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"The Immunomodulatory Markers in Patients With Chronic Urinary Tract Infection and Typhoid Fever"

A graduation project submitted to the college of pharmacy /Babylon University as partial fulfillment of the requirement of the BSc degree in pharmacy

By

Rula Dhiaa

Rand Mohemmed

Zahraa Saleem

Supervised by

Prof. Dr. Hussam Wahab Al-Humadi

Assist. Prof. Dr. Rafal Jalil Al-Saigh

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بسِـــــمِ اللـَّهِ الرَّحْمَـٰنِ الرَّحِيمِ

يرْفعِ اللَّه اللذِينَ آمَنوُا مِنكُمْ وَاللذِينَ أوُتوُا الْعِلْمَ دَرَجَاتٍ واللَّه بِمَا تعْمَلوُنَ خَبيِر

صدق الله العلي العظيم

الاية ﴿11﴾ سورةالمجادلة

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علامات تعديل المناعة لدى مرضى التهاب المسالك البولية المزمنة وحمى التيفوئيد

الخلاصة:

الخلفية: الحمى المعوية التي تسببها السالمونيلا التيفية هي مشكلة صحية عامة عالمية. المرضى الصغار الذين يتعافون من حمى التيفوئيد يصابون بحالة حاملة مزمنة (3-5٪) تعمل كمستودع للعدوى من خلال التخلص المستمر من البكتيريا في البراز والبول مما يتسبب في بيلة جرثومية من حمى التيفود مع حالات مزمنة من عدوى المسالك البولية (UTIبسبب ل S. Typhi. يرتبط إفراز الواسمات المسببة للالتهاب بما في ذلك إنترلوكين 6 (IL-6وعامل نخر الورم ألفا (TNF-αوفيتامين د (VDبشكل شائع بهذه الحالات المزمنة. لذلك ، هدفنا هو التحقيق في مستويات VD وبعض علامات تعديل المناعة (IL-6 و TNF-αفي هذه الحالات المزمنة من التهاب المسالك البولية بسبب S. Typhi.

الطريقة: تم تضمين 41 مريضًا بالغًا لديهم مستويات عالية من IgM و IgG من S. Typhi مع التهاب المسالك البولية المزمنة (المجموعة المستهدفة) مقارنة بـ 32 حالة سلبية لكل من IgM و IgG من S. Typhi المصابة بالتهاب المسالك البولية المزمنة (المجموعة الضابطة) في المستشفيات العامة في محافظة بابل. تم جمع البيانات الوصفية للمرضى والمجموعة الضابطة من قبل باحث مدرب جيدًا بعد استبيان منظم. بالتوازي ، يتم جمع الدم المحيطي لتحديد IL-6 و TNF- α و VD. ثم تم دراسة تقييم العلاقة بين البيانات السريرية والوصفية ومستويات الواسمات المعدلة للمناعة إحصائياً.

النتائج: غالبية المرضى المستهدفين كانوا من الإناث (56٪) و 71٪ كانوا يقيمون في المناطق الريفية. كان 47 ٪ منهم يعيشون في حالة اجتماعية واقتصادية متوسطة ، علاوة على ذلك ، كان 47 ٪ من المجموعة المستهدفة يعانون من فقر الدم المزمن. بعد ذلك ، كانت هناك زيادة كبيرة في مستويات IL-6 و TNF- α في المجموعة المستهدفة مقابل مجموعة التحكم (P <0.001)، مع انخفاض مستوى VD بشكل ملحوظ مقابل التحكم (P <0.05).

الخلاصة: نسبة عالية من المجموعة المستهدفة لديها مستوى منخفض جدا من VD يرتبط في الغالب بمرض نشط. علاوة على ذلك ، تشير زيادة مستويات IL-6 و TNF-α إلى تأثير عكسي على علاج التهاب المسالك البولية والذي يرتبط ارتباطًا وثيقًا بنتيجة المرض.

Abstract

Background: Enteric fever caused by *Salmonella Typhi* is a global public health problem. Little patients recover from the typhoid fever develop a chronic carrier state (3-5%) acting as reservoir of infection by continued shedding of bacteria in faeces and urine causing a bacteriuria episode of typhoid fever with chronic cases of urinary tract infection (UTI) due to *S. Typhi*. The secretion of proinflammatory markers including interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and Vitamin D (VD) is commonly associated with these chronic conditions. Therefore, our aim to investigate the levels of VD and some immunomodulatory markers (IL-6 and TNF-α) in these chronic cases of UTI due to *S. Typhi*.

