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## Role of IL-8 in urinary tract infection among diabetic and non-diabetic patients

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### Abstract

Urinary tract infection (UTI) is one of the most common problems among women and men. The infection is mostly bacterial and occurs when a microorganism invades the lining of the urinary tract. Patients with diabetes are at increased risk of infection, especially UTIs. A high level of sugar leads to a change in the immune function of diabetic patients, which is closely related to many innate and adaptive immune system defects, which may lead to an increased risk of developing a urinary tract infection. The change in immune function may appear in the form of a decrease in the levels of some motors such as IL-8. Urinary and blood samples were collected from 73 UTI patients who had diabetes and 45 UTI patients who did not have diabetes. The bacterial types that cause UTI were diagnosed in both groups and compared with the control group (20 samples for healthy people), and a study was conducted the relationship of the high level of this immune cytokine with the type of bacterial infection. The level of interleukin-8 in this study was  $(3.5 \pm 16.80)$  pg/ml compared to the control samples, which amounted to  $(1.45 \pm 9.48)$  pg/ml, while its level in diabetic patients with urinary tract infection was  $(3.77 \pm 15.86)$  pg/ml. ml, compared to the control samples  $(2.11 \pm 9.64)$  pg/ml. The most pathogenic cause of UTI patients and diabetics was *E.coli* (28.50%), and its percentage was in UTI patients without diabetes (31.42%).

**Keywords:** Interlukine-8, Urinary tract infection (UTI), diabetes mellitus

### Introduction

Urinary tract infection is a bacterial infection that mostly affects the urinary system, when bacteria enter the urinary tract and then into the bladder and multiply in the urine (Komala *et al.*, 2013) <sup>[9]</sup>. Urinary tract infection is one of the most common microbial problems among women, men and children. Studies indicate that about (10-20%) of females suffer from urinary tract infection (Lee AC *et al.*, 2013) <sup>[11]</sup>. Symptoms appear on the patient when there is a growth Bacteriophages have more than 105 bacteria cells/1ml of urine (Mukherjee *et al.*, 2015) <sup>[10]</sup>. Diabetes mellitus: Diabetes mellitus (DM) is one of the most common endocrine disorders characterized by elevated blood sugar levels (2020 *et al.*, Shah) <sup>[12]</sup>. Diabetes can be caused by either a lack of insulin or the action of insulin or both (Sachinathana *et al.*, 2018) <sup>[13]</sup>. There are three types of diabetes, type 1 diabetes and type 1 diabetes called insulin-dependent diabetes (type 1 diabetes occurs As a result of beta-cell destruction, absolute insulin deficiency occurs (Parajapati, 2018) <sup>[14]</sup>. The known and common symptoms are increased frequency of urination, frequent thirst, increased hunger and weight loss. Other symptoms may include blurred vision, fatigue, and general weakness, wound healing. It is estimated that type 1 diabetes accounts for 5-10% of all cases of diabetes (World Health Organization 2017). Type 2, Type 2 diabetes is defined as “a metabolic disorder characterized by chronic hyperglycaemia with defective carbohydrate, fat and protein metabolism resulting from defects in insulin secretion or insulin action (Reed *et al.*, 2021) <sup>[15]</sup>. Type 2 diabetes occurs 2 primarily as a result of obesity and lack of exercise, some people are at greater genetic risk than others, type 2 diabetes accounts for about 90% of diabetes cases (Rafiq *et al.*, 2020) <sup>[17]</sup>. Type II DM, as these biomarkers have been found to increase in the onset of DM and remain elevated throughout the duration of the disease, and this also enhances the involvement of inflammatory factors in the pathogenesis and complications of DM (Mussa *et al.*, 2012) <sup>[21]</sup>. Type 3 is gestational diabetes: Women with gestational diabetes are at increased risk of complications during pregnancy and at delivery, and they and their children are also at increased risk of developing diabetes later on (WHO, 2017). Cytokines: These are small proteins, which play a major role in regulating and directing leukocyte migration, angiogenesis, tumorigenesis and immune responses to

