

## The Possibilities of Tuberculosis Selected Interleukins and Neurotransmitters among Displaced Iraqi Peoples

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### ABSTRACT

**Background:** Tuberculosis is one of the world's oldest and most important disseminating infectious disease that still accounts for a high morbidity and mortality among adults particularly in developing countries including Iraq which suffered from migration and displacement of millions of peoples due to series of wars ended by ISIS terror and invasion. Tuberculosis might cause changes in activity of neuro modulating factors as well as in the behaviour of the immunological responses including cytokines at the same time. **Methodology:** In the current study there were 50 patients (18 displaced and 32 non displaced TB patients) and 40 healthy control. Interleukins-6, interleukin-10, acetylcholine and serotonin were estimated in the serum of those patients examined. **Results:** interleukin (IL-6), interleukin (IL-10), serotonin (ST) and acetylcholine (ACH) concentrations showed various differences individually and/or together with respect to patients origin and gender in comparison to non-TB control individuals. **Conclusions:** Serum cytokines IL-6, and IL-10 were elevated. Neurotransmitter like serotonin and acetylcholine were significantly correlated with each other and with cytokines studied which revealed a synergistic effect of tuberculosis on the values estimated of both cytokines and neurotransmitters.

### INTRODUCTION

In the first half of the twentieth century, tuberculosis was widespread among the Iraqi population. Iraq has a great burden of tuberculosis (TB) and ranks 44<sup>th</sup> World-wide among countries with a high TB burden and 5<sup>th</sup> among the countries of the Eastern Mediterranean Region.

According to the world health organization(1nb), there were 20000 incident cases of TB in Iraq during 2010, while according to the 2012 report of the Ministry of Health (MOH), the incidence rate of TB in Iraq was 45/100,000, with 13,860 new TB cases and 1140 of previously treated cases(2). Iraq is situated in 8<sup>th</sup> rank in Eastern Mediterranean Region Organization (EMRO) according to incidence of TB in 2011, and there were estimated 15000 incident infections of the all new and relapse cases of TB in Iraq (1). Iraq is an upper middle-income country in the Eastern Mediterranean Region (EMR). The region accounts for 25% of the global burden in 2014. TB is a public health priority in Iraq and the estimated deaths due to TB are more than 4000 annually(3). Moreover, it was suggested that there might be a relation between TB and immunological parameters like interleukins and neurotransmitters as part of its pathogenesis, e.g. IL-6 stimulates the secretion of IFN- $\gamma$  for the activation of macrophages ( 4 ).

Therefore, thought that IL-6 to be play a role in controlling *M. tuberculosis* infection during the initial stages( 5 ). However, low level IL-10 production by activated macrophages can trigger antimicrobial response and prevent tissue damage, therefore; modification of the IL-10 level during TB therapy might benefit for the rapid clearance of bacteria( 6 ). *Mycobacterium tuberculosis* (Mtb), through host generated IL-10 to suppress the immune response by this way is often able to stay in the host for its entire lifetime, if not treated properly( 7 ). On the other hand, serotonin (5-hydroxytryptamine, 5-HT) is one of the most powerful neurotransmitters, it is an important mediator of interactions between the nervous and immune system ( 8 ). Serotonin causes vascular contraction and proliferation, potentiates synthesis of IL-6 that plays a protective role in mycobacterial infection ( 9 ). Recently, it has been shown that 5-HT has negative immunoregulatory effects through suppression of the stimulated production of INF- $\gamma$ /IL-10 production ratio, which reflects the pro-inflammatory capacity of immunocytes producing these cytokines ( 10,11 ). Acetylcholine (Ach) was the first neurotransmitter to be identified. It is the most abundant neurotransmitter in the brain. Synthesized by enzyme choline acetyltransferase, the enzyme that converts choline to acetylcholine ( 12 ). Reports have mentioned tuberculosis as a cause of aggravation of symptoms in diagnosed patients of myasthenia gravis which is an autoimmune disorders characterized by presence of antibodies against acetylcholine receptors, which binds to and blocks the acetylcholine receptors thereby preventing the normal neuromuscular junction transmission( 13 ). Screening for tuberculosis is obligatory before initiating immunosuppressive agents in the

treatment of myasthenia, as tuberculosis can outbreak in the immune suppressed state( 14 ).Tuberculosis is an important factor to be kept in mind during the administration of myasthenia patients, it can be a stimulate factor for crisis( 15 ).

