

## **EVALUATION OF INNATE IMMUNE RESPONSE AND BACTERIAL ISOLATES RATHER THAN *M TUBERCULOSIS* IN TUBERCULOSIS PATIENT.**

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**ABSTRACT:** *The work was applied on 109 TB Patient admitted to the Babylon centre of tuberculosis and chest disease. Sputum cultures of tuberculosis patients reveals major types of bacteria, Streptococcus pyogenes, staphylococci. Aureus, Moraxella catarrhalis, Pseudomonas aeruginosa, Neisseria meningitidis, Streptococcus viridans, and Serratia marcescens Haemophilus influenzae. Streptococcus epidermidis aureus from high rate of these isolates. The study aims to evaluate the parameters of cell mediated immunity including IL6 and phagocytosis for tuberculosis patients. Blood samples were collected from patients and control groups to estimate phagocytosis, interleukin-6 (IL-6) by ELISA (Enzyme linked immunosorbent assay) method. The cell mediated investigation showing that there is a significantly increased ( $p < 0.05$ ) in phagocytosis activity of neutrophils in TB patients (21%) compared to control group (11.85%) and significantly increased ( $p > 0.05$ ) of IL-6 level in the TB patients (388.05) IU/ml compared to control group (82.83) IU/ml. The results revealed that rural-urban ratio was higher in rural than in urban for TB patients, furthermore the T.B infection was higher in male than female.*

**KEYWORDS:** TB patients, IL6, phagocytosis, Bacterial isolate.

### **INTRODUCTION**

Tuberculosis (TB) is considered one of the most important infectious diseases in the world and its incidence is on rise (Reza *et al.*, 2007). This form leads to a global cause of morbidity and death (Dye, 2006). TB infection is acquired by the inhalation of droplets containing *Mycobacterium Tuberculosis* bacilli (MTB). Tuberculosis is an immunological disease and the clinical manifestations of tuberculosis are the functions of the immune status of the host (Singh, 2000). There are a number of factors that make people more susceptible to TB infection worldwide; the most important of these factors is HIV (Davies *et al.*, 2006; Chaisson and Martinson, 2008). Co-infection with HIV is a particular problem in Sub-Saharan Africa, due to the high incidence of HIV in these countries. Smoking more than 20 cigarettes, a day also increases the risk of TB by two to four times (Restrepo, 2007).

### **MATERIALS AND METHODS**

#### **Patients:**

Bacteriological study includes culturing of sputum specimens with selective and differential media. Biochemical investigations were done for bacterial identification (McFadden, 2000). A total of 109 TB patients consisting of 79 males and 30 females were involved in this study.

Their age range was from (9–60) years. Case information was taken for each patient include; name, sex, age, residency, duration of infection, and duration of therapy. All tuberculous cases were clinically diagnosed by a specialist clinician. Those patients were admitted to Babylon Center of Tuberculosis and Chest Diseases.

### **Control:**

A total of 40 apparently healthy subjects (25 males and 15 females) were involved as controls group. The age range of controls was matched to the patients (10 – 60) years.

### **Blood samples:**

Three ml of blood were collected by vein puncture into two sterile test tubes , in one of them 2 ml of blood were put and left for (2 – 4) hours , then the upper layer ( serum ) was collected in clean test tube and stored at – 20 C° until using it in serological tests and determination of IL6 . The second sample of blood 1 ml was putted in another test tube containing anticoagulant EDTA , Differential Leukocyte Count and used for assessment of phagocytic activity (Lewis *et al.*, 2001).

### **Enzyme- linked Immunospot Test**

In vitro test which used enzyme –linked immunospot technology which measures, Mycobacterial antigens number or enzyme- linked immunosorbent assay (ELISA) technology (Quanti FERON). IL6 production in whole blood (Lazarevic *et al.*, 2005).