Method: 41 Adults patients with high levels of IgM and IgG of *S. Typhi* with chronic UTI (target group) were included in comparison to 32 negative to both IgM and IgG of *S. Typhi* with chronic UTI (control group) at general hospitals of Babylon Province. Descriptive data for patients and control group were collected by well-trained researcher following a structured questionnaire. In parallel, peripheral blood collected to determine IL-6, TNF- α, and VD. Then the assessment for the association between clinical and descriptive data and immunomodulatory markers levels was investigated statistically.

Results: The majority of target patients were females (56%) and 71% were resident in rural areas; 47% of them were living in middle socioeconomic state, moreover, 47% of target group had chronic anemia. Following to that, there was highly increased in IL-6 and TNF-α levels in target group versus control ($P<0.001$), with significant low VD level versus control ($P<0.05$).

Conclusion: A high proportion of target group have very low VD level mostly associated with active disease. Moreover, the increase of IL-6 and TNF-α levels suggests an inverse impact on curing of UTI which closely associated with the outcome of the disease.

Keywords: Typhoid Fever, *S.typhi*, Chronic UTI, Immunomodulatory markers, IL-6, TNF-α, Vitamin D.

INTRODUCTION

Typhoid fever is a systemic infection with the bacterium *Salmonella enterica* serotype *typhi*. This highly adapted, human-specific pathogen has evolved remarkable mechanisms for persistence in its host that help to ensure its survival and transmission. Typhoid fever is an important cause of illness and death in the overcrowded and unsanitary urban conditions. The provision of clean water and good sewage systems lead to a dramatic decrease in the incidence of typhoid fever. Reliable data from which to estimate the burden of disease in these areas are difficult to obtain, since many hospitals lack facilities for blood culture and up to 90 percent of patients with typhoid are treated as outpatients. Community-based studies have consistently shown higher levels of typhoid than public health figures The introduction of chloramphenicol for the treatment of typhoid fever in 1948 transformed a severe, debilitating, and often fatal disease into a readily treatable condition. The emergence of resistance to chloramphenicol and other antimicrobial agents has been a major setback [1].

Typhoid is usually contracted by ingestion of food or water contaminated by fecal or urinary carriers excreting *S. enterica* serotype *typhi*. In endemic areas, identified risk factors for disease include eating food prepared outside the home, such as ice cream or flavored iced drinks from street vendors, drinking contaminated water, having a close contact or relative with recent typhoid fever, poor housing with inadequate facilities for personal hygiene, and recent use of antimicrobial drugs [2].

Pathogenesis

The pathogenesis of typhoid fever depends upon a number of factors, including infectious species, virulence, host's immunity, and infectious dose. The larger the infectious dose, the shorter the incubation period, and the higher the attack rate. Typhoid fever is more severe in debilitated and immunocompromised patients such as those on glucocorticoid therapy, and those with altered phagocyte function (i.e., patients with malaria and sickle cell anemia). Salmonella is an acid-sensitive bacteria except for a few resistant strains, so typically it is destroyed in the stomach by gastric acid unless a large dose is ingested [3]. In patients with achlorhydria,

intake of antacids and antihistamines, colonization of Salmonella occurs even with smaller doses. Food and beverages also act as buffers against gastric acid that facilitates bacteria reaching the small gut [4].

The virulence of Salmonella is determined by typhoid toxin, or Vi antigen (polysaccharide capsule), liposaccharide O antigen, and flagellar H antigen. Strains positive for Vi antigen have an attack rate twice that of Vi negative strains, even for the same dose of micro-organisms. One of the main differences between *Salmonella typhi* and non-typhoidal salmonella (NTS) is the presence of Vi antigen in *Salmonella typhi* but absent in NTS. The main role of the Vi antigen is to act as an antiphagocytic agent preventing the action of macrophages, thus shielding the O antigen from antibodies that confer the serum resistance. The flagellar H antigen provides bacterial mobility and adherence upon the gut wall mucosa. Invasion of the gut wall is assisted by flagella, and the type III secretion system is capable of transferring bacterial protein into enterocytes and M cells (specialized epithelial cells that serve as antigen-presenting cells in gut mucosa or lymphoid tissue) or by direct penetration of mucosa [5].

Bacteria attached to M cells are absorbed by pinched off cytoplasm containing bacteria and extruded into the luminal space. In this process, M cells are damaged, and the basal lamina is exposed. It provides easy access to pathogens for the invasion, which worsens the condition [6]. The cystic fibrosis transmembrane conductance regulator (CFTR) is said to be important in the uptake of Salmonella; so, patients with abnormal CFTR protein are resistant to typhoid. The transferred proteins activate the host cell Rho GTPases, which trigger the actin rearrangement so that bacterial protein uptake takes place in the phagosomes where the bacteria can grow. This special characteristic of the bacteria helps them to remain viable in a pool of host immunity. Salmonella also produces a molecule that stimulates the epithelial release of chemoattractant eicosanoid, which sequesters neutrophils into the lumen and potentiates mucosal damage [7].

