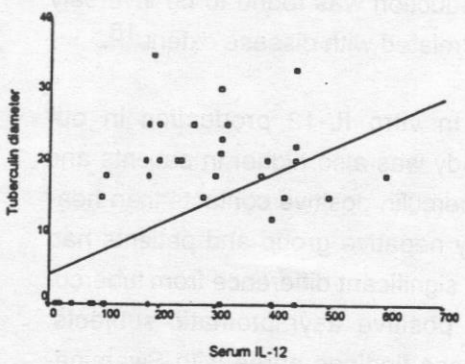

Fig. (3) : Correlation between tuberculin diameter & serum IL-12

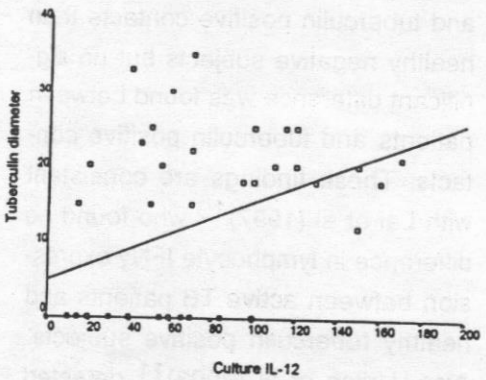

Fig. (4) : Correlation between tuberculin diameter & culture IL-12

DISCUSSION

Gamma interferon is a cytokine produced mainly by T lymphocytes, natural killer cells and macrophages and enhance cell mediated immunity which is necessary for protection against intracellular pathogens. IL-12 secretion by antigen presenting cells was found to be a potent inducer of IFN gamma production and is also critical for development of Th1 type response [8,9]. This study was carried out to highlight the role of IFNγ and IL-12 in different clinical forms of tuberculosis and to determine their relation to hypersensitivity and disease susceptibility.

In Vitro study in this work revealed a significantly higher IFNγ in patients and tuberculin positive contacts than healthy negative subjects but no significant difference was found between patients and tuberculin positive contacts. These findings are consistent with Lai et al (1997)[10] who found no difference in lymphocyte IFNγ expression between active TB patients and healthy tuberculin positive subjects. Also, Urlich et al (2003)[11] detected higher IFNγ production by PBMCs in asymptomatic infected subjects with TB than healthy non infected but this cytokine was minimally detected in blood samples from patients with active TB. Similar findings were also obtained in other studies[2, 12, 13, 14].

Whether IFNγ production in TB patients is similar to or lower than asymptomatic tuberculin positive subjects depend on the disease severity. Dlugovitzky et al (1999)[15] found that patients with mild TB showed a preferential production of IFNγ over IL-4 upon specific antigen stimulation and patients with moderate disease appeared compatible with a mixed production of both cytokines coexisting with a higher synthesis of TGF-β than mild patients. Patients with advanced disease showed the least IFNγ production with higher IL-4 and TGF-β. Similarly, PHA stimulated IFN gamma production was found to be inversely correlated with disease extent [16].

In vitro IL-12 production in our study was also higher in patients and tuberculin positive contacts than healthy negative group and patients had no significant difference from tuberculin positive asymptomatic subjects. These findings agree with Swaminathan et al (1999)[17] who found that the production of IL-12 from PBMCs was similar in patients and healthy tuberculin reactors. Also, multi-drug resist-

ant TB patients were similar to healthy tuberculin reactors in their IL-12 P70 producing capacity [18].

The depression of in vitro cytokine production in TB was found to be a transient phenomenon that reverse after 2 weeks of therapy [11,3]. The underlying mechanism for this depression of in vitro IFN gamma and IL-12 by PBMCs in TB patients is unclear. However, inhibitory cytokines production may be etiologic factors. Both IL-10 and TGF-β, which increase in active TB, inhibited proliferation and IFN production by CD4+ T cells [1]. Also, neutralization of IL-10 increased the in vitro production of IL-12 in TB patients about two folds [19]. Other mechanisms may also contribute to cytokine depression. Nuclear extracts of T cells from most TB patients showed markedly reduced expression of proteins that bind to proximal IFN gamma promotors compared with healthy tuberculin reactors [20]. In addition, increased apoptosis of PBMCs in tuberculous patients may be also acting [21].

Unlike the in vitro cytokine production, serum levels of both IFNγ and IL-12 in our study were significantly higher in patients than contact tuberculin reactors and healthy non reactor groups and contact tuberculin reactors had a higher level than tuberculin negative healthy subjects. These results are in agreement with previous studies which detected increased serum IFNγ and IL-12 in active TB patients than controls [22] and increased serum IFNγ in tuberculous pleurisy than other causes of effusion [23].

The increased serum levels of IFN gamma and IL-12 in TB patients despite the in vitro peripheral lymphocyte anergy could be explained by the increased production of these cytokines at the site disease [2]. This explanation is supported by finding of significant increase in the percentage of BAL cells expressing mRNA for IFN gamma and IL-12 in active versus inactive pulmonary TB subjects [5, 24].

In this study there was no significant difference between pulmonary or extra-pulmonary TB patients regarding serum or in vitro cytokine levels. Consistent with these findings Verbon et al(1999)[22] found that IFNγ or IL-12 levels did not correlate with TB localization whether pulmonary or extrapulmonary.

Our study revealed a significant

positive correlation between the studied cytokines and tuberculin diameter, a finding that go with other studies as Fjallbrant et al (2001)[25] who detected greater lymphocyte transformation response and higher IFNγ in tuberculin positive than negative subjects. Also, Wilsher et al (1999)[26] found that Mantoux size correlated with lymphocyte proliferation and IFNγ production. Thus, tuberculin anergy may reflect an inappropriate immune response to MTB with high percentage of IL-4, IL-10 positive lymphocytes and low IL-12 and IFNγ positive lymphocytes suggesting a Th2 biased immune response [27].

The correlation of IFNγ and IL-12 with tuberculin test size which is the hallmark of delayed hypersensitivity confirms the protective role of these cytokines in immune defense against MTB. This is supported by the finding that interferon receptor deficiency leads to a predisposition to mycobacterial infection and impairs the formation of mature granuloma [28]. Also, IL-12 receptor beta-1 chain deficiency was found in three unrelated individuals with severe idiopathic mycobacterial infection [29].

Conclusion : Serum interferon gamma and IL-12 increased significantly in tuberculous children correlating with tuberculin size denoting a role in the immune response to TB. The relatively depressed in vitro production of these cytokines by patients' PBMCs may be a possible factor in disease severity.

Recommendations : Adjuvant cytokine therapy especially with IFNγ and IL-12 in treatment of resistant or advanced tuberculosis is recommended in future researches.

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