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Evaluation of TLR-2 sera levels in a sample of Iraqi pulmonary TB patients

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Abstract

The non-specific response of immunity has developed as the initial barrier for human protection from invading pathogens, which comprises certain pathogen recognition receptors (PRR) for instance toll-like receptors (TLRs). Toll like receptor 2 (TLR 2) is capable of recognizing pathogen associated molecular patterns (PAMP) coded by *Mycobacterium tuberculosis*. To evaluate TLR 2 level in sera of pulmonary tuberculosis (TB) patients. About 120 subjects, involving 80 patients with pulmonary TB including 40 multiple drug resistance (MDR), 20 recently diagnosed pulmonary TB (RD) and 20 recurrent TB patients named as old cases (OC), in addition to 40 apparently healthy individuals were studied as control group. Sera from 68 TB patients and 20 healthy controls were obtained for measuring TLR 2 levels by enzyme linked immunosorbent assay (ELISA). The present result revealed that serum levels of TLR 2 showed there is no significant differences between each patient's group and control except OC group given that, has decreased significantly ($p<0.05$). The mean ±SD of TLR 2 level in MDR, RD, OC and controls were 4.0± 2.4, 3.4±1.4, 2.2±0.9 and 4.1± 3.0 ng/ml, respectively. The results exhibited that during treatment of tuberculosis, patients with pulmonary tuberculosis showed elevated TLR 2 concentration, which looks probably in charge of regulating inflammation and infection. Consequently, this study proposes that killing of MTB could occur in the time of disease management because of effective treatment, in addition to activation and releasing of different immune system mediators via TLR 2.

Keywords: TB; TLR 2 ; TLR 2 serum level; ELISA.

تقييم مستويات TLR 2 في مصول مرضى التدرن الرئوي العراقيين

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الخلاصة

تعتبر المناعة الطبيعية الخط الدفاعي الاول ضد الاحياء المجهرية، والتي تتضمن مستقبلات خاصة لتمييز الممرضات TLR. TLR-2 قادر على تمييز انماط الجزئيات الخاصة بالممرضات والتي تعبر عنها بكتريا التدرن الرئوي. لذلك هدفت هذه الدراسة للتحري عن تأثير تركيز TLR 2 في مصول مرضى التدرن الرئوي العراقيين والسيطره. شملت هذه الدراسة 120 شخص عراقيا عربيا وتوزعوا الى مجاميع كما ياتي: 80 مريضا مصاب بالتدرن الرئوي (20 شخصا منهم مشخصين حديثا، 20 مريض مشخصين سابقا(اصابة متكررة) ، 40 مريض مقاوم لادوية متعددة بالاضافة الى 40 شخصا من الاصحاء ضاهريا كمجموعة سيطرة. تم جمع عينات من 68 مريضا و 20 من الاصحاء (السيطرة) لغرض قياس تركيز الواسم TLR-2 في المصل باستخدام بتقنية الامتزاز المناعي المرتبط الانزيم. اظهرت تراكيز الواسم TLR-2 في المصل تغايرات غير معنوية بين مجاميع المرضى والسيطره عدا مرضى التدرن المشخصين قديما حيث ظهروا انخفاظا معنويا ($p< 0.05$) في تركيز الواسم بالمقارنة مع السيطرة. كانت مستويات الواسم TLR-2 لمجموعة التدرن المقاوم، المشخص حديثا، متكرر الاصابة والاصحاء (4.0± 2.4, 3.4±1.4, 2.2±0.9 and 4.1± 3.0 ng/ml) على التتابع. اظهرت نتائج الدراسة الحالية انه خلال العلاج، يكون هناك زيادة

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بتركيز الواسم TLR 2لدى مرضى التدرن الرئوي والذي يظهر كمسؤول عن تنظيم الاصابة والالتهاب. لذلك ممكن يكون هناك قتل لبكتريا التدرن خلال فترة العلاج نتيجة للتاثير المباشر للمعالجة وكذلك تنشيط العديد من وسائط الاستجابة المناعية من خلال الواسم . TLR 2

Introduction

Tuberculosis (TB) is a contagious disease with chronic progress, and its causative agent is the intracellular bacterium *Mycobacterium tuberculosis* [1]. Toll-like receptor 2 (TLR 2) is the principal receptor for MTB constituents, distinguishing lipoarabinomannan its originator, phosphatidylinositol mannoside and 19-kDa lipoprotein [2].

