

Antibiotic susceptibility of *Escherichia coli* isolated from renal failure patients and relation with some antioxidants and toll-like receptor₄

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ABSTRACT

Chronic kidney disease (CKD) is a common disorder that is increasing around the world. It's related to the progression of end-stage renal disease (ESRD) ,This study included examining the sensitivity of *E. coli* isolates to antibiotics using the Vitec system, and collection of blood samples from patients suffering from kidney failure and urinary tract infections who were attending the Imam Hussein medical city / dialysis unit and a urology consultant for age groups between (20-70) years for both sexes to determine the concentration of the Toll-like receptor. as well as the determination of superoxide dismutase (SOD) activity and glutathione concentration Through the results of estimating the toll-like receptor 4(TLR₄), it became clear that the age group between (40-60) years showed the highest immune response, as the concentration of this immune receptor reached 1.115 ng / ml. The results of the statistical analysis confirmed the existence of significant differences $P > 0.05$ in the concentration of this receptor in the age group between (20-40) years, while the remaining two age groups did not show any significant differences. By estimating the activity of enzyme antioxidants in the groups under study and comparing it with the activity of the enzyme in healthy people, it became clear from the results that the highest activity of the SOD enzyme was recorded in healthy people, which amounted to 15.35 units / ml . while it was noted that there was a significant decrease in the activity of this enzyme in all Patients with renal failure and patients with renal failure accompanied by urinary tract infections. The results of estimating the concentration of glutathione (GSH) in the groups under study showed a high concentration of this compound in healthy people 9.7 micromol / ml, while a significant decrease in its concentration was observed in both patients with renal failure and patients with renal failure who had urinary tract injuries.

Keywords: Chronic kidney failure ,Urinary tract infection ,Superoxide dismutase ,Glutathione ,*Escherichia coli* , Toll-like receptor₄

1-INTRODUCTION

Chronic kidney failure has become one of the important problems facing medicine in the twenty-first century, as one of the international statistics indicated that about 10% of the world's population is exposed to this disease, and another statistic confirmed that the number of infected people worldwide reached 843.6 million people as of 2017. (Kovesdy ,2022 ; Jager *et al* .,2019). The kidneys are exposed to infection with some types of microorganisms, either through direct invasion or indirectly, as bacteria, viruses, Mycobacteria, fungi and protozoa participate in the events of this infection. Which includes adhesion, which is achieved by the presence of two types of cilia (P fimbriae, Type 1 fimbriae) first, their production of toxins second, their production of iron withdrawal systems (siderophores) third, the presence of a capsule fourth, and their wall containing lipopolysaccharide fifth As well as its ability to move VI. (Prasad and Patel ,2018; Parves and Rahman ,2018).

E.coli bacteria are the most important types European Journal of Molecular & Clinical Medicine ISSN 2515-8260 Volume 7, Issue 09, 2020 76 76 that cause urinary tract infection. (Al-Wazni and Hadi ,2015). Where Its diseases are due to its possession of many virulent factors, including iron chelates Siderophores, cystic necrotizing factor, colisin, possession of surface structures such as flagella, capsule, and lipopolysaccharides (LPS), as well as possession of cilia(Pilli or Fimbreae), which helps it adhere to the host's tissues, giving it the ability to produce the biofilm (Zowawi et al .,2015; Terlizzi et al .,2017). as such Excel bacteria Coli Having multiple antibiotic resistance (MDR) Multing Resistance (Laird ,2016) By possessing resistance enzymes, including enzymes β -lactamases, and Other enzyme confer resistance to antagonists aminoglycosides and for quinolones. In addition to its possession of other resistance mechanisms such as changing the permeability of the cell membrane, changing the target site, inhibiting the manufacture of proteins, and its possession of efflux pumps, as it gives bacteria resistant to antibiotics such as Microlides, Novobiocin and Rifamcin (Kapoor et al .,2017).

The exposure of the body to infection with microorganisms generates an immune response that shows its signs through a number of mechanisms that allow the identification of invading microorganisms, as Toll-like receptors (TLR) can distinguish the molecular patterns associated with the pathogen (Pathogen Associated Molecular Pattern, PAMPS) Therefore, it represents the first line of defense against bacterial infections. The immune receptor TLR4 belongs to this type of receptor and has long been known to be the sensitive receptor for the lipopolysaccharide (LPS) of Gram-negative bacteria (Molteni *et al* .,2016) .Many chronic diseases, including chronic renal failure, are associated with an increased state of oxidative stress, represented by an imbalance between oxidants and antioxidants in the body, by increasing reactive oxygen species (ROS), which is one of the Types of free radicals) Therefore, the body resorts to curbing the effect of free radicals by providing antioxidants in the body, which are classified into two types: enzymatic and non-enzymatic. The enzyme (Superoxide dismutase SOD) belongs to the first type of it in view of the role it plays in neutralizing the increase in free radicals in many pathological conditions, while glutathione belongs to the non-enzymatic antioxidants (Rosa *et al* .,2021 ;Farhood *et al* .,2019; Aziz *et al* .,2016).

