Role of IL-6 in urinary tract infection among diabetic and non- diabetic patients

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ABSTRACT

Urinary tract infection is the presence of microorganisms mostly bacteria in the urinary tract, one of the reasons that increases the risk of infection is diabetes, which is the most common metabolic disorder that is increasing at an alarming rate all over the world, due to high blood sugar caused by weak immunity leading to This results in damage to neutrophil function and decreased antibacterial activity in urine and thus bacteriuria.The study included 118 samples (73 samples for diabetic patients and suffering from urinary tract infection, and 45 samples for UTI without diabetes), where the level of interleukin-6 was measured in these two groups and compared with the control group (20 samples), and the level of this immune cytokine was compared at Infection with different bacterial species.

The level of interleukin-6 was high in patients with urinary tract infection who did not suffer from diabetes, which reached (24.1 ± 8.75) ng/ml compared to the control samples whose level was (0.763 ± 4.18) ng/ml and with a significant difference (0.01>p). . There is a significant increase for IL-6 in patients with urinary tract infection without diabetes (2.58±9.77) compared to the control samples whose level reached (0.551±3.79) at a significant level (P>0.01).

The highest level was when infected with E.coli and its level was (3.92 ± 17.51) ng/ml in normal UTI patients and (2.571 ± 13.43) ng/ml in diabetic patients.

Introduction

Urinary tract infection means the presence of microorganisms in the urinary tract, most often bacteria [1].The urinary tract is characterized by its natural defense mechanisms that prevent urinary tract infections [2] .The term urinary tract infection refers to the presence of at least 105 bacteria cells / 1 ml in a single urine sample [3]. *E.coli* bacteria are the main cause of urinary tract infections, as they constitute mostly (90-50%) of the isolated species due to their superficial structures that help them attach to the epithelial cells of the urinary tract, followed by less common types such as *Enterobacter spp*, *Proteus spp* and the *Klebsiella spp*[4]. In addition to other species such as, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis* and *Staphylococcus aureus* [5] .

Multiple and unique potential mechanisms of diabetes may contribute to the increased risk of UTI in diabetic patients [6]. Type 1 diabetes appears at an early age, mainly due to the destruction of the pancreas by autoimmunity, and thus results in complete insulin deficiency [7].

Type 2 diabetes (T2D) is a metabolic disorder that is the most common and clinically important and which has recently become a global epidemic and a major problem faced by health care in different parts of the world[8] . Diabetes is defined as an immune disease that leads to a defect in insulin signaling and the selective destruction of insulin-producing beta cells and this is where cytokines play an instrumental role[9]. Chemokines play a major role in inflammatory processes through the regular migration of leukocytes to sites of damaged tissues[10].

Interleukin IL-6 is a cytokine with multiple functions and directions, involved in the regulation of immune

responses, acute phase responses and inflammation. It is produced by endothelial cells, fibroblasts, monocytes, and macrophages in response to various stimuli (IL-1, IL-17 and TNF- a) During systemic inflammation, in innate immunity IL-6 directs leukocyte migration and activation[11]. IL-6 inhibits insulin-dependent insulin receptors by interfering with insulin sensitivity and enhances glucose production and stimulates LDL and triglyceride production in the liver[12] .

Material and method

This study was conducted in some hospitals in Salah al-Din Governorate (Salah al-Din General Hospital, Balad General Hospital, Dhuluiya General Hospital, Samarra General Hospital) for the period from October 2020 to March 2021, at ages ranging from (20-60) years. Patients were placed in sterile plastic containers. Patients were recommended that the sample be collected from the midstream urine (Clean-Catch-Midstream Urine) and information regarding age, gender and whether there was a previous urinary tract infection (UTI) was recorded in the questionnaire.

The samples were cultured by taking a sterile loop of the bacteria vector (loop) from urine samples on saline mannitol agar medium, blood agar medium and MacConkey agar medium. The plates were incubated under aerobic conditions at a temperature of 37°C for (24-48) hours, all the colonies of single isolates were transferred to the appropriate medium. Her then diagnosed colonies phenotypically

[13] A portion of the bacterial colonies were taken and stained with Gram stain, then examined under a light microscope using an oil lens with a final magnification of X100 to observe the response of cells to the dye, their shape and arrangement and to distinguish between Gram positive and negative cells. Biochemical tests were used to diagnose positive and negative bacteria. Then use the Vetik 2 compact system to give a final diagnosis of the bacterial isolates.

Measurement of the level of interleukin-6 in the sera of patients with urinary tract infection (UTI).