### **NBT dye reduction test:**

NBT test was performed by the method of Park *et al.* (1968), with the subsequent modification of Freeman and King (1972 ). 0.1ml of blood was mixed with 0.1ml of NBT solution in a well of micro titer plate. The mixture was mixed gently and covered to ensure humidity, and incubate at 37C° for 15min. follow this with an equal period at room temperature. Smear was then made and immersed with Gemsa stain and left for 5min. then rinsed with Gemsa stain buffer and allowed to dry. The slide was examined under oil immersion. 100 PMN was counted and then percentage of PMNs reduce NBT was recorded.

### **Statistical Analysis**

T-test ( $p < 0.05$ ) were carried out according to Niazi (2004 ).

## **RESULTS AND DISCUSSION**

### **Bacteriological study**

Bacteriological study of Sputum of Tuberculosis patients reveals many bacterial isolates, this study concerned with many types of bacteria *M. catarrhalis* and *S.aureus*. Numbers of bacterial isolates varies with type of specimens and virulence efficacy , *S.aureus* forms highest rate of these isolates 19.5 % while *M. catarrhalis* form 18.3 % for all bacterial isolates ( table 1). In addition bacterial including *Ps.aeruginosa* 8.9% , *St.pyogens* 15.8%, *S. macescence* 0.5% , *K. pneumonia* 7.6% , *S.epidermidis* 12.7% and *St.virdanse* 1.1% These isolates characterized by their highly ability to cause enteric infection in human and the

symptoms of infection appears with certain days as a results of their toxins activity (Brook *et al* , 2004) .

Bacterial types	No. of isolates %
<i>St.pyogens</i>	15.8
<i>S.aureus</i>	19.5
<i>M. catarrhalis</i>	18.3
<i>Ps.aeruginosa,</i>	8.9
<i>S. macescence</i>	0.5
<i>H.influenzae</i>	6.1
<i>K. pneumonia</i>	7.6
<i>N.menegenitites</i>	9.5
<i>S.epidermidis</i>	12.7
<i>St.virdanse</i>	1.1
<i>Totale</i>	100

**Table 1. Bacterial isolates from tuberculosis infected patients.**

### **Distribution Tuberculosis with Age, Sex and Geographical**

Only three TB case was diagnosed in a 9 years old young child, the number increased in the age group of >10 years and the same in >20-30, table (2). However, the number increased significantly in the age group of > 51-60 to reach 22.01 % of the total number of TB patients. The number of cases were maintained in most at the same level in the age groups of >41-50 years (18.3%) and >31-40 years (22%). Only 10% with the >60 years age group.

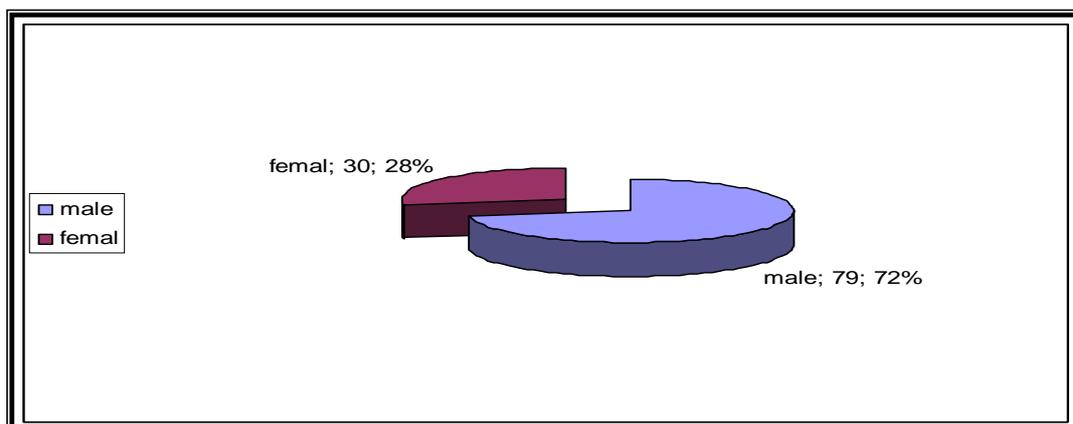
The results that were expressed in table (2) revealed the wide age range for TB patients. This suggests that all age groups were susceptible to infection with TB. This result was agreement with what have been reported by (CDC, 2009) when illustrated that people of all ages; all nationalities and all incomes can get tuberculosis. Zaman *et al.*, (2006) had reported that the age groups studies were also different between studies and covered all ages. The result in table (1) reveals that the most predominant age groups (51-60) years of TB patients. This finding was matched with (WHO, 2006) that reported TB is mainly a disease of older people or of the immunocompromised .The result was matched with that recorded by (Butcher *et al.*, 2001), who mentioned that the aging process has significant and deleterious effects on immune responses in human, resulting in increased susceptibility to bacterial infection because compromised functioning of innate immune responses, at least as much as reduced adaptive responses.

