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Validity of Serum Toll-Like Receptor-2 (TLR-2) in Patients with Type 1 Diabetes Mellitus

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Abstract:The role of the Toll Like Receptor (TLR)/innate immune activation in type 1 Diabetes Mellitus (T1DM) is somewhat controversial. The aims of this study were to determine the serum levels of TLR-2 in apparently healthy subjects' sera and T1DM patients and its efficacy as a specific diagnostic marker. Thirty T1DM patients and ten apparently healthy subjects were included. Serum level of TLR-2 was detected by sandwich ELISA technique. Results demonsrate that the mean age is 40.1±16.4 and 27.2±7.5 years for T1DM cases and healthy group respectively. The data of the present study revealed a simple difference between groups regarding ages, since there was no significant differences in serum TLR-2 mean value between these groups (p=0.35); therefore this study dealt with these groups as T1DM group in general regarding serum TLR-2. The difference in the mean of serum TLR was slightly higher in T1DM group (1.46) in comparable with healthy controls (1.07). The small area under the Receiver Operating Characteristic (ROC) curve (ROC area=0.61) which is not significantly different from the 0.5 ROC area related with an equivocal test. Between each male and female within the cases there was no obvious or statistically significant difference ($p \geq 0.85$) in mean serum TLR with 1.49, 1.42 respectively.
Albeit, not significant statistically, this study demonstrated a noticeable increase in serum TLR among DM cases compared to healthy controls. One reason behind such observation is that there is a decline in count of cells that carry the TLR receptors among diabetics, which will ameliorate the increase in TLR related with the disease. Furthermore, the contrasting roles of inflammation in T1DM may thus be accounted the capacity of innate pathways to initiate the both immunity by *B* cell damage and immunoregulation.

Key words: TLR-2, type 1Diabetes Mellitus, Validity.

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Introduction

Type 1 diabetes mellitus (T1DM) is an organ specific autoimmune disease causing by T cell mediated damage of insulin generating pancreatic beta cells. The progress of T1DM favors genetically predisposed individuals (1). Reports showed that about 285 million patients in the world affected with diabetes mellitus and the incidence may be doubled by 2030 (2). Diabetes mellitus is more common in Asia and Africa (3).

Toll-like receptors (TLRs) are a group of glycoprotein located mostly in the cellular membranes and represent a sense danger signals, acting as a key molecules in bridging innate and adaptive immune responses (4, 5, 6)

Additional environmental factors (infections) is important in the progress of this disease which has been reported and it`s role in triggering or preventing T1DM is not understood (7). However, this finding contrast with other studies and the role of TLR/innate immune activation in T1DM is somewhat controversial. Hence, additional studies are needed to elucidate the role of TLR activation (1).

Olivieri *et al*. (2013) reported that the age-related changes phenomenon is moderately assumed.

The acquired immunity during aging display a drop in ability of the body to resist the infection, while serum levels of inflammatory cytokines are improved with age. The source of age-related systemic chronic inflammation, named inflammation, was primary attributed to the progressive activation of immune cells over period named inflammaging (8).

The mechanism of this disease was begun when the inflammatory procedures induced upon anti-infectious immunity allow the presentation of β cell antigens directly to the T cells auto-reactive. Pro-inflammatory cytokines cause the up-regulation of class I major histocompatibility molecules (MHCI) on β cells, and may thereby "unmask" them for recognition by CD8+ T cells (9). In addition, affiliated destruction to β cells with motivation of Antigen-Presenting Cells (APCs) by the infection may simulate the presentation of β cell antigens to these cells like CD8+ T cells (10).

Finally, the relationship between TLR activation and individual host immune response signatures is a key for understanding autoimmune disease risk factors and pathogenesis. This knowledge will facilitate the progress of overall and individualized therapeutic strategies to better prevent and manage autoimmune disease (1).

Materials and Methods

Patients Study Groups

Thirty T1DM patients with age range 17-76 years were randomly divided into three groups contain age (<35, 35-49, 50>) attending Baghdad Medical City Teaching Hospital, during the period from December 2011 to July 2012.

Ten apparently healthy were chosen as healthy control groups respectively. For these 2 study groups, the blood samples were collected to evaluate the serum level of Toll like receptor-2 by using sandwich ELISA technique.

Kits and Reagents

Toll like receptor-2(TLR-2)

Elisa kit was a sandwich enzyme immunoassay for in vitro quantitative measurement of TLR-2 in human serum, plasma and other biological fluids (Toll like Receptor -2, 3013).

Statistical Analysis

Analysis was computer aided using IBMSPSS version 21computer software (IBM Statistical Package for Social Sciences). ROC analysis was used to calculate the magnitude of difference between cases and controls attributed to serum TLR (11).

Results and Discussion

The results presented here were based on the analysis of a random sample of 30 T1DM and 10 healthy control individuals. The data of the current study revealed a simple difference in the

mean of the TLR-2 serum level among age group cases with DM, since there was no statistically significant differences in serum TLR-2 mean value between these 3 groups (p=0.35) (Table 1); therefore this study was dealt with these 3 groups as DM cases in general regarding serum TLR-2.

