DETECTION LEVELS OF TLR2 WITH BACTERIAL SPECIES IN UTIS PREGNANT WITH AND WITHOUT DIABETES

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ABSTRACT

Gestational diabetes mellitus (GDM) or GDM (gestational diabetes mellitus) is a dangerous obstetric condition that affects 5–10% of pregnancies around the world and Any degree of glucose intolerance that begins or manifests during pregnancy is referred to as gestational diabetes. It's characterized by a lack of insulin response to compensate for pregnancy's insulin resistance. 159 urine and blood samples were collected for pregnant women suffering from urinary tract infection with and without gestational diabetes during periods. The study included the collection of a urine sample and a blood sample from pregnant women with ages ranging from (45-15) years from the office of the gynecological consultant at Salah Al-Din General Hospital in the city of Tikrit as well as from the evening interventions and women’s clinics under the supervision of the doctor and between December / 2020 until February / 2021. Vitek 2 Compact System used for detection bacterial species and ELISA technique used to assess TLR-2. Results of our study show the bacterial isolates Staphylococcus aureus and Klebsiella pneumonia recorded highest percentages in UTIs pregnant with DM (36.36% and 27.27%) and without DM (25% and 21.87%) respectively. E. coli scored low percentage in UTIs pregnant with DM (13.64%) than without DM (28.13%). TLR-2 is non-significant positive correlation with Age (r= 0.101), Pregnancy time (r=0.171) and Pus cells (r=0.355) in UTIs pregnant without DM. TLR-2 is non-significant positive correlation with Age (r= 0.087), and non-significant negative correlation with Pregnancy time (r=-0.308) and Pus cells (r=-0.001) in UTIs pregnant with DM. Conclusions of our study showed Staphylococcus aureus and E. coli are more prevalence in pregnant with UTIs and DM and without and DM. TLR-2 is predict factor for UTIs pregnant women with DM.

Keywords; TLR-2, GDM, UTIs, Pregnant and immunity, Bacteria and pregnant.

I. INTRODUCTION

Urinary tract infection UTI, it means the present of bacteria which are multiplication in urine with in urinary drainage system in diabetic women, urinary tract infections are caused by sweat urine, which serves as a breeding ground for bacteria (Kunin, 1994). The high incidence of urinary tract infection with diabetic patients can causes rapid parenchymal involvement, pyelonephritis was found more incidence in premeno paused diabetic women more than non diabetic, diabetic women are more liable to UTI due to decreased of neutrophil response and decrease of cytokinesin urine and decreased of leukocyte concentration (Chandel et al., 2012). In nondiabetic pregnant women, the prevalence of S-UTI ranges from 3% to 10.1 percent, while it can reach as high as 27.6% in diabetic pregnant women(Mulu et al., 2013).

Immune dysfunction has been linked to hyperglycemia, which affects neutrophil chemotaxis, macrophage function, and phagocytic responses, rendering diabetic patients more susceptible to infections and other comorbidities (Berbudi et al., 2020). Increased risk of hypertension and pre-eclampsia (PE), macrosomia, premature birth, and stillbirth associated with GDM diagnosis will pose additional health risks, as evidenced by the increased risk of hypertension and pre-eclampsia (PE), macrosomia, premature birth, and stillbirth associated with GDM diagnosis.

GDM, or gestational diabetes mellitus, is a serious obstetric complication that affects about 5–10 percent of pregnancies worldwide and is defined as any degree of glucose intolerance with onset or first recognition during pregnancy. It is characterized by an insufficient insulin response to compensate for the insulin-resistant state of pregnancy (Kampmann et al., 2015). The pathogenesis of GDM is not entirely understood, with one popular
theory relating abnormal placental hormone expression to maternal metabolic abnormalities and insulin malfunction (Behboudi-Gandevani et al., 2019). GDM may develop as a result of the maternal immune system's abnormal adaptation to pregnancy, as well as elevation of circulating inflammatory markers connected to innate immunity, resulting in immune pathway dysregulation and endothelial dysfunction and vasculopathy (Lorenzo-Almorós et al., 2019).