**Table ( 2) Tuberculosis infection distribution according to age group**

Age group (years)	TB patients
<10	3:109(2.7%)
11-20	16: 109 (14.6%)
21-30	16: 109 (14.6%)
31-40	22: 109 (20.1%)
41-50	20: 109 (18.3%)
51-60	24: 109 (22.01%)
>60	8: 109 (7.3%)
<b>Age range (years)</b>	<b>9-60</b>

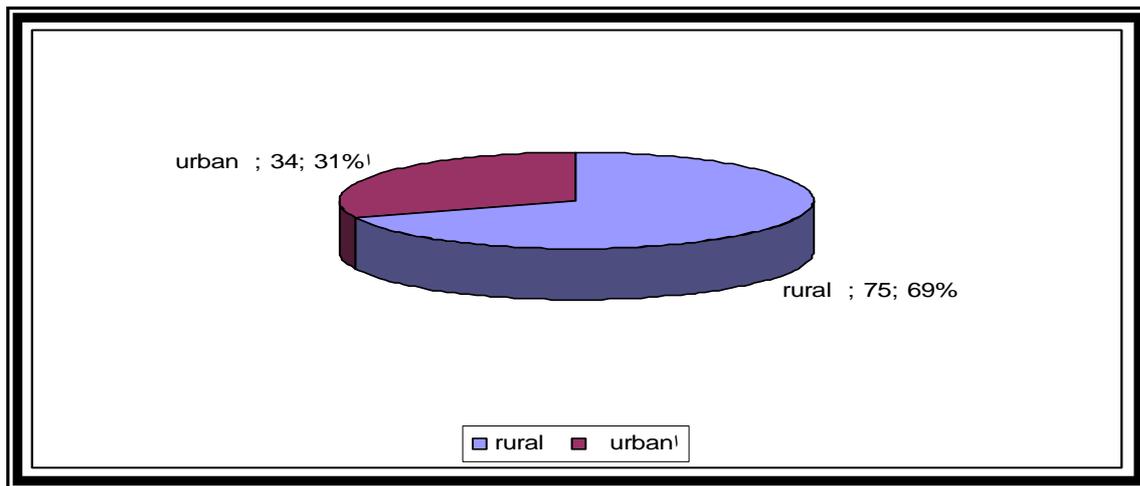
In this study, the TB patients consisted of 79:109 (72%) males and 30:109 (28%) females, figure (1). For TB patients showed that male–female ratio was higher in males than in females. This finding was matched with that recorded by (Al- Saadi, 2004) who mentioned that the rate of TB in male was higher than female for TB patients attending Tuberculosis center in Babylon city, Iraq.

This difference is partly due to the fact that men have less access to diagnostic facilities in some settings, but the broader pattern also reflects real epidemiological differences between men and women, both in exposure to infection and in susceptibility to disease. ( Davies *et al.*, 2006 ).

**Figure ( 1) Sex distribution for TB patients**

The geographical distribution of 109 TB patients included in this study is illustrated in figure ( 2). TB patients were classified according to the home address into rural 75:60 (68.8%). While the patients' habitat in urbanite 34:109 (31.1%). For TB patients, showed that rural-urban ratio was higher in rural than in urban. This finding was matched with that recorded by (Bhatt *et al.*, 2009) who mentioned that tuberculosis cases were commonly found in rural areas 59%.