Bacteria induce proliferation of Payer patches via recruitment of lymphocytes and mononuclear cells and induce necrosis and eventually, ulceration that complicates the symptoms. Pathogens reach the reticuloendothelial system via both lymphatic

system and bloodstream, including other multiple organs, most commonly gallbladder in almost all cases. The early bacteremic phase (24 hours to 72 hours) is asymptomatic and transient as these bacteria are phagocytosed by macrophages and monocytes in the reticuloendothelial system called primary bacteremia [8]. The capacity of pathogens to grow in these immune cells makes them characteristic, and intracellular multiplication of bacteria in the reticuloendothelial system enforces them to re-enter the bloodstream causing continuous bacteremia for several days and weeks known as secondary bacteremia. Secondary bacteremia is the phase in which disease symptoms manifest. Like in other gram-negative bacteria, an endotoxin has an important role in the pathogenesis. The lipopolysaccharide induces the shock-like reaction, and endotoxemia leads to vascular hyperactivity and catecholamine release, which causes focal necrosis and haemorrhage [9].

Obviously, *S. typhi* Vi-positive strains are more infectious and more virulent than Vi-negative strains of *S. typhi*. High gastric acidity is one important barrier against invasion of *S. typhi* and a low gastric pH is therefore an important defence mechanism. Aging, gastrectomy, proton-pump inhibitors or antacids leads to achlorhydria and facilitates typhoid infection. In the small intestine, the bacteria first adhere to mucosal cells and then invade the mucosa following which they rapidly penetrate the mucosal epithelium via either microfold cells or enterocytes and arrive in the lamina propria, where they rapidly elicit an influx of macrophage that ingest the bacilli but do not generally kill them. Some bacilli remain within the macrophage of the small intestinal lymphoid tissue and some microorganisms translocate to the intestinal lymphoid follicles and the draining mesenteric lymph nodes and by which they enter the thoracic duct and the general circulation [10]. 7 to 14 days is usually the incubation period of typhoid. After that there is an interaction between host immunologic mediators and bacterial factors leading ultimately to the necrosis of Peyer’s patches [8].

The Epidemiology of Typhoid fever

In many parts of the developing world, typhoid fever continues to present an important public health challenge, with 16.6 million cases and 600,000 deaths

reported annually. With regards to its clinical manifestations, it has been reported previously that these can differ markedly in different parts of the world where typhoid fever is endemic. In South America and parts of Southeast Asia (e.g., Malaysia and Thailand), typhoid fever manifests as a relatively mild illness with low fatality rates and minimal neurologic complications. In contrast, in sub-Saharan Africa and Indonesia, severe, and often, fatal disease is frequently seen with higher mortality and is often accompanied by neurologic involvement such as delirium and coma. The reasons for these differences in disease severity are not known but may be related to differences in health care facilities, host immune responses, genetic factors, and also perhaps to differences in strains of Salmonella typhi circulating in areas of endemicity. Few studies have been performed to determine if variations in clinical presentation are related to strain differences. Previous studies have, in fact, shown little correlation between strain characteristics and disease severity [11].

Chronic bacteriuria associated with typhoid fever

Bacteriuria due to *Salmonella typhi* usually occurs following recent typhoid fever or in chronic carrier states. Data from 18 patients with S. typhi bacteriuria, seen during 5 years, were analyzed. Fourteen patients had localized urinary tract infection due to *S. typhi*. Four others had bacteriuria, probably associated with typhoid fever. Localized abnormalities of the urinary tract and kidneys and also systemic diseases were found to predispose patients to *S. typhi* bacteriuria. Local abnormalities encountered included urolithiasis (n =53), prostatic hypertrophy (n=51), and tuberculosis (n=51). One renal transplant recipient and another with lupus nephritis had *S. typhi* bacteriuria. One had associated strongyloidosis, and another was pregnant [12]. Recovery of *S. typhi* from urine is a rare event, even in areas endemic for this infection (8–10%). *S. typhi* can be isolated from urine following a recent episode of typhoid fever, in chronic carrier states involving the urinary system, and occasionally following localized urinary tract infection (UTI) due to *S. typhi*. The former two conditions are likely to be asymptomatic. The last situation is extremely rare, tends to be chronic, and occurs in individuals with structural or functional abnormalities of the urinary tract [13]. Acute symptomatic