## MATERIALS AND METHODS

Isolation and identification of *Mycobacterium tuberculosis*

Sampling, isolation and identification of acid-fast bacilli were done as mentioned elsewhere ( 16 ).

Estimation of interleukins and neurotransmitters:

These parameters were estimated according to instructions of manufacturer ( Bioassay Technology Laboratory ( BT Lab ), Korea .

## RESULTS

### Effect of tuberculosis on interleukin 6 (IL-6)

Table ( 1 ) shows that ELISA result of the present study revealed that there was a high level of serum IL-6 concentration of TB patients (mean=189 pg/ml) in comparison with the non-TB control individuals group (mean=100 pg/ml). Statistically, there was very highly significant difference between IL-6 and tuberculosis incidence ( $p = 0.0009$ ).

**Table 1. Effect of tuberculosis on interleukin 6 (IL-6) among patients of Kirkuk district.**

Parameter	Subjects	Number	Ranges (Min-Max)	Mean (pg/ml)	Standard Deviation	SE Mean	T-value
Interleukin 6 (IL-6)	TB patients	50	65 – 740	189	118	17	3.43
	Controls	40	7 – 520	100.3	126	20	

**Two-sample T-Test. T= 3.43, p-value = 0.0009**

### Effect of tuberculosis on interleukin- 6 according to the gender and origin of patient

Table 2 shows very highly significant differences between serum interleukin 6 concentration in male TB patients (mean=166.3 pg/ml, 165.5 pg/ml) and female TB patients (mean=137 pg/ml, 250.2 pg/ml) of displaced and non-displaced groups respectively ( $p=0.00001$ ).

**Table 2. Effect of tuberculosis on interleukin 6 (IL-6) according to the gender and origin of patient**

Subjects	Gender	TB- patients		Non-TB Control	Total
		Displaced	Non-displaced		
(IL-6) (pg/ml)	Male	166.3 ( 10 )	165.5 (16)	100.3(40)	432.1
	Female	137.0 (8)	250.2 (16)	0(0)	387.2
Total		303.3(18)	415.7(32)	100.3(40)	819.3

$\chi^2 = 118.297$ , P-value = 0.00001 ; ( ), number of patients

### Effect of tuberculosis on interleukin 10 (IL-10)

Table 3 shows that ELISA result of the present study revealed that there was a high level of serum IL-10 concentration of TB patients (mean=163 pg/ml) in comparison with the non-TB control individuals group (mean=81 pg/ml). Statistically, there was highly significant difference between IL-10 and tuberculosis incidence ( $p = 0.0033$ ).

**Table3. Effect of tuberculosis on interleukin 10 (IL-10)**

Parameter	Subjects	Number	Ranges (Min-Max)	Mean (pg/ml)	Standard Deviation	SE Mean	T-value
Interleukin 10 (IL-10)	TB patients	50	10 - 985	163	159	22	3.03
	Controls	40	16 - 370	81	95	15	

Two-sample T-Test. T= 3.03, p-value = 0.0033

### Effect of tuberculosis on interleukin -10 according to the gender and the origin of patient

Results of the present study were presented in Table 4 which shows that there were very highly significant differences between serum interleukin 10 (IL-10) concentration in male TB patients (mean=138.5 pg/ml, 175 pg/ml) and female TB patients (96.6 pg/ml, 195.3 pg/ml) of displaced and non-displaced groups respectively, ( $p=0.00001$ ).

**Table4. Effect of tuberculosis on interleukin 10 (IL-10) according to the gender and origin of patient**

Subjects	Gender	TB- patients		Non-TB Control	Total
		Displaced	Non-displaced		
(IL-10)	Male	138.5(10)	175.0(16)	81(40)	394.5

(pg/ml)	Female	96.6(8)	195.3(16)	0(0)	291.9
Total		235.1(18)	370.3(32)	81(40)	686.4

$\chi^2 = 76.029$ , P-value = 0.00001 ; ( ) , number of patients

**Correlation between factors; interleukin 6 (IL-6) and interleukin 10 (IL-10) according to origin of patient among patients with incidence of tuberculosis of Kirkuk district.**

Table 4.21 shows that the effect of tuberculosis incidence on interleukin 6 (IL-6) and interleukin 10 (IL-10) serum concentrations together in TB patients group according to the different origin 538.4 pg/ml in displaced and 786 pg/ml in non-displaced TB patients. Statistically, there were very highly significant differences between patients origin and IL-6 and IL-10 concentrations together in comparison both groups results with that of non-TB control individuals 181.3 pg/ml. (p = 0.00001).