The higher incidence of TB among rural could be attributed to non-medical factors such as poor quality of life, poor housing, and overcrowding, population explosion, under nutrition, lack of education , large families , early marriage , lack of a wareness of causes of illness . All these factors interrelated and contributed in the occurrence and spread of tuberculosis. Other studies found that tuberculosis cases finding rate in urban 52% and 28% in rural (Hussain *et al.*, 2004).



**Figure ( 2) Geographical distribution for TB patients.**

#### **Assessment of phagocytosis:**

Nitroblue tetrazolium (NBT) reduction dye was used to assess phagocytic activity of polymorphonuclear cells (PMNs).The reduction was graded on the basis of the number of neutrophils with intracellular deposit of formazan stippling per 100 neutrophils counte .

NBT test is a non-specific cytochemical test of neutrophil cytoplasmic membrane function, and membrane changes may be induced in vivo not only by endotoxin but also by tissue breakdown products and acute phase proteins (Gordon *et al.*, 1975).

The mean value of phagocytosis in TB patients is high in age51-60 and reach 21 % while in controls were 11.857 table (3). It is clear that the phagocytosis increases significantly in TB patients .This finding was matched with that recorded by(Martineau *et al.*, 2007) who mentioned that neutrophils isolated from the peripheral blood of pulmonary tuberculosis (PTB) patients also exhibit reduced phagocytic activity in vitro.

**Table ( 3) Phagocytic activity of polymorph nuclear phagocytic cell (PMNs) for TB Patients .**

Age group (years)	Groups	NBT% (M ± SD)
<10	Patient	20.242*± 1.858
	Control	15.914± 1.524
11-20	Patient	13.200*± 1.652
	Control	10.9500± 1.118
21-30	Patient	17.371*± 1.342
	Control	11.214 ± 1.228
31-40	Patient	14.312*± 1.159
	Control	9.025± 1.109
41-50	Patient	17.442*± 3.896
	Control	11.014 ± 2.370
51-60	Patient	21.000*± 2.094
	Control	11.857 ± 1.431
60-70	Patient	13.600*± 1.021
	Control	9.385 ± 1.025

\*Standard deviation

**Concentration of Interleukin (IL6)**

Interleukin (IL6 ) concentration high in The age group 60-70TB patients was 388.05 IU/ml, while in controls was 82.83 IU/ml. Statistical analysis show shows Interleukin concentration no significant differences between TB patients and controls at 0.05 ( $p < 0.05$ ). The results indicates the presence of reduced cell mediate immunity in TB patients depending on the fact that IL6 as a cytokine regulator of CMI is increases dramatically to at least 10 fold over the concentration in the normal state in which there is no antigenic stimulation (Doan *et al.*, 2008). This finding was matched with (Ribeiro-Rodrigues *et al.*, 2006) who mentioned that Th2 –cells, as a source for IL6 ; responses were suppressed by MTB during active TB. While Hougardy *et al.*, (2007) mentioned that it is not surprising that some microbial

**Table (4) Concentration of Interleukin (IL6) IU/ml for TB Patients and Controls**

Age group (years)	Groups	IL6 IU/ ml ( M ± SD)
<10	Patient	*111.50 ±2.37
	Control	86.60±2.37
11-20	Patient	* 364.1 ± 25.09
	Control	82.83 ± 3.76
21-30	Patient	*246.09± 29.6
	Control	94.50± 4.50
31-40	Patient	*905.4± 4.80
	Control	88.07± 2.63
41-50	Patient	*867.7± 21.68
	Control	82.14± 3.23
51-60	Patient	244.08*± 20.41
	Control	93.78± 2.9
60-70	Patient	*388.05± 35.57
	Control	82.83±2.31

\*Standard deviation

**CONCLUSION**

The study concluded that males are more susceptible To TB infection than female and the immunological assay such as IL6, NBT In addition the bacteriological isolation M.tuberculosis could be a good indicator for T.B infection.

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