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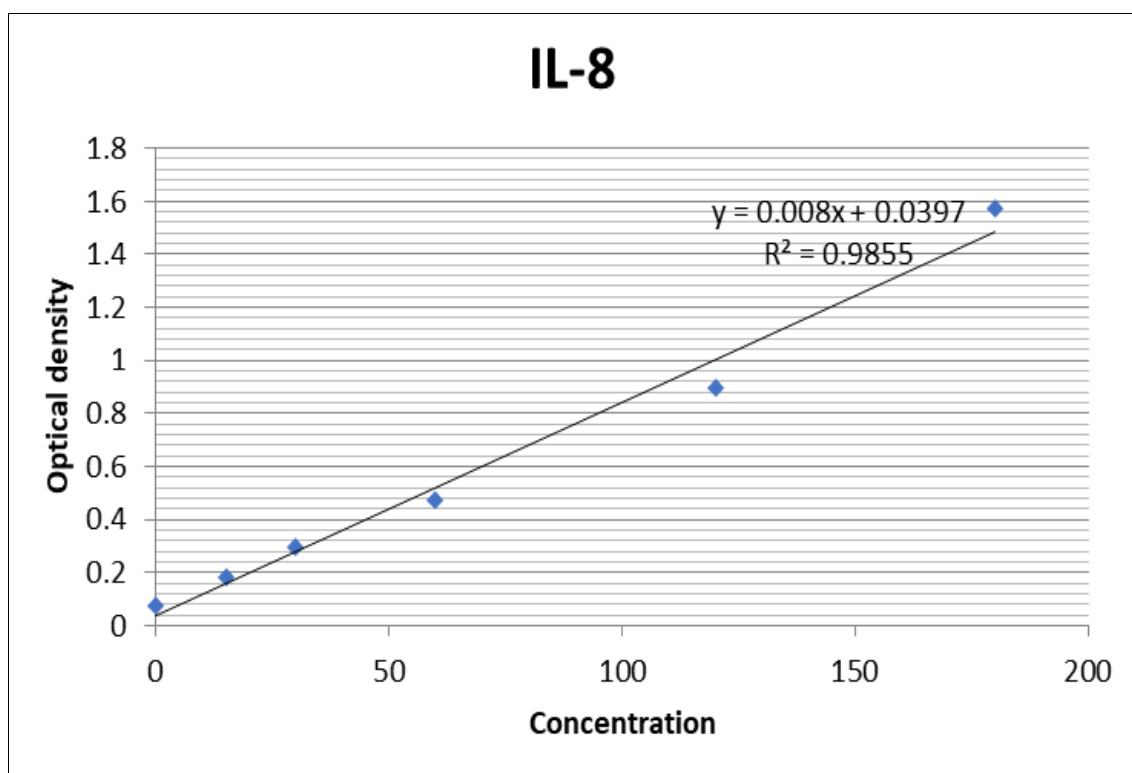
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microbial infection (Kofanova *et al.*, 2018) [20]. Interleukin-8 (IL-8): It is a pro-inflammatory polypeptide belonging to the CXC chemical family, and it is secreted by many cells, including fat cells, monocytes/macrophages, T lymphocytes, endothelial cells and human cells (Motawa *et al.*, 2018) [16]. Among its functions are Multiple IL-8 also enhances macrophage directing in adipose tissue that causes local and systemic inflammation and is a link between dysfunction and conditions associated with insulin resistance (Marino F *et al.*, 2015).

### Materials and methods

(118) blood and urine samples were collected from patients with symptoms of urinary tract infection, patients with diabetes, and patients who attended Balad General Hospital, Samarra, Salah al-Din and Dhuluiya, for both sexes, at ages (20-60) years, for the period from October 2020 to March 2021. The sample was collected by drawing venous blood and placed in sterile tubes and then placed in a centrifuge at a speed of 3500 rpm for 10 minutes, then separated the serum by a micropipet and then kept in sterilized Abendorf tubes at a temperature (20) C for use in Immunological tests later. Samples were collected from the middle urine because it is less contaminated after making sure to wash the area well and put the sample in a sterile plastic container and close the container directly to avoid contamination. The

samples were planted by using a transfer lug after sterilizing it with flame, where the filling of the lug was taken and planted on the culture media, and the dishes were incubated under air conditions at a temperature of 37 °C for a period of (24-48) hours. The characteristics of colonies growing on mannitol saline agar medium, blood agar medium and Macconkey medium were observed in terms of size and color in addition to colony shape and their effect in the medium such as pigment production, blood analysis, mannitol fermentation, urease production, citrate consumption and whether it is fermented or not fermented with lactose sugar. A portion of the bacterial colonies were taken and stained with gram stain and examined under a light microscope. Biochemical tests were used, and a device was used to diagnose isolated bacterial samples. (Vitek 2 compact system) (In the sera of IL-8 UTI patients, the level of interleukin-8 was measured). Using the Sandwich-ELISA method as a working principle, the Microelisa template in this kit is pre-coated with an IL-8-specific antibody. Samples are added to the pits in the plate and combined with the Horseradish IL-8 (HRP) conjugated antibody specific to IL-8. To each pit and incubated, the components are washed with the washing solution, pits containing IL-8 antibodies and IL-8 conjugated HRP will appear blue and their intensity varies with different concentration.