Table 1: The difference in mean TLR concentration by age group among cases with type 1 Diabetes Mellitus

	Age group (years)			
TLR2 concentration	**<35**	**35-49**	**50>**	**P (ANOVA)**
Range	(0.287 - 3.079)	(0.613 - 4.699)	(0.44 - 2.9)	0.68(NS)
Mean	**1.28**	**1.54**	**1.67**	----
SD	0.84	1.21	0.9	----
SE	0.233	0.384	0.341	----
N	13	10	7	----

No positive trend is detected, even after multiple linear regression was used the case-control differences after regulating for age will remain not significant.
The ranges, mean value and standard deviation of each the age and TLR concentration in diabetic mellitus patient and control groups are shown in table 2.

Table 2: The case-control difference in TLR means concentration by age groups among controls and T1DM group

	Study group		
Age (years)	**Controls**	**Cases (DM)**	**P (t-test)**
Range	(12 - 37)	(12 - 76)	0.02
Mean	**27.2**	**40.1**	----
SD	7.5	16.4	----
SE	2.38	2.99	----
N	10	30	----
TLR conc.			0.24(NS)
Range	(0.361 - 2.231)	(0.287 - 4.699)	----
Mean	**1.07**	**1.46**	----
SD	0.58	0.97	----
SE	0.183	0.177	----
N	10	30	----

The cases group was equally distributed among males and females with a mean age of 40.1 years (+/-16.4). The healthy control groups had a mean age of 27.2 years (+/-7.5), table 2.

As shown in (table 2), the mean (1.46) TLR was slightly higher in cases with age range (12 - 76) compared to healthy controls (1.07) with age range (12 - 37),

but the difference was too small to be of statistical significance ($p \geq 0.24$).
The effect of Type 1 Diabetes Mellitus in causing elevation in serum TLR2 concentration was of very small magnitude as shown by (figure 1,2) the small area under the ROC curve (ROC area=0.61) which is not significantly different from the 0.5 ROC area associated with an equivocal test.

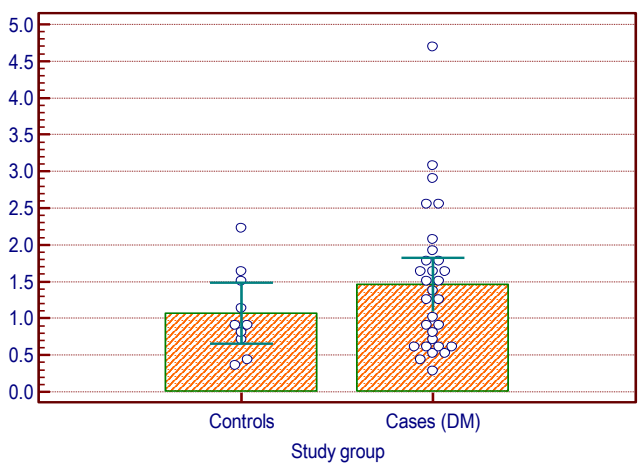

Figure 1: Dot diagram with error bars showing the case-control difference in mean (with its 95% confidence interval) serum TLR2 concentration.

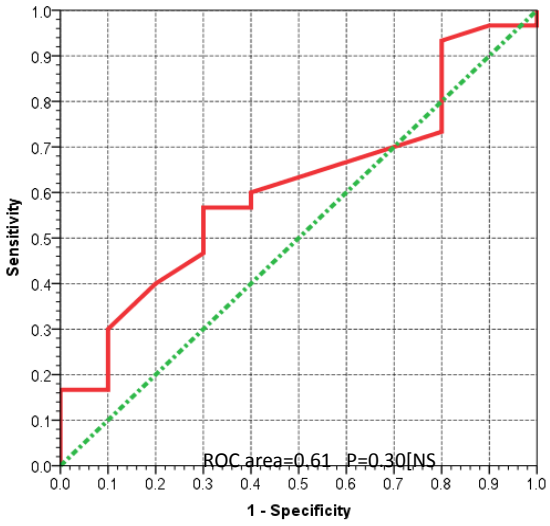

Figure 2: ROC curve showing the tradeoff between sensitivity (rate of true positive test results) and 1-specificity (rate of false positive) test results for serum TLR2 concentration when used as test to diagnose DM differentiating it from healthy controls.

Among the cases group there was no obvious or statistically significant difference in mean serum TLR between males (1.49) and females (1.42),with ($p \geq 0.85$) as shown in (table 3).

Table 3: The difference in the ranges, mean value and standard deviation of the serum TLR-2 in blood of diabetic mellitus patient either (male or female) among cases with DM.

	Gender		
TLR conc.	**Female**	**Male**	**P (t-test)**
Range	(0.613 - 2.555)	(0.287 - 4.699)	0.85(NS)
Mean	**1.42**	**1.49**	----
SD	0.66	1.23	----
SE	0.171	0.318	----
N	15	15	----

No positive trend is observed, Even when multiple linear regression was used the case-control differences after adjusting for gender will remain not significant ($p \geq 0.85$).