Because DM suppresses the immune system, enhancing the progression of acute cystitis to acute pyelonephritis and renal abscess, UTI is the most commonly observed maternal infection in pregnant women with DM, in addition to the anatomical and physiological changes seen in the renal tract during pregnancy. The prevalence of urinary tract infections (UTIs) in diabetic pregnant women has gotten little attention. This article, however, is the first in the Middle East and one of the few in the world to compare the prevalence of UTI in GDM (Al-Bash et al., 2016).

Although the role of immune dysregulation in GDM pathophysiology is unknown at this time, an aberrant maternal immune response contributes to secondary GDM effects such as cardiovascular and metabolic problems in both the mother and the child. This is based on discoveries showing immune cell infiltration of visceral adipose tissue induces pathological disruption of insulin signaling, contributing to type 2 diabetes patients' insulin resistance. (Rocha et al., 2020).

When compared to pregnant women who are in good health, individuals with GDM had a greater prevalence of vaginal infection. Both DM and atypical Lactobacillus species in the vaginal area were linked to poor pregnancy outcomes(Zhang et al., 2018). While E. coli is the most common bacterium found in UTIs, additional aggressive pathogens found in diabetic UTIs include gram-positive bacteria such as staphylococcus aureus (James and Hijaz, 2014).Tessema et al., (2020) The following were the high distributions of bacteria that cause UTIs: The microorganisms with the highest prevalence were Escherichia coli 16 (69.6%), Staphylococcus aureus 2 (8.7%), Klebsiella pneumoniae 2 (8.7%), Enterobacter aerogenes 2 (8.7%), and Pseudomonas species 1 (8.7%), (4.3 percent ). Many factors which are increased risk of urinary tract infection in diabetics which include age, duration of diabetes mellitus metabolic control (Al-Bidhani, 2018).

Pattern recognition Receptors are an important aspect of the innate immune system because they act as a first line of defense against invading pathogens. The signaling cascade is activated when microorganism-derived When pathogen-associated molecular patterns (PAMPs) or host-derived damage-associated molecular patterns (DAMPs) are detected, the expression of cytokines, chemokines, and interferons increases.(Afkham et al., 2019). One of the most important subgroups of PRRs is the Toll-like receptor (TLR) family, which serves as a link between innate and adaptive immunity. TLRs are expressed on a range of cell types, including fibroblasts, endothelial cells, and epithelial cells, as well as placental tissue (Firmal et al., 2020). Each TLR recognizes a different microbial product and activates a different signaling pathway, resulting in a different immune response. TLR2 activation in the lower female reproductive tract (FRT) may play a major role in the control of the ectocervix's innate immunological and inflammatory systems during pregnancy, interacting with other neuroendocrine variables, according to the findings (Lashkari et al., 2015). TLR2 homodimers and TLR2 heterodimers with TLR1 or TLR6 have previously been shown to activate innate immunity in response to the identification of damage-associated molecular patterns (DAMPs). Because several DAMPs are generated throughout the evolution of type 2 diabetes, it's possible that TLR2 is implicated (Sepehri et al., 2016). Yanai et al., (2016) reveal that maternal DM causes increased inflammatory activation in neonates via an innate immune response mediated by TLR5 or TLR1/2.

Our research aims to identify bacterial and immunological alterations in pregnant women with gestational diabetes.

II. MATERIALS AND METHODS

Samples collection

103 urine and Pregnant women's blood samples were taken. suffering from urinary tract infection and gestational diabetes for periods ,The study included the collection of a urine sample and a blood sample from pregnant women with ages ranging from (45-15) years from the office of the gynecological consultant at Salah Al-Din General Hospital in the city of Tikrit as well as from the evening interventions and women’s clinics under the supervision of the doctor and between December / 2020 until February / 2021 . where the results of the first culture showed that 58 samples gave bacteria growth, while no bacterial growth appeared in 45 samples. And 56
urine samples and a blood sample for pregnant women suffering from urinary tract infection without gestational diabetes, where the culture results showed that 27 samples gave bacterial growth, while 29 samples did not show bacterial growth.