UTI is not a recognized manifestation of *S. typhi* infection. The information available currently on bacteriuria due to *S. typhi* is based on a few reports available on the role of *S. typhi* in causing UTI, that *S. typhi* is shed in urine following a recent typhoid fever as part of the natural history of this disease or in chronic carrier states [14]. Proteinuria and leukocyturia also present in those patients indicate local inflammation of the urinary tract. Microbiologically, *S. typhi* was isolated in large numbers from urine of all these patients. Therefore, it is logical to believe that the bacteriuria was due to local infection of the urinary tract by *S. typhi*. This UTI could have resulted from a previous unrecognized blood infection, especially in patients with fever. For patients who are chronic carriers of *S.typhi*, developing an acute UTI due to the same organism is another possibility. Whatever the reasons, bacteriuria was the only manifestation of *S. typhi* infection in these patients, and the infection was diagnosed because of the investigations done for UTI [15].

The symptoms, as in earlier reports, were related mainly to the lower UTI that patients had abdominal pain. Fever, the most consistent feature of typhoid fever with dysuria. When fever was present, there was no specific pattern [16].

Leukocyturia, proteinuria, and significant bacteriuria in all patients suggests local infection, probably microabscess formation in the kidney, rather than pure excretion. Conditions predisposing to this infection were also varied that up to 50% of patients with *S. typhi* UTI had urinary calculi [17].

Fifty-six percent had single episodes of acute UTI. This condition occurred both in patients with local abnormalities of the urinary tract and in those without such abnormalities. However, patients who develop *S. typhi* bacteriuria should be evaluated carefully for urinary tract abnormalities [18].

TABLE 1. Summary of findings for 18 patients with *S. typhi* bacteriuria

Characteristic	No. of patients (%)	Relevant features[a] (*n*)
Symptoms		
Dysuria	12 (67)	Abdominal pain (1)
Abdominal pain	3 (17)	
Fever	13 (72)	>1 mo (5), 1 wk–1 mo (5), <1 wk (3)
Laboratory findings		
Pyuria	17 (94)	
Proteinuria[b]	10 (91)	
Positive blood culture[c]	1 (14)	Pregnancy
$>10^5$ CFU of *S. typhi*/ml of urine	12 (67)	
Associated conditions		
Urolithiasis	3 (17)	Staghorn, multiple, ureteric
Bladder abnormality	3 (17)	BPH, tuberculosis, post-TUR
Renal	2 (11)	Lupus nephritis and posttransplant (both immunosuppressed)
Systemic	5 (28)	JRA, pregnancy, strongyloidosis, hypocalcemia following thyroidectomy, hepatitis

[a] BPH, benign prostatic hypertrophy; TUR, transurethral resection of prostate; JRA, juvenile rheumatoid arthritis.
[b] Not done for seven patients.
[c] Done for seven patients only.

Diagnosis

The diagnosis of typhoid is usually made in the developing world from clinical criteria. In areas of endemic disease, fever without evident cause that lasts for more than one week should be considered typhoid until proven otherwise. However, malaria, deep abscess, tuberculosis, amoebic liver abscess, encephalitis should also be considered for differential diagnosis. Over and above, the following complications of typhoid should be kept in mind as they are often confusing factors during diagnosis and treatment:

- **Abdominal**: Gastrointestinal perforation, gastrointestinal haemorrhage, Hepatitis, Cholecystitis (usually subclinical).
- **Cardiovascular**: Asymptomatic electrocardiographic changes, Myocarditis, Shock.
- **Neuropsychiatric**: Encephalopathy, delirium, psychotic states, cranial or peripheral neuritis, Guillain- barre syndrome, meningitis, impairment of coordination.