**Table 4.21 Correlation between cytokine factors; interleukin 6 (IL-6) and interleukin 10 (IL-10) according to gender and origin of patient among patients with incidence of tuberculosis of Kirkuk district.**

Parameter		Gender	TB patients		Non-TB Control	Total
			Displaced	Non-displaced		
Cytokines	IL-6 (pg/ml)	Male	166.3 (10)	165.5(16)	100.3 (40)	432.1
		Female	137.0(8)	250.2 (16)	0(0)	387.2
	IL-10 (pg/ml)	Male	138.5(10)	175.0 (16)	81(40)	394.5
		Female	96.6(8)	195.3 (16)	0 (0)	291.9
Total			538.4	786.0	181.3	1505.7

$\chi^2 = 195.9$ , p – value = 0.00001; ( ) , number of patients

**Effect of incidence of tuberculosis on serum serotonin (ST) among patients of Kirkuk district.**

ELISA result of the human serum serotonin (ST) concentration of TB patients of the present study. Statistically, showed that there was non-significantly difference between tuberculosis incidence and ST concentration in comparison between patients serum ST level (mean=294 pg/ml) with the non-TB control individuals group (mean=260 pg/ml), (p = 0.37) (Table 4. 22).

**Table 4.22 Effect of incidence of tuberculosis on serum serotonin (ST) among patients of Kirkuk district.**

Parameter	Subjects	Number	Ranges (Min-Max)	Mean (ng/ml)	Standard Deviation	SE Mean	T-value
Serotonin (ST)	Patient	50	15 - 897	294	201	28	0.89
	Control	40	15 - 574	260	156	25	

**T-Test. T= 0.89, p-value =0.37**

**Effect of incidence of tuberculosis on serum serotonin (ST) among patients of Kirkuk district according to the gender and origin of patient.**

Table 4.23 shows a very highly significant difference between serum serotonin concentration in male TB patients (235.6 pg/ml, 251.5 pg/ml) and female TB patients (mean=205.8 pg/ml, 247.5 pg/ml) of displaced and non-displaced groups respectively, in comparison with the non-TB control individuals group (mean=260 pg/ml), (p=0.00001).

**Table 4.23 Effect of incidence of tuberculosis on serum serotonin (ST) among patients of Kirkuk district according to the gender and origin of patient.**

Parameter	Gender	TB patients		Non-TB Control	Total
		Displaced	Non-displaced		
Serotonin (ng/ml)	Male	235.6(10)	251.5(16)	260(40)	747.1
	Female	205.8(8)	247.5(16)	0(0)	453.3
Total		441.4(18)	499.0(32)	260(40)	1200.4

$\chi^2 = 173.694$ , p – value is = 0.00001; ( ), number of patients

**Effect of incidence of tuberculosis on serum acetylcholine (ACH) among patients of Kirkuk district according to the origin of patient.**

ELISA result of the human serum acetylcholine (ACH) concentrations of TB patients (mean=203.8 pg/ml) revealed that there were non-significant differences between tuberculosis incidence and ACH concentrations in comparison with that of non-TB control individuals group (mean=208.5 pg/ml), (p = 0.80) (Table 4.24).

**Table- 4.24 Effect of incidence of tuberculosis on serum acetylcholine (ACH) among patients of Kirkuk district according to the origin of patient.**

			Ranges	Mean	Standard	SE	
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Parameter	Subjects	Number	(Min-Max)	(ng/ml)	Deviation	Mean	T-value
Acetylcholine (ACH)	TB patient	50	15 - 400	203.8	92.0	13	- 0.25
	Control	40	46 - 400	208.5	86.3	14	

**Two-sample T-Test. T= -0.25, p-value = 0.80**

**Effect of incidence of tuberculosis on serum acetylcholine (ACH) among patients of Kirkuk district according to the gender and origin of patient.**

Table 4.25 shows a very highly significant difference between serum acetylcholine(ACH) concentration in male TB patients (179.6 pg/ml, 193.9 pg/ml) and female TB patients (mean=154.3 pg/ml, 250.3 pg/ml) of displaced and non-displaced groups respectively, in comparison with the non-TB control individuals group (mean=208.5 pg/ml), (p=0.00001).