**Identification of Bacterial species**

1. The isolates were initially diagnosed based on the phenotypic characteristics. This diagnosis is considered a preliminary diagnosis, as the characteristics of the developing colonies were observed in terms of shape, color, texture, colony surface, transparency, and decomposition pattern on the blood agar medium.

2. Then it was diagnosed microscopically through Gram staining to observe the cells' response to the dye, their sizes, shapes and assemblies.

3. The diagnosis was made using modern devices, using the Vitek 2 Compact System, in order to diagnose bacterial isolates to the level of the type.

**Detection of TLR-2 by ELISA technique**

The 3 mL of fasting venous blood placed in gel tubes transferred to a centrifuge (Eppendorf, Germany), centrifuged at 2000g for 10 min to extract serum, and stored at -80°C for use. The expression levels of TLR2 in serum and tested by commercial human TLR2 ELISA kit (ab227897, Abcam, UK), according to instructions. The optical density at 450 nm was measured with a spectrophotometer. Results are expressed in pg/mL based on a comparison of the standard curve for each cytokine kit.

**Statistical analysis**

All statistical analyzes were performed using SPSS (Statistical Package For The Social Sciences) (version 25).

Continuous data were subjected to a normal distribution test, and accordingly, the distributed data crossed a normal distribution with the arithmetic mean ± standard deviation and under comparison using the Analysis of Vaviance. As for the data that did not have a normal distribution, it was expressed by the median and the range, and it was compared using the Kraskal Wallis test with respect to the marginal data. The Receiver Operating Charteristic (ROC) curve was used to evaluate the diagnostic value of TLR2 protein in distinguishing between UTI patients with and without diabetes. For the purpose of revealing the association of TLR2 protein with the demographic and clinical characteristics of patients and healthy subjects, a test was adopted Pearson link for this. The P value was considered significant if it was less than 0.05.

### III. RESULTS

**1-Bacterial isolates from UTIs pregnant with and without DM**

Results of our study show the bacterial isolates Staphylococcus aureus and Klebsiella pneumonia recorded highest percentages in UTIs pregnant with DM (36.36% and 27.27%) and without DM (25% and 21.87%) respectively. E. coli scored low percentage in UTIs pregnant with DM (13.64%) than without DM (28.13%). Staphylococcus hominis and Staphylococcus haemolyticus recorded high percentage in UTIs pregnant with DM (v and 9.09%) than without DM (3.12% and 3.12%) respectively. P. aeruginosa, Proteus mirabilis, C. freundii and E. cloaca don’t appear in UTIs pregnant with DM than without DM (3.12%, 6.25%, 3.12% and 6.25%) respectively. The differences between bacterial isolated and UTIs pregnant with and without DM were non-significant (p>0.05). Gram positive bacteria recorded high percentage (59.09%) than Gram negative bacteria (40.91%) in UTIs pregnant with DM. While in UTIs pregnant without DM, Gram negative bacteria recorded high percentage (68.75%) than Gram positive bacteria (31.25%) with significant different (p<0.05) (table 1).

<table>
<thead>
<tr>
<th>Bacterial types</th>
<th>With DM</th>
<th>Without DM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>9(28.13%)</td>
<td>3(13.64%)</td>
<td>0.345</td>
</tr>
<tr>
<td>Klebsiella pneumonia</td>
<td>8(25%)</td>
<td>8(36.36%)</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus hominis</td>
<td>7(21.87%)</td>
<td>6(27.27%)</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus haemolyticus</td>
<td>1(3.12%)</td>
<td>3(13.64%)</td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
<td>1(3.12%)</td>
<td>2(9.09%)</td>
<td></td>
</tr>
</tbody>
</table>

**Table (1) bacterial isolates from UTIs pregnant with and without DM**
2-Relation of TLRs-2 to personal characters of pregnant women without DM.

Our results show the TLR-2 is non-significant positive correlation with Age (r= 0.101), Pregnancy time (r=0.171) and Pus cells (r=0.355) in UTIs pregnant without DM (table 2).

Table (2) pearson correlation between TLRs-2 and personal characters of pregnant women without DM.