Studies have revealed that the recognition of mycobacterial products by TLRs causing NF-kB stimulation and accordingly to gene transcription that yields pro-inflammatory cytokines, such as IL-12, TNF-α, IL-1 and nitric oxide [3].

The recognition of mycobacteria by TLRs enhance phagocytosis by alveolar phagocytes and IL-12 production by macrophages and dendritic cells. IL-12 causes natural killer cells and Th1 responses that leads to IFN-γ release [4, 5].

TLRs are expressed by immune and non-immune cells differentiate pathogen associated molecular patterns on microorganisms. Signal that produced from toll like receptor activating affords a fast protective response against bacteria [6]. The interfering between *M. tuberculosis* or other pathogen with the TLRs has been investigated in both human and mice. In respect to TLR 2 , researches have revealed that infection with MTB causing increased TLR 2 regulation in tuberculosis patients monocytes [7].

M. tuberculosis causes increase of Toll Like Receptor 2 ligands expression, for example 19-kDa lipoprotein and the LprA lipoprotein. The interference between toll like receptor 2 and 19-kDa lipoprotein, LprA of mycobacteria, or live *M. tuberculosis* cause numerous consequences on the TLR 2 morphology and functional expression regulation by macrophages and dendritic cells (DCs), for instance, the expression of HLA-DR in response to interferon gamma [8].

Furthermore, more reports have focused on the function achieved by TLR 2 in non-specific immunity in tuberculosis patients. Whether *M. tuberculosis* may modify the response to acquired immunity through TLR 2 directly, still unclear. In definitely, the results that derivatives of *M. tuberculosis* and TLR 2 ligands have on expression of TLR 2 by T helper $CD4^+$ cells and their clinical outcomes need additional investigation [9].

TLR 2 is taken part in immunity against infection of MTB. The capability of toll like receptor 2 to contribute in the white blood cells triggering by MTB and different responses mediation activated by these cells propose that protein of TLR may work discrete functions in the mycobacteria immune responses [10]. A study showed that TLR 2 existence is unessential for increasing sufficient inherited resistance to aerobic infection with *M. tuberculosis* [11].

A role of toll like receptor signal in host resistance to *M. tuberculosis* is extra reinforced by the observation that mice lacking in MyD88, a main adaptor molecule essential for signaling actions by most TLR/IL-1R family participants that improved predisposition to pathogenic aerosol infection [12]. Numerous studies explored the main role of MyD88 in resistance to mycobacteria, but it was hard to explore this role to the function of a TLR signal. Thus, in mice lacking in TLR 2 although in some cases exhibiting specific defects in immune response to mycobacteria, which display only slight rises in predisposition to low dose aerogenic challenge [11, 13]. This vulnerability is supplemented by changes in pro-inflammatory cytokine and nitric oxide synthase 2 expression and the response to pulmonary granulomatous [14].

The machinery of soluble form of toll like receptor 2 (sTLR 2) making comprising: endocytosis of the cell surface receptor, alteration into sTLR 2 by exocytosis in an internal acidic compartment, and sTLR 2 releasing by exocytosis successively, or its maintenance in an intracellular pool [15]. In spite of the up-modulation of sTLR 2 subsequent cell activation *in vitro*, in vivo contact to *M. tuberculosis* which recognized at least in part by TLR 2 leads to an obvious down-modulation of serum sTLR 2 [16].

The purpose of the current study is to assess the effect of TLR 2 level in sera of pulmonary tuberculosis (TB) patients on TB infection in three categories of patients, which are newly diagnosed, recurrent cases and multiple drug resistant.