This ELISA kit uses the Sandwich-ELISA method. The Microelisa plate available in this kit was pre-coated with an antibody specific to IL-6. Standards or samples are added to the existing pits and combined with the specified antibody. An IL-6-specific antibody (HRP) is then added to each pit and incubated. Components are washed. TMB substrate solution is added to each pit. Only those pits containing IL-6 antibodies and IL-6 conjugated HRP will appear

It is blue and the intensity of the color varies depending on the concentration.

Results and Discussion

The results of bacterial isolation for 73 people with symptoms of UTI and with diabetes, and 45 people with symptoms of UTI without diabetes, showed that the number of samples that gave bacterial growth on the culture media used was 35 samples out of the total samples for people with UTIs and they have diabetes, while 35 samples appeared for people with UTIs who do not have diabetes. After conducting biochemical tests, the following bacterial types were diagnosed:

Escherichia coli, Klebsiella. pneumoniae Pseudomona aeruginosa, Staphylococcus aureus, Enterococcus faecalis, Staphlococcus. saprophyticus ,

The current study showed that the percentage of isolates of ***Escherichia coli*** from patients with urinary tract infection who have diabetes is (28.50%), and it was the most prevalent pathogen, followed by ***Staphylococcus aureus*** bacteria, which recorded an isolation rate of (25.70%) as shown in Table (4-1).

Table (4-1) Percentage of bacterial types that cause urinary tract infection for diabetics and non-diabetics

Insulation Groups	*Staphlococcus Saprophyticus*	*Pseudomonas aeruginosa*	*Klebsiella. pneumoniae*	*Enterococcus faecalis*	*Staphylococcus aureus*	*Escherichia coli*
Diabetics with UTI	14.20%	8.50%	5.70%	17.10%	25.70%	28.50%
Patients with UTI without diabetes	11.42%	11.42%	8.57%	14.28%	22.28%	31.42%

With regard to patients with urinary tract infection without diabetes, the percentage of E.coli was recorded (31.42%), which is the most common cause of urinary tract infection, while the percentage of ***Staphylococcus aureus*** was recorded, it was isolated (22.85%). It is noted from the percentages in Table (4-1) that Gram-negative bacteria were the predominant and the most common E. coli bacteria in persons with urinary tract infection without diabetes, ***E. coli*** contains many factors associated with virulence, including Adhesives, toxins, iron acquisition agents, lipopolysaccharides, polysaccharide capsules, gases, plasmids and other

mobile genetic elements [14]. Gram-positive bacteria were observed in greater proportions in people with diabetes, represented by ***Staphylococcus aureus***. It is known that people with diabetes suffer from weak immune systems, which facilitates the opportunity for bacteria to spread, and because of its virulence factors that enable it to infect the host's body systems and cause infection in vulnerable families. Those with weak defenses that are frequently exposed to antibiotics such as weakened immunity, diabetes, and other anatomical abnormalities of the urinary tract) [15].

Interleukine-6 kinetics measurement in serum of patients with urinary tract infection.

Samples	Interleukine-6
Samples of patients with urinary tract infection	9.77 ± 2.58
Control	3.79 ± 0.551
Samples of patients with diabetes and suffering from urinary tract infection	8.75 ± 1.24
Control	4.18 ± 0.763

The results of the current study appear when investigating the level of immune cytokines.

IL-6 in the serum of UTI patients and diabetics, as Table (4-2) shows, there is a significant increase for interleukin-6 in UTI patients without diabetes, which reached a level of (24.1±8.75) ng/ml compared to samples The control, which had a level of (0.763±4.18) ng/ml, with a significant difference (0.01>P). IL-6 is a sensitive physiological marker of subclinical systemic inflammation, and is associated with insulin resistance and diabetes[16]. It is noted in the above table that there is a significant increase for IL-6, as its level reached in patients with urinary tract infection without Incidence of diabetes to (2.58 ± 9.77) compared to the control samples, which reached a level of (0.551 ± 3.79) at a significant level (0.01 P>). (Min Yang) study showed that the level of IL-6 in blood was significantly higher in DM patients than in healthy controls, where its level was (30.3±21.5) pg/ml compared to healthy controls (2.6±1.2) pg/ml and with a significant difference (0.01>P) and that IL-6 had a significant association with the inflammatory marker CRP or serum ferritin [17].

Figure (4-1) Interleukin-6 levels, depending on the type of bacterial infection
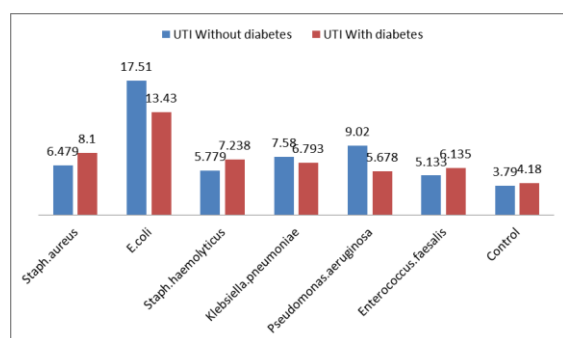

Fig. (4-1) shows the level of IL-6 in the serum of patients with urinary tract infection and diabetes patients, according to the bacterial infection, and as shown, the highest level was when infected with E.coli and its level was (3.92 ± 17.51) ng/ml in patients with UTI normal and (2.571±13.43) ng/ml, in diabetic patients.