There was no statistical significant differences in serum TLR-2 mean value between 3 groups of cases age ($p=0.35$); therefore this study dealt with these groups as T1DM group in general regarding serum TLR-2.

The cellular modifications during ageing are moderately assumed; however immune system dysfunction is concerned with the age-related weakening in health and the function of elderly macrophages is declined, there is a signal for weakened downstream signaling actions, including activation of negative and inhibition of positive modulators of TLR induced signaling events (12).

In this point the present study ($P = 0.35$) be in agreement with Olivieri *et al*. (8) in their conclusions which reported that the Toll-like receptors and co-effecter molecules do not illustrate a reliable age-dependent change through the perfect systems, one can see that there are no association between groups of ages and the concentration of the TLRs (Table 1).

The current study showed that the mean of serum TLR was somewhat higher in cases (1.46) compared to healthy controls (1.07), with the difference was too small to be of statistical significance ($p \geq 0.24$). Serum levels of TLR-2 in this study is in agreement with further studies like that of Wen et al (13) who suggested that Toll-like receptors-2 and also MyD88 was responsible for occurence of T1DM.

As appear in Dot diagram the case-control difference in mean of serum TLR2 concentration; the outcome of TLR2 in causing T1DM consequence and then elevation in serum TLR2 concentration was of a small enormity. Furthermore, the small area under the ROC curve (ROC area=0.61) which is not significantly different from the 0.5 ROC area associated with an equivocal test.

The type 1 diabetes mellitus onset is related to the autoimmune process proceeding such as the exhaustion of memory CD4+ cells and the imperfect natural killer activity could briefly weaken host resistances against many infectious illnesses (14).

Low level of the complement factor 4, decreased the cytokine response after stimulation in humoral innate immunity have been defined in diabetic cases. In the other hand, like in cellular innate immunity many studies show decreased functions of phagocytosis, chemotaxis,

diabetic macrophages and killing of diabetic polymorphonuclear cells comparing with cells of controls (15). These findings here were disagreed with studies conducted by Filippi and von Herrath (7) who reported that the inflammation and infections in humans might protection a defensive role in T1DM; especially, in NOD mice the disease can be prevented by infection with a number of viruses (13).

However, this finding contrasts with other studies, and the role of TLR/innate immune activation in T1DM is somewhat controversial, Studies have linked TLR2 mediated signaling as a contributing factor to the induction of diabetes. For example, secondary necrosis in apoptotic beta-cells activated APCs through TLR2 signaling. Activated APCs in turn primed islets specific CD4+ T cells which contributed to the initiation of T1DM (1).

The alteration in TLR activity with gender, age, and immunosuppressive agents is still essentially unknown. This study (P = 0.35) was in agreement with the result of Khan et al. [16] who reported that the TLR activity did not modify significantly between the ages of 2 and 67 years. This may be due to genetic causes like that of "MicroRNAs (miRNAs), a class of gene for immune system, regulated by direct targeting the components of the TLR signaling system, activation of TLRs pathway and finally, direct activation of the RNA-sensing TLRs, like TLR-8, in humans"(16). During aging TLR signaling is vary by this gene, and impaired miRNAs/TLR signaling interaction in immune system cells. Interestingly, the weakening appears enhanced in presence of the age-related diseases, such as cardiovascular diseases, diabetes, and cancers (8).

On the other hand, this study (p= 0.85) was in agreement with the study of Khan *et al.* (16) who reported that the TLRs activities were not significantly different between the genders, But also, this study suggests that sex differences in TLR responses may elucidate that the female dominance to many autoimmune disorders. Furthermore, a considerate the special effects of immunosuppressive mediators on TLR-pathway activity should allow more focused treatment for autoimmune complaints.

Generally, variety of the equilibrium between immunoregulation and autoimmunity, and thus prevention of T1DM, might rely on the double function of the innate immune players involved in the disease and also, depending on timing and whether β cell antigens are existed, TLR-mediated special effects will differentially move the progress of autoimmunity (7). As, TLR2 signaling, activation of APCs/T cells and production of inflammatory cytokines each functions may lead to promote autoimmune processes after β cell antigens are present the other hand may appear to counter autoimmunity by improving and stimulating CD4+CD25+ Tregs and conferring DCs with tolerogenic possessions (17).

The Treatment of prediabetic patients' with a synthetic TLR2 agonist diminished T1DM and amplified the number and function of CD4+CD25+ Tregs, also conferring dendritic cells (DCs) with tolerogenic properties. TLR2 ligation also encouraged the extension of Tregs upon culture with DCs and improved the proses of protection from the disease (17).

Finally, the current study identifies new mechanisms by which TLR2 helps immunoregulation and controls autoimmune diabetes in healthy or

diseased hosts. And also, should assistance to appreciate T1DM causes and grow novel immunobasic therapeutic interferences (17).

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