**Table 4.25 Effect of incidence of tuberculosis on serum acetylcholine (ACH) among patients of Kirkuk district according to the gender and origin of patient.**

Parameter	Gender	TB patients		Non-TB Control	Total
		Displaced	Non-displaced		
Acetylcholine (ng/ml)	Male	179.69(10)	193.9(16)	208.5(40)	582.0
	Female	154.3(8)	250.3(16)	0(0)	404.6
Total		333.9(18)	444.2(32)	208.5(40)	986.6

$\chi^2 = 192.093$ , p – value is = 0.00001; ( ), number of patients

**Correlation between factors of neurotransmitters serotonin (ST) and acetylcholine (ACH) according to the gender and origin of patient among patients with incidence of tuberculosis of Kirkuk district.**

Table 4.26 shows the effect of tuberculosis incidence on serotonin (ST) and acetylcholine (ACH) serum concentrations together in TB patients group according to the different origin 775.3pg/ml in displaced and 943.2pg/ml in non-displaced TB patients. Statistically, there were very highly significant differences between patients origin and ST and ACH concentrations together in comparison both groups results with that of non-TB control individuals 468.5 pg/ml, (p = 0.00001).

**Table 4.26 Correlation between factors of neurotransmitters serotonin (ST) and acetylcholine (ACH) according to gender and origin of patient among patients with incidence of tuberculosis of Kirkuk district.**

Parameter		Gender	TB patients		Non-TB Control	Total
			Displaced	Non-displaced		
Neurotransmitters	Serotonin (ng/ml)	Male	235.6(10)	251.5(16)	260(40)	747.1
		Female	205.8(8)	247.5(16)	0(0)	453.3
	Acetylcholine (ng/ml)	Male	179.6(10)	193.9(16)	208.5(40)	582.0
		Female	154.3(8)	250.3(16)	0(0)	404.6
Total			775.3	943.2	468.5	2187

$\chi^2 = 398.342$ , p-value = 0.00001 ; ( ), number of patients

**Correlation between cytokines interleukin 6(IL-6) and interleukin 10 (IL-10) and neurotransmitters serotonin (ST), acetylcholine (ACH) according to the gender and origin of patient with incidence of tuberculosis of Kirkuk district.**

Effects of cytokine IL-6, IL-10 and neurotransmitter hormones ST and ACH in correlation with incidence of tuberculosis in the present study TB patients according to the patients origin and gender. Results showed that there were an elevation of all serum ( IL-6, IL-10, ST and ACH) concentrations in both groups (1313.7 pg/ml , 1729.2 pg/ml) displaced and non-displaced respectively in comparison with that of non-TB control individuals group(649.8pg/ml). Statistically, there were a very highly significant differences between effects of previous factors and the patients origin and gender in comparison with that of non-TB control group, (p=0.00001) (Table 4.27).

**Table 4.27 Correlation between cytokines interleukin 6 (IL-6), interleukin 10 (IL-10) and neurotransmitters serotonin (ST), acetylcholine (ACH) according to the gender and origin of patient with incidence of tuberculosis**

Parameter		Gender	TB patients		Non-TB Control	Total
			Displaced	Non-displaced		
Cytokines	IL-6 (pg/ml)	Male	166.3(10)	165.5(16)	100.3(40)	432.1
		Female	137.0(8)	250.2(16)	0(0)	387.2
	IL-10 (pg/ml)	Male	138.5(10)	175.0(16)	81(40)	394.5
		Female	96.6(8)	195.3(16)	0(0)	291.9
Neurotransmitters	Serotonin (ng/ml)	Male	235.6(10)	251.5(16)	260(40)	747.1
		Female	205.8(8)	247.5(16)	0(0)	453.3



	<b>Acetylcholine</b>	<b>Male</b>	179.6(10)	193.9(16)	208.5(40)	582.0
	<b>( ng/ml)</b>	<b>Female</b>	154.3(8)	250.3(16)	0(0)	404.6
<b>Total</b>			1313.7	1729.2	649.8	3692.7