**Fig 1:** Standard curve for interleukin- 8

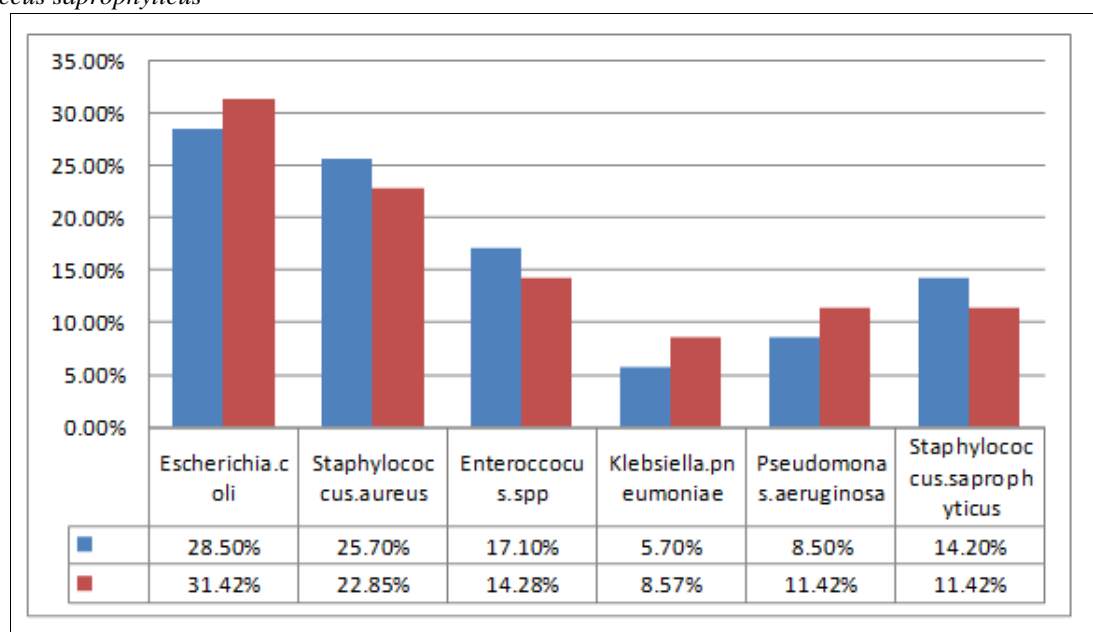
### Results and Discussion

The results of bacterial isolation of 73 samples of people with symptoms of urinary tract infection and people with diabetes, and 45 samples of people with symptoms of urinary tract infection and non-diabetics, showed that the number of samples that gave bacterial growth on the culture media used 35 samples of the total samples for people who

They suffer from a urinary tract infection and have diabetes, while 35 samples appeared for people with a UTI who do not have diabetes.

After conducting biochemical tests, the following bacterial types were diagnosed:

*Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Faecalis faecalis*,

*Staphylococcus saprophyticus*

**Fig 2:** The bacterial species that cause urinary infection in diabetic and non-diabetic patients.

The current study showed that the percentage of *E.coli* isolates from patients with urinary tract infection who had diabetes was (28.50%) as the most prevalent pathogen, and the percentage of isolates of *Klebsiella pneumoniae* was (5.70%). The percentage of isolated bacilli positive for *Faecalis* gram stain. *.faecalis* is (17.10%), as these results are close to a study conducted to determine the distribution of the spread of different bacteria that cause urinary tract infection among diabetic patients, where the percentage of *E.coli* isolate (49.01%), which is the main cause of infection, while *Klebsiella pneumoniae* was (8.3%) and *Faecalis faecalis* (13.84%) (Jha *et al.*, 2014). Bacteria were recorded. *Pseudomonas aeruginosa* isolated rate (8.50%) The percentage was similar to that obtained by a previous study where it was (8.69%) for patients diagnosed with urinary tract infection (Kama *et al.*, 2020). While the percentage of *Staphylococcus saprophyticus* bacteria reached (14.20%), most of the studies that obtained isolation rates for this bacteria indicated a very low isolation rate, while other studies in Uganda obtained isolation rates of up to (51.4%) (Barbara. *et al.*, 2017), these differences in the isolated percentages of bacterial pathogens could be due to differences in study design as well as sample size. While patients with urinary tract infection without diabetes recorded the percentage of *E.coli* (31.42%), and it was the most common cause of UTI in this group, Hammoudi's results were in support of the results of the current study, as the percentage of *E.coli* isolation was (22.2%). While *Staphylococcus aureus* bacteria were isolated (22.85%) and this percentage was close to what was reached by a study in the city of Basra, where the percentage of Staph. aureus (17.78%) was *Pseudomonas aeruginosa* (Hadi *et al.*, 2014) [1]. was (11.42%) These results were similar to the results of other studies, including those that obtained an isolation rate

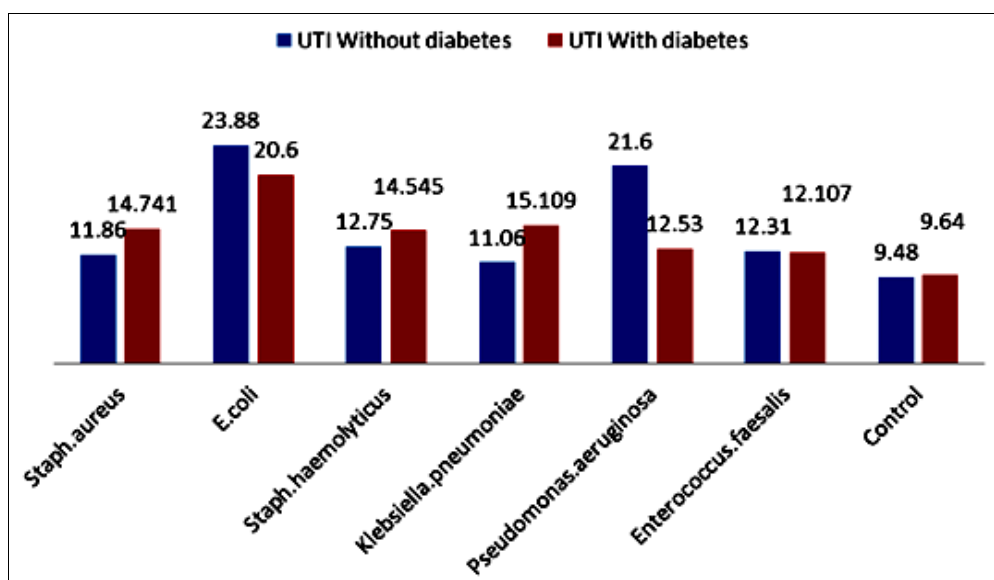
(12.5%) (Priyadharsini *et al.*, 2014) [6]. The current study obtained an isolation rate of *Staphylococcus saprophyticus* (11.42%), although this pathogen is considered less common in urinary tract infection, but it is considered pathogenic to some extent. (Jhora and Paul, 2011) [4]. *Klebsiella pneumoniae* bacteria are common. pneumonia is among the most common causes of urinary tract infection, as its percentage in this study reached (8.57), another study came with a similar percentage, which was (8.2%) (Shilpi *et al.*, 2013) [5]. In Figure (4-1), Gram-positive bacteria were observed in greater proportions in diabetics. It is known that people with diabetes suffer from weak immune systems, which facilitates the opportunity for bacteria to spread, and because of the virulence factors they possess, which enable them to infect the host's body systems and cause infection, Increased resistance to therapeutically important antimicrobial agents and genetic evolution of microbes may provide a greater advantage to bacteria allowing them to induce infection in vulnerable hosts with weak defenses frequently exposed to antibiotics such as weakened immunity, diabetes, and other anatomical abnormalities of the urinary tract) (Kudinha, 2017) [7]. Whereas in UTIs without diabetes, Gram-negative bacteria were dominant and the most common being *E.coli*, *Escherichia coli* contains many factors associated with virulence, including adhesives, toxins, iron-acquiring factors, lipopolysaccharides, and polysaccharide capsules. Polymorphs, gases, plasmids and other mobile genetic elements (Sarowska *et al.*, 2019) [8]. The rate at which strains of *E. coli* are becoming resistant to the vast majority of antibiotics is increasing worldwide. In addition, intestinal bacteria contain a gene(s) that confer resistance to nearly all antibiotics.