- **Respiratory:** Bronchitis Pneumonia (Salmonella enterica serotype typhi, Streptococcus pneumoniae).
- **Hematologic:** Anaemia, Disseminated intravascular coagulation (usually subclinical), thrombocytopenia, haemolytic uremic syndrome.
- **Others:** Focal abscess, pharyngitis, miscarriage, relapse, chronic carrier, influenza, dengue, leptospirosis, infectious mononucleosis, brucellosis, rickettsial diseases etc. should be considered [19].
- **Routine blood tests**: 15-25% patients show leucopoenia and neutropenia. Leucocytosis found in intestinal perforation and secondary infection. In younger children, leucocytosis is common association.
- **Liver function tests:** These may be deranged. Although significant hepatic dysfunction is rare, some studies and case reports showed there was hepatic derangement simulating acute viral hepatitis and also present as hepatic abscess.
- **Blood culture**: This is the standard diagnostic method; it is positive in 60 to 80 per cent of patients with typhoid. Culture of the bone marrow is more sensitive, around 80 to 95 per cent patients, even in patients taking antibiotic for several days, regardless of the duration of illness. Blood culture is less sensitive than bone marrow because there is lower number of organism in blood than bone marrow. The sensitivity of blood culture is higher in the first week of illness, increases with the volume of blood cultured (10- 15ml should be taken from school-children and adults, 2- 4 ml are required from toddlers and preschool children). Toddlers have higher level of bacteraemia than adult.
- **Other cultures**: The sensitivity of stool culture depends on the amount of faeces cultured, and the positivity rate increased with the duration of illness. Stool cultures are positive in 30 % of patients with acute typhoid fever. Urine culture have got 0-58% sensitivity.
- **Felix-Widal test**: The classic Widal test is more than 100 years old. It detects agglutinating antibodies to the O and H antigens of *S. enterica serotype typhi*. The levels are measured by using doubling dilutions of sera in large test tube [20]. Although easy to perform, this test has moderate sensitivity and specificity. Its reported sensitivity is 70 -80 % with specificity 80 -95 %.

It can be negative in up to 30% of culture proven typhoid fever, because of blunted antibody response by prior use of antibiotic. Moreover, patients with typhoid may show no detectable antibody response or have no demonstrable rise in antibody titre. Unfortunately, S. enterica serotype typhi shares these antigens with other salmonella serotypes and shares these cross-reacting epitopes with other Enterobacteriaceae. This can lead to false positive results. If paired serums are available a fourfold rise in the antibody titre between convalescent and acute sera is diagnostic. Considering the low cost of Widal test, it is likely to be the test of choice in many developing countries. This is acceptable, as long as the results of the test are interpreted with care, on the background of prior history of typhoid, and in accordance to appropriate local cut-off values for the determination of positivity.

- **New diagnostic tools:** ELISA test detect IgM and IgG antibodies, detect IgM and IgG antibodies against antigen of *S. typhi* which is superior to culture method in sensitivity (93%) and has high negative predictive value.
- **Recently DNA probes and polymerase-chain-reaction (PCR)** have been developed to detect *S. enterica serotype typhi* directly in the blood [21].

Treatment of typhoid fever

General management: Supportive measures are important in the management of typhoid fever, such as oral or intravenous hydration, the use of antipyretics, and appropriate nutrition and blood transfusions if indicated. More than 90% of patients can be managed at home with oral antibiotics, reliable care and close medical follow-up for complications or failure to respond to therapy. However, patients with persistent vomiting, severe diarrhoea and abdominal distension may require hospitalization and parenteral antibiotic therapy [22].

Antimicrobial therapy: The fluoroquinolones are widely regarded as optimal for the treatment of typhoid fever in adults. However, the emergence of MDR strains has reduced the choice of antibiotics in many areas. There are two categories of drug resistance: resistance to antibiotics such as chloramphenicol, ampicillin and trimethoprim-sulfamethoxazole (MDR strains) and resistance to the fluoroquinolone drugs.The so-called nalidixic-acid-resistant *S. typhi* (NARST) is a

marker of reduced susceptibility to fluoroquinolones compared with nalidixic-acid-sensitive strains. There have been also a few reports of resistance to third-generation cephalopsorins at the same time [23].

Ciprofloxacin, ofloxacin, perfloxacin and fleroxacin have generally proved effective. Chloramphenicol, despite the risk of agranulocytosis in 1 per 10 000 patients, is still widely prescribed in developing countries for the treatment of typhoid fever with high rate of relapse (57%) and the frequent development of a carrier state in adults [24]. Of the third-generation cephalosporins, oral cefixime (1520 mg per kg per day for adults, 100200 mg twice daily) has been widely used in children in a variety of geographical settings and found to be satisfactory [25]. Other agents, e.g. cefodoxime, have proved successful against typhoid fever [25]. Recent data on the use of azithromycin in children indicate that it may be safely given as an alternative agent for the treatment of uncomplicated typhoid fever [26]. Although there are no data indicating that azithromycin is unsafe for pregnant or nursing women [26].

Management of carriers

An individual is considered to be a chronic carrier if he or she is asymptomatic and continues to have positive stool or rectal swab cultures for *S. typhi* a year following recovery from acute illness. Overall, some 15% of typhoid fever patients become chronic carriers. The rate of carriage is slightly higher among female patients, with cholelithiasis. If cholelithiasis is present, the patient probably requires cholecystectomy in addition to antibiotics in order to achieve bacteriological cure. Clearance of up to 80% of chronic carriers can be achieved with the administration of ciprofloxacin twice daily for 28 days. A screening technique to identify carriers among food handlers and in outbreak investigations by detection of IgM antibodies are very high in chronic *S. typhi* carriers [27].