Materials and Methods

Study subjects

The research comprised of 80 patients 50 males and 30 females aged from 14 to 76 years, classified as 20 recently diagnosed patients, 20 recurrent TB infection (old cases) and 40 multiple drug resistant patients (MDR), who were undergoing anti-TB treatment at the national reference laboratory of tuberculosis (Baghdad, Iraq) during February 2017 - July 2017.

The exact condition for reception was existence of at least one of: (i) positive gene Xpert MTB/RIF result for sputum samples which reflected as more sensitive and more specific tool for MTB diagnosis and identifying its resistance or sensitivity to rifampicin. (ii) Medical and X-ray examination demonstrating pulmonary tuberculosis and one positive result culture for *M. tuberculosis* as possible for three discrete sputum analyses. (iii) Clinical and radiological finding specifying enhancement in supposed patients tuberculosis pulmonary type with practical management. Acquired immune deficiency syndrome (AIDS) patients or those having immunosuppressive drugs were excluded.

The control subject comprised of 40 of apparently healthy individuals 20 males and 20 females aged from 21 to 51 years, employed from the University of Anbar and Usul Al-din University College. The precise rules of sampling were the lacking of lung lesions on X-ray of chest with no history of TB disease. All the people of both the patients and control are citizens of Iraq.

This study supposed that Iraqi people have common ways of contact to mycobacteria, since the styles of its spread are chiefly through large air droplets and small droplet nuclei particles. The review institutional board approved this study, and recorded learned agreements were taken from all subjects before blood collection.

TLR 2 detection by ELISA procedure

Serum levels of human Toll Like Receptor 2 (TLR-2) level in serum was determined by using ELISA kit, Cat No. MBS26890/ Mybiosource/ Canada according to manufacture instructions at University of Baghdad/College of Sciences/ Biology department.

Statistical analysis

All statistical analyses were conducted using statistical package for social science (SPSS) program version 17 (SPSS INC., Chicago IL, USA). Results were presented as mean ± SD. ANOVA, t-test, the least significant difference ($LSD_{0.05}$) and correlations coefficient (r) were used to analyse the results and comparisons between two groups. Differences were considered significant when $P \leq 0.05$ [29].

Results

Serum level of TLR-2

The results of current study showed non-significant differences between patients and control groups. However, old cases group was decreased significantly ($p<0.05$) in comparison to control group. The TLR 2 serum levels mean ±SD for MDR, RD, OC and controls were 4.0± 2.4, 3.4±1.4, 2.2±0.9 and 4.1± 3.0 ng/ml, respectively, as shown in Figure-1.