<table>
<thead>
<tr>
<th>TLR-2</th>
<th>Variables</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.653</td>
<td>Age</td>
</tr>
<tr>
<td>0.446</td>
<td>Pregnancy time</td>
</tr>
<tr>
<td>0.104</td>
<td>Pus cells</td>
</tr>
</tbody>
</table>

3-Relation of TLRs-2 to personal characters of pregnant women with DM.

Conducted results show the TLR-2 is non-significant positive correlation with Age (r= 0.087), and non-significant negative correlation with Pregnancy time (r= -0.308) and Pus cells (r= -0.001) in UTIs pregnant with DM (table 3).

Table (3) pearson correlation between TLRs-2 and personal characters of pregnant women with DM.

<table>
<thead>
<tr>
<th>TLR-2</th>
<th>Variables</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.635</td>
<td>Age</td>
</tr>
<tr>
<td>0.087</td>
<td>Pregnancy time</td>
</tr>
<tr>
<td>0.995</td>
<td>Pus cells</td>
</tr>
</tbody>
</table>

4-Relation TLRs-2 with bacterila types in pregnant women without DM.

Results of our study show the highest median of TLR-2 scored in E. coli isolate (647.05) and lowest recorded in Staphylococcus hominis (424.46) with non-significant different (p>0.05) among levels of TLR-2 based on bacterias isolates in UTIs pregnant without DM. the median of TLR-2 in Gram positive bacteria (447.29) was high than Gram negative bacteria (433.0) with non-significant different (p>0.05) in UTIs pregnant without DM (table 4).

Table (4) relation TLRs-2 with bacterila types in pregnant women without DM.

<table>
<thead>
<tr>
<th>TLR-2 (ng/ml)</th>
<th>Bacterial types</th>
</tr>
</thead>
<tbody>
<tr>
<td>647.05(383.85-1568.8)</td>
<td>E. coli</td>
</tr>
<tr>
<td>463.0(358.83-504.37)</td>
<td>Staphylococcus aureus</td>
</tr>
<tr>
<td>433.0(353.12-461.56)</td>
<td>Klebsiella pneumonia</td>
</tr>
<tr>
<td>424.46(350.27-427.32)</td>
<td>Staphylococcus hominis</td>
</tr>
<tr>
<td>437.3(427.32-447.47)</td>
<td>Staphylococcus haemolyticus</td>
</tr>
<tr>
<td>0.345</td>
<td>valueP</td>
</tr>
<tr>
<td>447.29(350.27-504.37)</td>
<td>Gram positive bacteria</td>
</tr>
<tr>
<td>433.0(338.85-1568.8)</td>
<td>valueP</td>
</tr>
<tr>
<td>0.889</td>
<td></td>
</tr>
</tbody>
</table>

5-Relation TLRs-2 with bacterila types in pregnant women with DM.

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Results of our study show the highest median of TLR-2 scored in E. coli and Staphylococcus aureus isolates (367.4 and 367.4) and lowest recorded in Klebsiella pneumonia and Staphylococcus hominis (330.29 and 301.75) respectively with non-significant different (p>0.05) among levels of TLR-2 based on bacteriis isolates in UTIs pregnant with DM. The median of TLR-2 in Gram positive bacteria (421.61) was high than Gram negative bacteria (415.9) with non significant different (p>0.05) in UTIs pregnant with DM (table 5).

Table (5) relation TLRs-2 with bacteriia types in pregnant women with DM.

<table>
<thead>
<tr>
<th>TLR-2 (ng/ml)</th>
<th>Bacterial types</th>
</tr>
</thead>
<tbody>
<tr>
<td>367.4(387.49-530.05)</td>
<td>E. coli</td>
</tr>
<tr>
<td>367.4(398.9-481.54)</td>
<td>Staphylococcus aureus</td>
</tr>
<tr>
<td>330.29(247.54-1531.7)</td>
<td>Klebsiella pneumonia</td>
</tr>
<tr>
<td>301.75</td>
<td>Staphylococcus hominis</td>
</tr>
<tr>
<td>358.83</td>
<td>Staphylococcus haemolyticus</td>
</tr>
<tr>
<td>237.43</td>
<td>valueP</td>
</tr>
<tr>
<td>421.61(415.9-427.32)</td>
<td>Gram positive bacteria</td>
</tr>
<tr>
<td>410.2</td>
<td>valueP</td>
</tr>
<tr>
<td>415.9(410.2-421.61)</td>
<td>Gram negative bacteria</td>
</tr>
<tr>
<td>0.550</td>
<td>valueP</td>
</tr>
</tbody>
</table>