**Table 1:** Interleukine-8 kinetics measurement in serum of patients with urinary tract infection and patient with diabetes.

Samples	Interleukine-8
Samples of patients with urinary tract infection	16.80 ± 3.5
Control	9.48 ± 1.45
Samples of patients with diabetes and suffering from urinary tract infection	15.86 ± 3.77
Control	9.64 ± 2.11

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**Fig 3:** shows the level of IL-8 in the sera of UTI patients who suffer from diabetes, UTI patients who do not have diabetes, and according to the bacterial infection.



As shown in the above figure. For UTI patients without diabetes, the highest level of IL-8 When infected with *E.coli*, it reached ( $5.04 \pm 23.88$ ) pg/ml, the production of toxins by colonizing *Escherichia coli* may cause an inflammatory response, which is a possible pathway for urinary tract infection symptoms, which leads to fluctuations that result in an increase in Cytokines, including IL-8 (Bien *et al.*, 2012) <sup>[24]</sup>, followed by *Pseudomonas aeruginosa*, where kinetics levels reached ( $4.92 \pm 21.6$ ) pg, either by *Staph. aureus* kinetics level was as follows ( $1.489 \pm 11.86$ ) pg/ml, while for *Klebsiella. pneumoniae* the IL-8 level reached ( $1.291 \pm 11.06$ ) pg/ml, and in those infected with *Staphylococcus saprophyticus* it reached IL-8 level ( $1.164 \pm 12.75$ ). (Pg/ml, and the level of this kinetics was when infected with *Faecalis. faecalis* ( $2.25 \pm 12.31$ ) pg., when infected with *Faecalis. faecalis*, in UTI patients with diabetes, diabetic patients have higher levels of cytokines compared to normal individuals and this elevation may be related to activation of macrophages, increased oxidative stress, or the introduction of macrophages. Cytokines (Shelbaya *et al.*, 2012) <sup>[26]</sup>. In Figure (4-2) above, the level of IL-8 in patients with urinary tract infection and suffering from diabetes was ( $3.59 \pm 20.6$ ) pg/ml, who were diagnosed with *E.coli* bacteria, where the highest percentage of kinetics was recorded, in several studies it was found that measuring IL-8 in urine along with serum CRP and lymphocyte neutrophil ratio (NLR) could indicate the pathogen. Among adults with diabetes, higher levels of UIL-8 (median: 2120 pg. /ml) indicate beta-lactamase. That results in *Escherichia coli* infection (Horváth J *et al.*, 2020) <sup>[25]</sup>. While the bacteria *Klebsiella. pneumonia* recorded ( $0.347 \pm 14.545$ ) pg, while the level of IL-8 was recorded in *Staphylococcus aureus* bacteria ( $1.511 \pm 14.741$ ) pg/ml. The level of this kinetic has reached for those infected with staph bacteria. Haemolyticus ( $1.269 \pm 14.545$ ) pg/ml, while for those infected with, *Pseudomonas aeruginosa* the cytokinetic level of IL-8 reached ( $1.97 \pm 12.107$ ) pg/ml. whereas, the level of cytokinetic IL-8 in *Faecalis faecalis* bacteria reached ( $1.832 \pm 12.107$ ) pg/ml. These levels were compared with the result of the control group, whose level of IL-8 was ( $2.11 \pm 9.64$ ) pg/ml.

#### Conflict of Interest

Not available

#### Financial Support

Not available

#### Conclusions and Recommendations

The study showed that the most common Gram-negative and Gram-positive bacteria in urinary tract infections were *E.coli*, followed by *Staphylococcus aureus*, followed by *Staphylococcus aureus*. The highest level of interleukin-8 was reached when infected with *E.coli* bacteria for diabetic and non-diabetic patients, the study of immune cytokines and their relationship to urinary tract infection and diabetes and the effect of each on the level of cytokines such as IL-10, IL-1 $\beta$  and IL-10 should be expanded, the sample size should be great to get accurate and comprehensive results.

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