$\chi^2=671.71$ , p-value is = 0.00001 ; ( ), number of patients

## DISCUSSION

Cytokines are critical for immunity against foreign invaders [220]. During tuberculosis cytokines play a various roles in host defence. The finding of present study shows that the serum IL-6 concentration was very significantly higher than that of non-TB control individuals (Table 4.17). This was in agreement with that demonstrated by Ieremenchuk *et al.* [221], from Ukraine, who reported the concentrations of IL-6 in all TB groups were significantly increased compared to control group, and also in agreement with Deventer *et al.* [222], who reported that IL-6 levels were significantly elevated in patients with TB during and after treatment compared with controls. Significant difference was found between gender and origin of TB patients and the level of IL-6 in their serum ( $p=0.00001$ ) (Table 4.18). ELISA result revealed that serum IL-10 concentrations in TB patients (mean=163pg/ml) were significantly ( $p=0.0033$ ) higher than that of non-TB individuals control (mean=81pg/ml) (Table 4.19). Very highly significant difference was found between serum IL-10 level in male TB patients (mean=138.5 pg/ml) and female TB patients (mean=96.6 pg/ml) among displaced group ( $p=0.00001$ ). and in relation to TB patients origin such difference was shown in comparison with non-displaced TB patients group, male (mean=175 pg/ml) and female (mean=195.3 pg/ml) (Table 4. 20). These findings were in disagreement with that carried out in Iraq by Auda *et al.* [223], who reported that no correlation was found between gender of TB patients and the level of IL-10 in their sera. In correlation effects of IL-6 and IL-10 were showed very highly significant difference between displaced group (serum level=538.4 pg/ml) and non-displaced group (serum level=786 pg/ml),  $p=0.00001$ . (Table 4.21). Cytokines play an important role in the pathogenesis of TB. Cytokines that elevated in serum were IFN- $\gamma$ , IL-6, and IL-10 [241]. In conclusion, proinflammatory cytokines play an important role in the pathogenesis of active pulmonary tuberculosis (APTb). Here anti-inflammatory cytokine (IL-10) is elevated in the serum of APTb patients. Measuring the serum levels of several cytokines may be useful for evaluating the activity of TB disease and

monitoring the clinical effect of anti-tuberculous treatment. Further studies are needed to address the role of cytokines in immunity to TB under natural conditions.

ELISA results of the each of neurotransmitter hormones serotonin (ST) and acetylcholine (ACH) concentration of TB patient's serum of the present study were shown in Tables 4.22, 4.24. Statistically, there were non-significant differences between tuberculosis incidence and serum level of each one of ST and ACH concentration in comparison with the non-TB control individual group ( $p=0.37$ ). Whereas, they showed very highly significant difference between ST and ACH serum concentration and patients gender (Tables 4.23, 4.25) ( $p = 0.00001$ ). The effect of neurotransmitter hormones serotonin (ST) and acetylcholine (ACH) in correlation together on incidence of tuberculosis. The results showed that there was an elevation of their serum concentration appeared in both groups of testing populations in comparison with the non-TB control individuals group. Statistically, there was very highly significant difference between the genders of each group themselves and different groups origin and in comparison both groups results with that of non-TB control individuals ( $p=0.00001$ ), (Table 4.26). However, there is no relevant information concluded elsewhere for comparison with the results presented here.

The effects of Pro-inflammatory cytokine (IL-6), anti-inflammatory cytokine (IL-10) and neurotransmitter hormones serotonin (ST), acetylcholine (ACH) in correlation together on incidence of tuberculosis among the present study population according to the patients origin and gender of the present study were reported. Results showed that there was an elevation of all serum (IL-6, IL-10, ST and ACH) concentration appeared in both groups of testing populations in comparison with the non-TB control individuals group. There were very highly significant differences between effect of each type of the above factors and the patients origin and gender in comparison both groups results with that of non-TB control individuals ( $p=0.00001$ ), (Table 4.27). The present analyses showed a difficulty in comparison as the first prospective study about tuberculosis incidence and evaluation of ST and ACH hormones effects on the TB disease, due to lacking of relevant data in literature.

## CONCLUSIONS

Serum cytokines IFN- $\gamma$ , IL-6, and IL-10 were elevated. Neurotransmitting hormones serotonin (ST) and acetylcholine (ACH) in correlation together, showed very highly significant difference between the genders of each group themselves and different groups origin.

## Acknowledgement

This study was presented as an e-poster of the EAACI, European Association of Allergy and Clinical Immunology which took place in Munich, Germany, 27-30 May 2018.

## DISCLOSURE

The authors have no financial conflicts of interest to declare

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