In view of the seriousness of renal failure, the study aimed to identify the most important bacterial species associated with this disease and their relationship to the levels of some antioxidants and determining the relationship between the high level of one of the immune receptors in the blood of patients with renal failure and the high severity of infection with the dominant bacterial type isolated in this study.

2-Material and methods

2-1-Specimen collection.

Blood samples were drawn from patients using medical syringes and placed in sterile plastic tubes free of anticoagulant materials, then the samples were transferred to the laboratory and after coagulation occurred, they were centrifuged at a speed of 4500 revolutions / min for 4 minutes, then the serum was withdrawn and placed in

tubes and kept in the freezer at a temperature -20°C , while the rest of the blood components were neglected (Garvey et al., 1977).

2-2-Testing the sensitivity of bacterial isolates to antibiotics

All work steps were carried out according to the instructions of the company that supplied Biomerieux, as follows:

- 1- 3 ml of saline solution (normal saline) are placed in sterile plastic tubes.
- 2- A number of purified colonies of bacteria to be diagnosed and activated should be transferred to a suitable medium for a period of 18-24 hours using a sterile transfer loop and mixed well until the solution becomes cloudy.
- 3- The turbidity of the suspension is measured using the Denis Chek device, which must be identical to the turbidity of MacFarland's solution (10^8 cells / ml).
- 4- The suspension is transferred to the holder of the device, and the special card is placed in the holder, followed by tying the card and the suspension through a very thin delivery channel, then the card code is entered by a scanner.
- 5- The holder is placed in a special vacuum chamber, as the bacteria are transferred to the card and distributed in the holes in it.
- 6- The connecting channel is cut off automatically, and the card is closed well to prevent any leakage, and it is transferred to the incubator and incubated at a temperature of $(35-37)^{\circ}\text{C}$.
- 7- The results of the diagnosis are read through a computer that contains a special program through which the wavelengths of the reactions are read and translated through color changes and turbidity as well as metabolites.

2-3-Determination of the level of Toll-like immune receptor (TLR4) in patients' serum by enzyme-linked immunosorbent (ELISA) method.

This assay was carried out according to the manufacturer's instructions (Bioassay), which is an enzyme immunoassay to measure the level of human TLR4 in patients' serum and titration solutions, depending on the specialized binding between it and the specialized antibodies to it that are coated to the holes of the 96-hole test plate.

1. Calibration and control solutions and samples were added to the holes designated for them, according to the map that was placed on a plate.
2. 50 microliters of the standard solution were added to its wells, while avoiding adding antibodies to the standard solution wells, given that the latter contains Biotinylated Antibody.
3. 40 microliters of each sample were added to the sample wells, followed by the addition of 10 microliters of TLR antibody (anti-TLR antibody) to the same wells, followed by adding streptavidin -HRP to the sample wells and the standard solution except for the control wells. The solution was mixed well and the plate was covered. With a suitable cover, it was placed in the incubator at 37°C for 60 minutes.
4. The plate cover was removed and washed well 5 times using buffer solution. This was followed by soaking the pits by adding 0.35 ml of the washing solution for a period of (0.5-1) minutes for each wash, and the plate was dried using filter paper or using other absorbent materials.
5. 50 microliters of A substrate solution was added to each well, followed by the addition of 50 microliters of substrate solution B to each well, and the plate was incubated after covering it well at 37°C for 10 minutes.

6. Add 50 microliters of Stop solution to each hole , noticing the color change in the holes to yellow.

7. The absorbance was read using an ELISA device at a wavelength of 450 nm, then the results were recorded in the form of an absorbance by referring to the standard curve shown in Figure 1 to determine the concentration of TLR4.