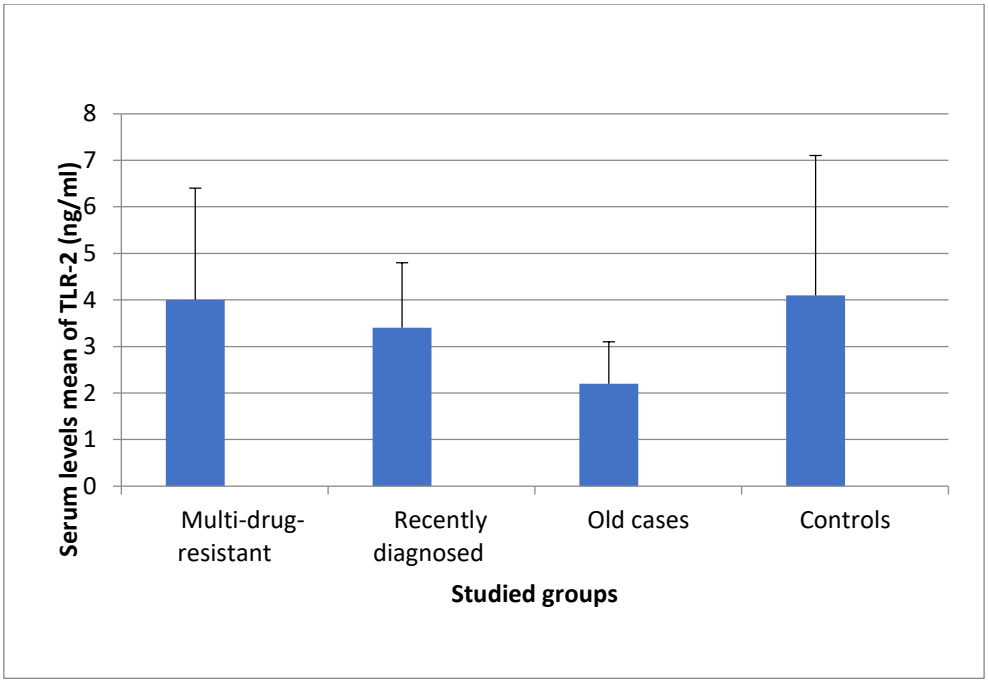

Figure 1-mean level of TLR-2 in TB patients and control.

Patient's distribution by age

As illustrated in Figure-2, there is no significant difference in the TLR 2 serum concentration within age classes (< 40 years and ≥ 40 years) in patients and control groups. The mean ± SD of TLR 2 levels in MDR, RD, OC and control less than 40 years were 4.0± 2.8, 3.1± 1.1, 2.2± 0.6 and 3.1± 2.0, respectively, while more than ≥ 40 years were 3.9± 1.7, 4.3±1.8, 2.14± 1.2 and 5.1± 3.5, respectively.

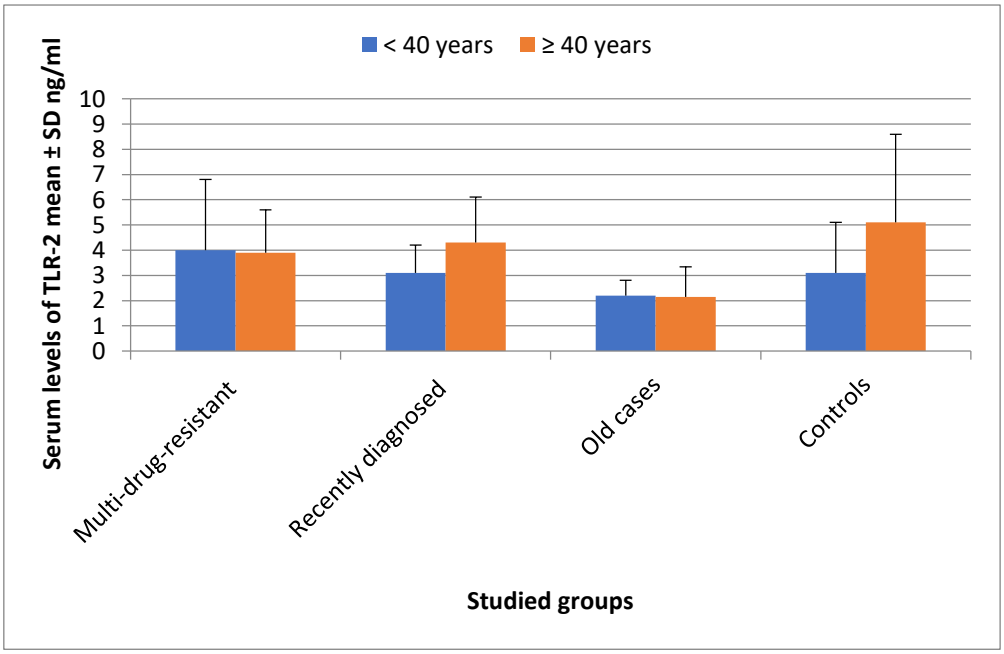

Figure 2-mean levels of TLR-2 in TB Patients and control distributed by age.

Patient's distribution by gender

As shown in Figure-3 the variation in serum TLR 2 levels between MDR and RD were not statistically significant. While the opposite picture was shown in OC patients and control (P< 0.05). The serum levels of TLR 2 mean ± SD in MDR, RD, OC and control male were 4.3 ± 2.8, 3.6± 1.6,

2.6± 0.6 and 4.9 ± 3.3, respectively, while in female were 3.4± 0.8, 3.3±1.2, 1.0 ± 0.9 and 2.6 ± 1.3, respectively.