The Immunological State of Typhoid Fever

During the host cell-pathogen interaction, a cytokine burst occurs in order to recruit the cells of the innate immune system and enhance the defense against pathogens. In UTIs, cytokines are mainly produced locally in the uroepithelial cell lining of

the bladder and secreted into the urine. Generally, creatinine correlation of the cytokine content to compensate for different dilutions of the urine is not considered necessary [28]. Mostly, interleukin 1- beta (IL-1B), interIeukin-6 (IL-6) and interleukin-8 (IL-8) are studied. IL-1B could be a promising marker for differentiation between upper and lower UTIs [29]. Studies performed on different cytokines are shown in Table 4. IL-6 and IL-8 are expressed rapidly after getting into contact with pathogens. IL-6 not only recruits immune cells, but also initiates gene cascades in order to produce antimicrobial peptides [30]. Due to its key role, it can be a predictor for UTIs and a marker of differentiation. IL-8 has a central role in all inflammatory processes. Although its elevated concentration is observed in UTIs and can be a predictor of acute pyelonephritis, its specificity is low. It raises in every kind of congenital urinary anomaly, except antenatal renal pelvic dilatation. Thus, IL-8 is not suitable for diagnosing UTIs when an anatomical disorder is present [31].

MATERIALS AND METHODS

Patients and Samples: This study was carried out at the general hospitals in Babylon Province, Iraq. The study was included 41 patients with bacteriuria due to *S. typhi*, in addition to 32 with bacteriuria without *S. typhi* positive.

Inclusion criteria: Eligible patients should have chronic UTI with positive IgM and IgG of *S. typhi* as target group compare with patients have chronic UTI without IgM and IgG of *S. typhi* as control group.

Ethics statement: Both groups provided written informed consent prior to inclusion into the study. No obligations or interventions put to interfere with standard care of patients. The study was approved by the National Ethics Review Committee at University of Babylon which in accordance to the Declaration of Helsinki.

Demographic and clinical variables

Descriptive data for patients and control were collected by trained researcher following a structured questionnaire. Age, gender, residency, economic and social

states and body mass index (BMI), in addition to anemic state were added as questionnaire to all subjects (patients and control).

IL-6, TNF-α and Vitamin D levels

Inflammatory cytokines and cell differentiation indices were measured by ELISA technique (elabscience company, USA). IL-6 is by E-EL-H0192 kit, TNF-α by E-EL-H2305 kit, and vitamin D by E-EL-H2359 kit. According to the manufacturer's protocol; each assay was run with known standards (provided with the kit) that were used to determine the quantity of cytokines in each sample in pg/ml and VD in ng/ml.

Statistical analysis

Descriptive measures and T-test analysis were used to examine associations and differences in Demographic and clinical variables, biochemical, and immunomodulatory markers. The significance level for all analyses was set at a probability (*P*) of less than or equal to 0.05. All analyses were performed by GraphPad Prism 5.3 for Windows (GraphPad Software, San Diego, CA, USA).

RESULTS

Demographic and clinical variables

The demographic characteristics of all patients included in the study are summarized in Table 1. The age range was from 17 to 72 years with mean for cases and controls were 38.3 and 32.4 years, respectively. The majority of the patients were females (56%) and 71% among all study sample were resident in rural areas while only 29% of them were in urban areas ($P<0.01$). Regarding the socioeconomic state, 47% of them were living in middle socioeconomic state, 37% were in low socioeconomic state and only 16% were living in good state. Most of study sample are married (78%).

The majority of cases have TB disease for more than one year (61%), 32% of them have disease between 6-12 months and only 7% have disease for less than 6 months (data not shown). Regarding the body weight, about 90% of study sample have body mass index (BMI) lower than 25 (relatively low BMI). In addition to the history taking from patients, 39% of them had chronic anemia with statistically significance vs control ($P<0.05$) (Table 1).

Immunomodulatory Markers

There was highly increased in IL-6 and TNF-α levels in target group in comparison with control ($P<0.001$) (Figure 1 and Figure 2 respectively), moreover, VD level was significantly decreased vs control ($P<0.001$) (Figure 3).