IV. DISCUSSION

One of the most prevalent illnesses affecting women is urinary tract infection (UTI). UTI is usually associated with vaginal infections and is caused by microorganisms that originate in the digestive tract (Czajkowski et al., 2021). Urinary tract infection (UTI) in pregnant women with diabetes mellitus (DM) is especially concerning because of the complications that can arise, which can be dangerous to both the mother and the baby. Diabetes mellitus has long been thought to increase the risk of a urinary tract infection (Al-Falahi and Al-Falahi, 2010). Mekapogu et al., (2016) show In diabetic pregnancy, Escherichia coli (25.0%), Staphylococcus aureus (22.5%), Coagulase negative staphylococci (CONS) (20.00%), and Klebsiella pneumonia (20.00%) were the most common isolates, while CONS (31.7%), E. coli (24.0%), and K. pneumonia (20.00%) were the most common isolates in non-diabetic pregnancy (16.5 percent ), and these results were consistent with our findings. According to the distribution of isolated bacteria throughout the research groups, E. coli was the most prevalent, accounting for 26.36 percent, followed by K. pneumoniae (17.27 percent), S. aureus and P. mirabilis (8.18 percent), and Enterobacter cloacae (1.81 percent) (Mardan and Alazzawy, 2020). Beksaç et al., (2019) founded the most frequent microorganism is Escherichia coli (56.7 percent ). Other commonly found bacteria in pregnant women without DM included Enterococcus faecalis (13.3%) and Klebsiella pneumonia (10%), which were similar to our findings. Knowing the uropathogens in a patient's population can help with patient management and future medical treatment planning. Preterm labor appears to be a significant concern in pregnancies including UTIs, fever, and urine issues (Beksaç et al., 2019).

In both pregnant women with and without diabetes mellitus, the overall prevalence of bacteriuria was low (DM) or gestational diabetes mellitus (GDM). There were no significant differences in bacteriuria or UTI across the groups. A routine ASB test and treat policy in pregnant women with diabetic mellitus (DM) or gestational DM is discouraged by data (GDM) (Schneeberger et al., 2017). In contrast to previous study, no significant changes between pregnant women with and without gestational diabetes, differences in the prevalence of asymptomatic bacteriuria (ASB) or the incidence of urinary tract infection (UTI) were discovered (GDM)(Schneeberger et al., 2017). Regardless of the strategy employed for glycemic management, In women with gestational diabetes mellitus (GDM) and pregestational diabetes mellitus, the prevalence of symptomatic urinary tract infection (S-UTI) is identical. (PGDM) (Al-Bash et al., 2016).

The most functioning molecules that contribute to the urinary tract defense system and UTIs are TLRs 2, 4, and 5. TLRs' ability to identify and recognize diverse portions of microbial components associated with the same infection is extraordinary (de Oliviera et al., 2012). Furthermore, the versatility of TLR molecules may allow for the identification of various microbes with various signaling pathways. Behzadi and Behzadi, (2016) show TLRs and their activity against microbial UTI causative agents may aid in the prevention, control, and treatment of UTIs at a higher quality level. In the same test, a screen of numerous common urinary tract infection (UTI)-related pathogens revealed substantial stimulation of TLR2-signaling. These findings suggest that two separate
TLR2-activity markers—urinary TLR2-stimulants and serum sTLR2 levels—are significantly higher in UTI patients compared to controls (Hossain et al., 2018). TLR antagonists and agonists have the ability to impact host defense processes, and some of these immunomodulating drugs may be able to overcome intrinsic TLR system disruptions to provide novel therapy options for UTI (Scherberich and Hartinger, 2008). Circulating leukocytes produce large levels of TNF at rest and show poor responses to TLR2 and TLR4 agonists as they age (Bailey et al., 2019). TLR2 and TLR4 activation in the lower female reproductive tract (FRT) may play a major role in the control of the ectocervix's innate immunological and inflammatory mechanisms during pregnancy, interacting with other neuroendocrine variables, according to the findings (Lashkari et al., 2015). TLR-2 levels rise with age, pregnancy duration, and the amount of pus cells in pregnant women without diabetes as their immunological health improves (Bailey et al., 2019).