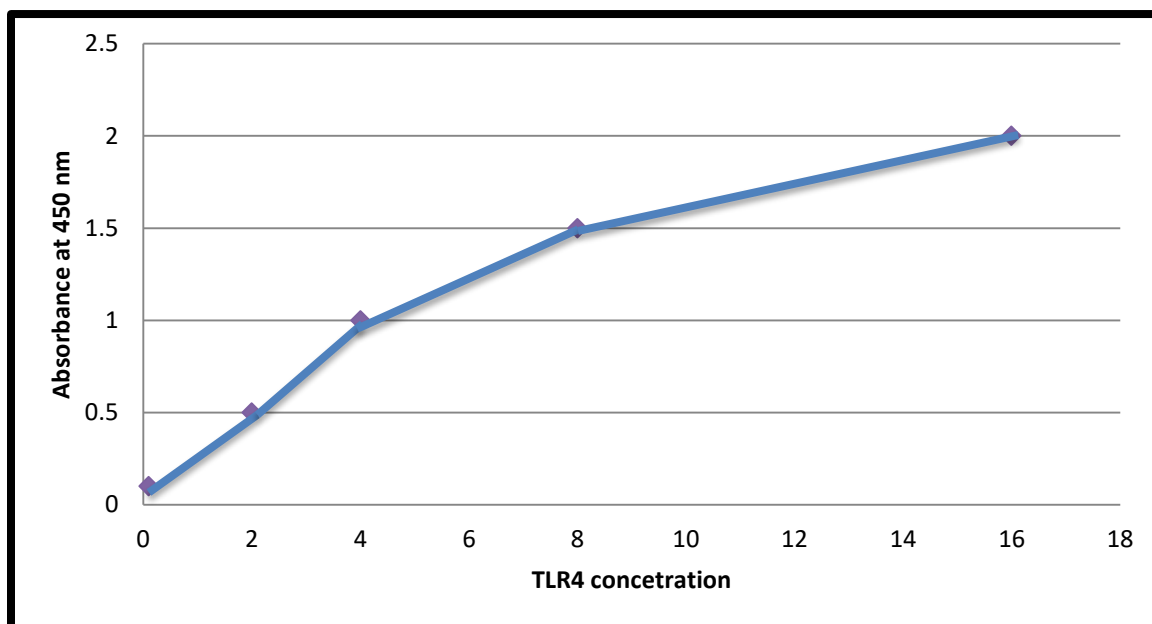


Figure 1: Standard curve for estimation of TLR4 immunoreactivity receptor concentration

2-4-Determination of Superoxide Dismutase (SOD) Enzyme Activity

The reaction mix, according to Marklund and Marklund (1974), consists of 50 µl serum, 2 ml of tris buffer, and 0.5 ml of pyragallol (0.2 mM), a substance that absorbs light at 420 nm. The ingredients in the control solution are identical, with the exception that dH₂O was used in place of the enzyme extract. dH₂O served as a blank. A unit of an enzyme is the amount of the enzyme that can prevent 50% of pyragallol oxidation. Using the following equation, SOD activity was estimated:

$$SOD \text{ Activity (unit)} = \frac{\frac{\%P}{50\%} \times R}{T}$$

Where:

- %P: percentage of the inhibition of pyragallol reduction
- * Note: %P of each sample is determined by comparing the sample's abs (X%) to the control's abs (100%)
- R: Total reaction volume (2.55 ml)
- T: Time of reaction in minutes (2 minutes)

2-5-Determination of Glutathione concentration

Glutathione concentration was determined according to the method of Beutler *et al.*, (1963) and Moron *et al.*, (1979). The acid soluble sulfhydryl groups form a yellow colored complex with dithionitrobenzene

(DTNB). Taken 100 μ l of serum, was mixed with 0.7ml of 0.2M sodium phosphate buffer (pH 8) and 2ml of 0.6 mM DTNB (prepared in 0.2M buffer , pH 8) . The yellow colored obtained was measured after 10 min at 412nm against a blank which contained 0.1 ml of 5%TCA in place of the supernatant. A standard curve was prepared using different concentrations (500-3000 μ g/ml) of GSH in 0.3 ml of 5% TCA as in figure 2. The GSH content was calculated with the help of this standard graph and expressed as micromoles/ml for blood.