DISCUSSION

Our experiments reveal a striking role of IL-6 in target group which *S. typhi* survives in resting macrophages. The macrophage is activated by cytokines to eradicate bacteria. Accordingly, even in immunocompetent individuals, *S. typhi* persists to grow and disease is developed after reactivation as a consequence of impaired immunity [32]. The previous studies suggested that different T-cell populations are required for protection against *S. typhi*. Th2 immune response has crucial role in progression of *S. typhi* infection while Th1 cells play a minor role in protection against typhoid fever. Further, a role of TNF-α in protection against tuberculosis has been demonstrated with cytokines as activated by macrophages that increase the resistance against *S. typhi*. The essential role of IL-6 in a protection against *S. typhi* as early immune response is to induce the inhibition of *S. typhi* growth with other cytokines like IL-1 and TNF-α, which are represented as potent proinflammatory cytokines. In another hand, IL-6 has been shown to promote differentiation of T cells especially to Th2 responses that stimulate cytotoxic functions with impaired Th1 cell development [33].

Table 1. Demographic variables of target and control groups.

Variables		**Target (n=41, %)**	**Control (n=32, %)**	***P value***
Age (yrs)	20-40	22 (54%)	15 (47%)	-
	40-60	13 (32%)	10 (31%)	-
	>60	6 (14%)	7 (22%)	-
Sex	Female	24 (59%)	17 (53%)	-
	Male	17 (41%)	15 (47%)	-
Residence	Urban	7 (17%)	14 (44%)	*P*<0.01
	Rural	34 (83%)	18 (56%)	-
Economic	Good	2 (5%)	10 (31%)	*P*<0.01
State	Middle	21(51%)	13 (41%)	-
	Poor	18 (44%)	9 (28%)	-
Social	Married	36 (88%)	21 (66%)	-
State	Non-Married	5 (12%)	11(34%)	*P*<0.05
BMI	16-20	12 (29%)	11 (33%)	-
	21-25	24 (59%)	12 (39%)	-
	26-35	5 (12%)	9 (28%)	*P*<0.05
Anemia		16 (39%)	9 (28%)	P<0.001

BMI: body mass index

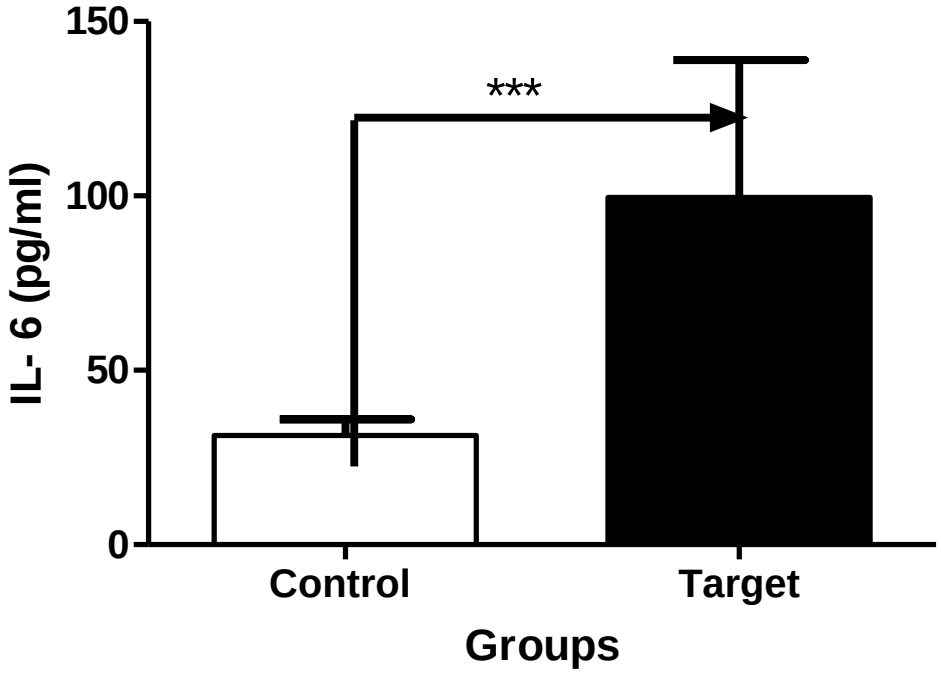

Figure 1: Variation in IL-6 concentration (pg/ml) among target and control groups. The number of asterisks (***) correspond to the level of the statistical significance (P < 0.001).