According to the findings, maternal DM causes excessive inflammatory activation in neonates via an innate immune response mediated by TLR5 or TLR1/2 (Yanai et al., 2016). TLR2 homodimers and TLR2 heterodimers with TLR1 or TLR6 have previously been shown to activate innate immunity in response to the identification of damage-associated molecular patterns (DAMPs). Because several DAMPs are generated throughout the evolution of type 2 diabetes, it's possible that TLR2 is implicated (Sepehri et al., 2016). TLR1 - TLR10 and MYD88 expression is dynamically regulated at the maternal level, implying that TLRs expressed in the endometrium and placenta may play a key role in modulating mucosal immune responses to promote the development and maintenance of pregnancy (Yoo et al., 2019). In pregnant with DM, the reflex relationship between TLR-2 and pregnancy yime and pus cells in UTIs returns to reduced immunological protein synthesis and decreased immune cell activity (Bailey et al., 2019).

TLR2 is a bacterial receptor that activates IL-8 in both Gram-positive and Gram-negative bacteria (Dziarski and Gupta, 2000). The findings revealed that cytokine production in whole blood after bacterial stimulation differed between pregnant and non-pregnant women, implying that changes in cytokine production may contribute to pregnant women's higher sensitivity. Furthermore, pregnancy had a different effect on whole blood cytokine production in response to E. coli stimulation. As a result, changes in cytokine production could explain why pregnant women react differently to different bacteria or their products (Faas et al., 2014).

It's currently unclear whether PRR that have a role in the development of innate immune responses involving secreted proteins also control other responses, such as cellular responses. Underhill et al., (1999) suggested that TLR2 elicits humoral innate immune responses in macrophages by recognizing bacteria that have been engulfed and present in phagosomes. TLR2 and TLR4 are now thought to be involved in phagocytosis of apoptotic cells and bacteria, although not at the engulfment stage, but at the phagosome maturation stage, but the data from the two groups is not entirely consistent (Watanabe et al., 2017). The findings imply that the S. aureus-induced pro-inflammatory cytokine response is not dependent on macrophages, and that TLR2 loss causes macrophages to release less IL-10, contributing to a dysregulated cytokine balance, bacterial clearance problems, and mouse death. As a result, TLR2 protects against S. aureus infection by modulating pro- and anti-inflammatory cytokine responses (Kohanawa et al., 2013). During early bacteremia, TLR2 downregulation and IL-6 and IL-10 amounts suggestive of immunological dysregulation may be linked to death from Staphylococcus aureus bacteremia (SAB). During the early stages of development, TLR2 expression and related cytokine reactions were measured. Although larger studies are needed, Staphylococcus aureus bacteremia (SAB) may be a possible prognostic factor in SAB (Kim et al., 2020). The strength of immune responses to bacteria is determined by the organism's type, dosages, location, and virulence genes. (Yoo et al., 2019).

V. CONCLUSIONS

1. Staphylococcus aureus and Klebsiella pneumonia recorded highest percentages in UTIs pregnant with DM and without DM respectively.

2. E. coli scored low percentage in UTIs pregnant with DM than without DM.

3. TLR-2 is positive correlation with Age, pregnancy time and Pus cells (r=0.355) in UTIs pregnant without DM.

4. In UTIs pregnant with DM, TLR-2 has a non-significant positive association with age and a negative correlation with pregnancy duration and Pus cells (r=-0.001).
5. The median of TLR-2 in Gram positive bacteria was high than Gram negative bacteria with non-significant different (p>0.05) in UTIs in pregnant women with and without diabetes mellitus

Recommendations

Soluble TLR-2 Concentration increases in non-diabetic pregnant women with UTI and could be used as additional assay for detection of UTI in these women. E. coli and staph aureus are the most common bacteria causes of UTI in pregnant women with and without diabetes respectively.

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