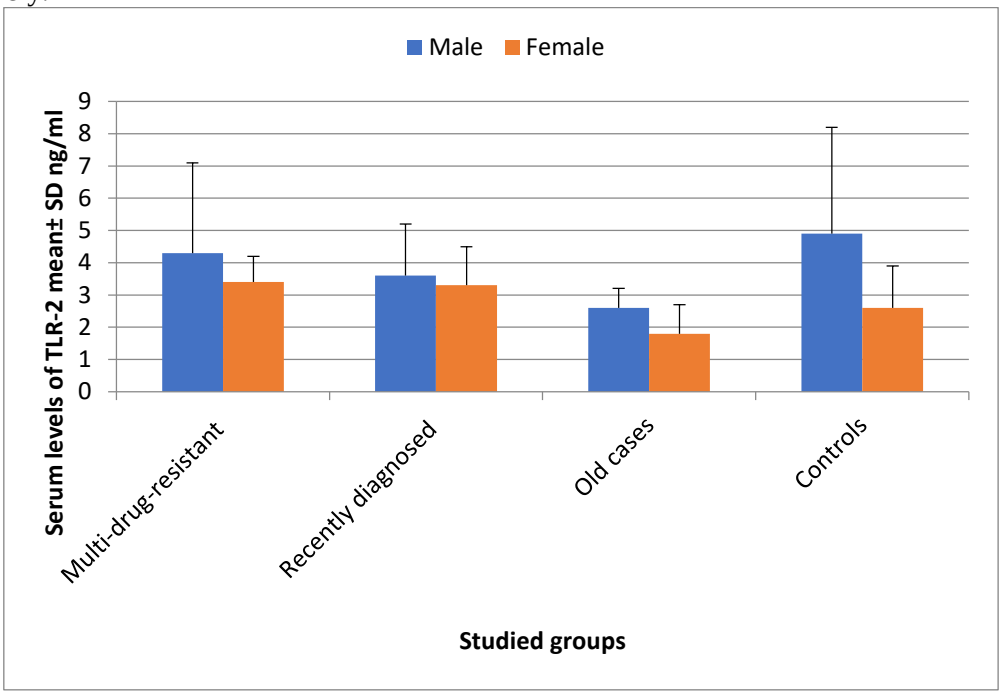

Figure 3-mean levels of TLR-2 in TB Patients and control distributed by gender.

Discussion

To our best knowledge this is the first local study evaluated the serum level of TLR 2 in pulmonary TB patients; recently diagnosed, recurrent TB infection, and in multiple drug resistance. We revealed that in recurrent TB patients, TLR 2 decreased significantly ($p<0.05$) than control.

Studies showed that for the recognition of various constituents of whole bacteria, numerous TLR may be wanted, and diverse cellular responses may depend on diverse TLRs. For recognition of mycobacteria, both TLR 2 & TLR4 are involved [17].

TLR signaling has been assumed to have a key role in the host resistance regulation to *M. tuberculosis* [13]. There are as well as other mycobacteria species comprise well-characterized TLR ligands that are strong *in vitro* stimuli of a number of cytokines of pro-inflammatory response, such as interleukin 12 (IL 12) and tumor necrosis factor (TNF) [18]. Another study conducted by Reiling *et al*. validated that TLR 2 presence is unnecessary for originating adequate non-specific immunity for aerobic *M. tuberculosis* infection [8]. Furthermore, it was found that derivative TLR 2 agonists of *M. tuberculosis* inhibit antigen processing and MHC II expression on macrophages [19]. Signaling via TLRs was linked for increasing specific immune responses [20]. Tuberculosis as a chronically persistent infection controlled by active predominantly mechanisms such as releasing of perforin which may be under TLR control [21].