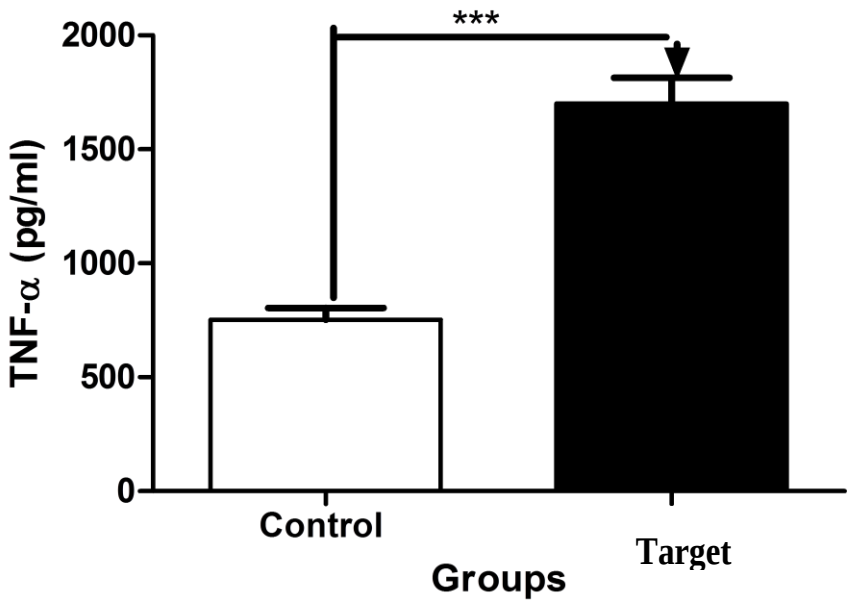

Figure 2: Variation in TNF-α concentration (pg/ml) among target and control groups. The number of asterisks (***) correspond to the level of the statistical significance (P < 0.001).

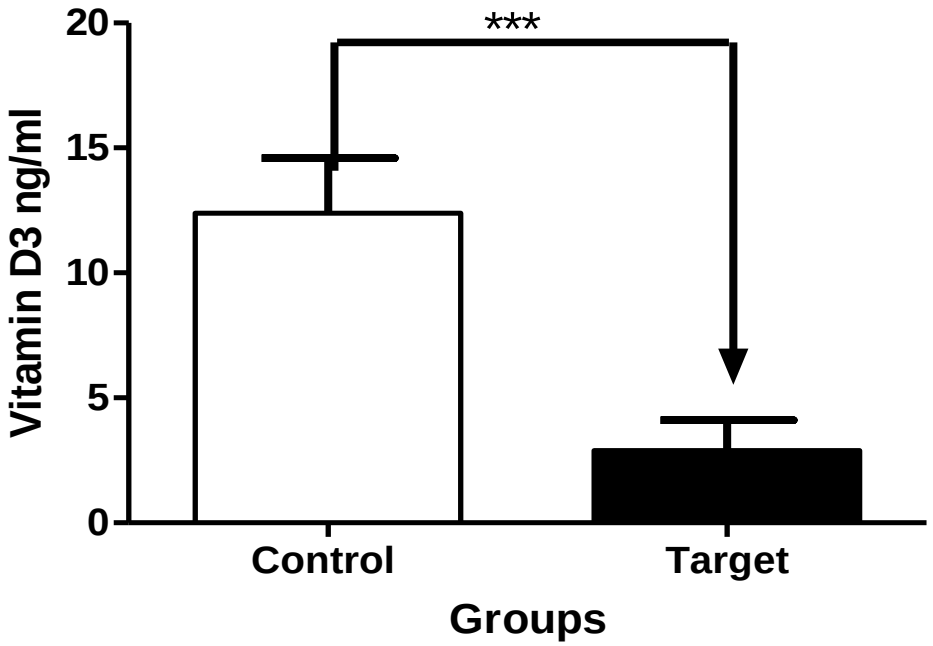

Figure 3: Variation in CD4 concentration (ng/ml) among patients with target and control groups. The number of asterisks (***) correspond to the level of the statistical significance (P < 0.001).

Similarly, the deficiency in VD are more obvious in latent MTB infection; the low VD in patients with typhoid fever have been reported that closely associated with clinical severity and more advanced of disease and the continuous decrease in VD level during treatment for typhoid fever is strongly associated with low response [34]. The VD level depletion is also related with wasting and weight gain with high proportion of BMI of patient's typhoid fever and cytokines level might also correlate with the degree of malnutrition leading to severe immune suppression state with peripheral blood lymphocytopenia [35].

The depletion in VD level and increase cytokines in peripheral blood could be due to pooling of T-cells to the site of infection, or due to hypermetabolism to typhoid fever [36].

In conclusion, our study has allowed correlating between the clinical variables with IL-6, TNF-α, and VD levels with some limitations in *S. typhi* diagnosis that was based only on sputum smear positive and we could not exclude the existence of other factors that might have an impact on the immunity state. Moreover, the study

might help to define the cytokines are critical marker to protect against *S. typhi* and facilitate the development of new immunologic means for prevention and therapy of *S. typhi*.

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