Infection with E.coli elicits innate and adaptive immune responses, characterized by the production of a number of proinflammatory mediators, including cytokines and chemokines, including interleukin-6 [18] (IL-6). Synthesis of IL-1α, IL-1β, IL-1ra, IL-6 and IL-8 at the protein level After stimulation with the E. coli strain, a different kinetic response to IL-6 was found, with peak levels already reached in two hours[19].

Pseudomonas.aerginosa followed with kinetic levels of (2.71 ± 9.02) ng/ml for normal patients and (1.567 ± 6.153) ng/ml for diabetic patients. P. aeruginosa has different acquired resistance mechanisms, and carbapenemase enzyme production is the most dangerous because this The enzyme can degrade all penicillins, cephalosporins, and carbapenems, exacerbating inflammation [20]. Pseudomonas aeruginosa promotes phagocytosis through the IL-6 signaling pathway, and the inflammatory response triggered by bacterial processing enhances the ability of these macrophages to control inflammation through increased proliferation [21].

Either Staph. aureus kinetic level was as follows (0.249 ± 6.479) ng/ml, and (0.991 ± 8.1) ng/ml, for both normal UTI patients and diabetic patients, respectively, while Klebsiella.pneumomiae IL-6 level reached (2.81 ± 7.58) (ng/ml (1.258 ± 6.793) ng/ml, and reached in those infected with Staph bacteria. Haemolyticus (0.454 ± 5.779) ng/ml in patients with urinary tract infection without diabetes, and its level was (0.405 ± 7.238) ng/ml in patients with diabetes, and the level of these kinetics was when infected with Enterococcus.faecalis bacteria (1.291 ± 5.133) ng/ml, in normal patients (0.827±6.135) ng/ml..

Recommendations and conclusions

The study showed that the most common type of Gram-negative and Gram-positive bacteria in urinary tract infections is E.coli. The level of interleukin-6 was elevated when infected with E.coli bacteria for non-diabetic patients.

The study of other types of other immune cytokines such as IL-10, IFN-β, IL-1β and 17-IL should be further studied, and their relationship to the different etiologies of urinary tract infections, as well as their role in differentiating between the severity and severity of diabetic UTIs in non-diabetic patients, should be further investigated. People with diabetes, studies should be conducted on a large number of patients, to check the levels of cytokines more comprehensively and better.

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دور IL-6 في عدوى المسالك البولية بين مرضى السكري وغير المصابين بالسكري

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الملخص

التهاب المسالك البولية هو وجود ميكروبات في الغالب بكتيريا في المسالك البولية، أحد الأسباب التي تزيد من خطر الإصابة هو مرض السكري، وهو الأكثر شيوعًا، وهو اضطراب التمثيل الغذائي الذي يتزايد بمعدل ينذر بالخطر في جميع أنحاء العالم، بسبب ارتفاع الدم السكر الناجم عن ضعف المناعة مما يؤدي إلى تلف وظيفة العدلات وانخفاض النشاط المضاد للبكتيريا في البول وبالتالي البيلة الجرثومية، وشملت الدراسة 118 عينة (73عينه لمرضى السكر ويعانون من عدوى المسالك البولية و 45 عينة لمرضى المسالك البولية بدون مرض السكري)، حيث تم قياس مستوى إنترلوكين 6 في هاتين المجموعتين ومقارنة بالمجموعة الضابطة (20 عينة)، كان مستوى الإنترلوكين 6 مرتفعًا في المرضى الذين يعانون من عدوى المسالك البولية والذين لا يعانون من مرض السكري والذي بلغ (24.1 ± 8.75) نانوغرام / مل مقارنة بعينات الضبط التي بلغت (0.763 ± 4.18) نانوغرام / مل وبفرق معنوي (P<0.01)، هناك دلالة على زيادة IL-6 في المرضى الذين يعانون من عدوى المسالك البولية دون مرض السكري (2.58 ± 9.77) مقارنة لعينات الضبط التي بلغ مستواها (0.551 ± 3.79) عند مستوى معنوي (P<0.01).

أعلى مستوى كان عند الإصابة ببكتيريا الإشريكية القولونية وكان مستواه (3.92 ± 17.51) نانوغرام / مل بشكل طبيعي في مرضى المسالك البولية و (2.571 ± 13.43) نانوغرام / مل في مرضى السكري.