A study of LeBouder *et al*. revealed that blood monocytes synthesize soluble forms of TLR 2, and in high level into human plasma and breast milk, the sTLR 2 releasing kinetic indicates increasing during cell activation [22]. In addition, they found that sTLR 2 reduction from serum leads to high responses of cells to bacterial lipopeptide, the TLR 2 ligand. They suggested that sTLR 2 has a role in immune regulation. Particularly, reduction of serum sTLR 2 was noticed in tuberculosis patients and these results agreed with the current study results. Lowering of sTLR 2 from serum increases sensitivity of cells to lipopeptide. Moreover, sTLR 2 liberating is modulated *in vitro* and *in vivo* by cellular activation & infection of MTB, respectively, and these sTLR 2 is able to cell activation modulation by bacterial lipopeptide. In contrast to the sTLR 2 up-modulation, after cell activation *in vitro*, *in vivo M. tuberculosis* exposure, which known at least in part by TLR 2 , caused in a noticeable down-modulation of serum sTLR 2 .

Such results may propose that the age is not risk factor that affect the serum level of the TLR 2 ; however, attention must be reflected in understanding these results because the sample size in patients

and controls may not allow a strong conclusion. In addition, age can be regarded as an effective factor when we have deal at age more than 60 years, because it is often there is immune functions dysregulation, and health decline and increased sensitivity to various infectious diseases are linked with ages advance [23], as well as the significant role of TLRs in immune response of host to TB at initial stage [24].

Unluckily, the patients and controls number in the present study at age older than 60 years could not permit a reliable statistical analysis, and therefore they were distributed into < 40 and ≥ 40 years. Accordingly, further studies are certainly required to shed light on this matter, and larger sample size and age group range has to be considered. The cellular variations during elderly are partly verified; however, dysfunction in host immunity is associated in the age-linked decline in health. The acquired immune response shows a functional weakening in capability to reply to new infections though serum cytokines levels are elevated with age. In spite of these age-related increases in immune cytokines, function reduction of aged macrophages. TLRs are critical in the macrophage response to pathogenic infection. Cross linking between the non-specific immune system and the acquired immune system via common receptors taken place. TLR assisted beginning of T cell and B cell responses increases progress of the acquired immune response, hence age related dysfunction of TLR might actually influence on the efficacy of both the inherited response and adaptive immunity [25].

In study of Scotland *et al*. they revealed that macrophage of female peritonea have TLR 2 expression significantly high, as well as TLR4 and TLR3 that recognize many microbes including different bacteria, yeast, viruses as well as damage associated molecular pattern (DAMPs) that elicited by injury cooperatively [26]. The raised TLRs expression on female tissue macrophage means that these cells have a remove and sense ability for pathogens, as demonstrated by their capability to ingest zymosan particles (TLR 2 agonist) effectively. There is no doubt, peritoneal female and male macrophage contact *in vivo* to live bacteria (by intraperitoneal uptake of GBS) that cause TLR activation generating sepsis less severely and low females bacteremia. These findings were linked with humoral responses to the strange antigenic challenge, and the proposal was that sex hormones might impact immune functions [27]. Immune resistance capability has revealed alterations between males and females, males gender are found to be more susceptible to infections, while females are at superior risk to progress autoimmune diseases [28].

In this study, the serum levels of TLR 2 in TB patients and control groups were higher in male than female, which is reverse to previous study, the sample size may be supports these findings[29].

Conclusions

This study found that during anti-TB management; pulmonary TB patients exhibited increased TLR 2 concentration, which looks possible in charge of regulation of infection and inflammation. So, these results propose that during anti-TB treatment, mycobacteria killing could happen due to therapy, which acts directly as well as the stimulation of mediators of the immune response through TLR 2 which are more and effective.

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Abbreviations

TLR 2: Toll like receptor 2; TB: tuberculosis; IFN: interferon; MTB: *Mycobacterium tuberculosis* bacteria; GBS: Guillain-Barré Syndrome, ELISA: enzyme linked immunosorbent assay, DAMPs: Damage associated molecular pattern.

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