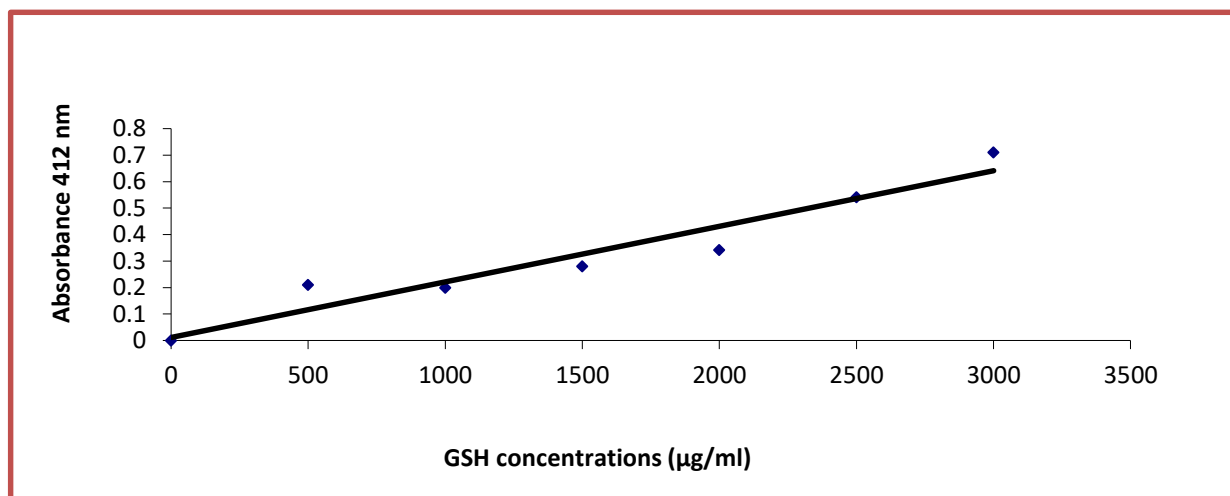


Figure 2: standard curve of GSH

3-RESULTS

3-1-Antibiotic susceptibility of *Escherichia coli* isolated from renal failure patients

By testing the sensitivity of *E.coli* isolates to antibiotics using the Vitec system, and the results shown in Figure 3, it was found that all isolates were 100% sensitive to Imipenem, 95.4% to Amikacin, 72.7% to Gentamicin and Cefepime, while the isolates were 90.9% resistant to Ticarcillin and Ticarcillin. Piperacillin, 63.6% for Sulfamethoxazole, and 50% for Ticarcillin-Clavulanic acid, Aztreonam, and Tobramycin.

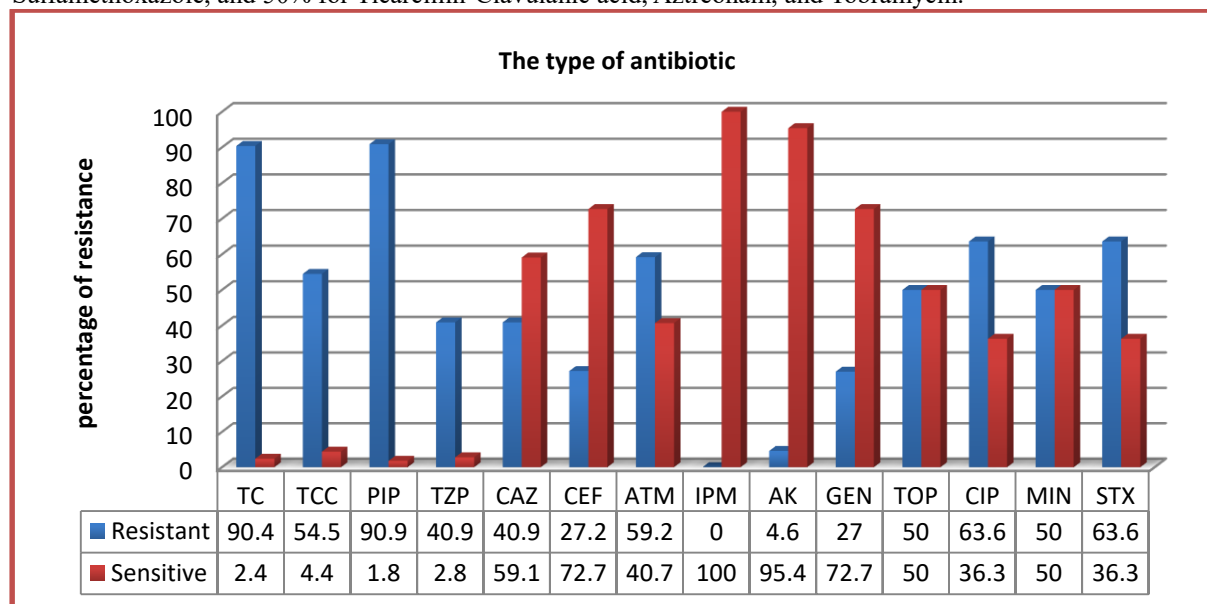


Figure 3: Percentages of antibiotic resistance of *E.coli* bacteria

3-2-Estimation of TLR4 concentration in patients with renal failure

The concentration of the TLR4-like immune receptor was studied from two aspects: the age group as well as the type of pathological injury related to renal failure. It is clear from the table 1 that the highest concentration of the immune receptor reached 1.115 ng / ml in patients with renal failure accompanied by urinary tract infection. The results of the statistical analysis confirmed the presence of significant differences at the level of probability $p < 0.05$ for healthy people and patients with renal failure accompanied by urinary tract infection, as well as those with urinary tract infection, while the renal failure group did not show any significant differences.

Table 1 : Toll-like immune receptor (TLR4) concentration in age groups and renal failure groups under study

age groups	control	Renal failure patients	Kidney failure patients + urinary tract infections	Patients with urinary tract infections	P value	LSD
	Concentration TLR ₄ (ng/ml) Rate $\pm$ standard deviation					
40 – 20	± 0.301 0.196	0.576 0.251 $\pm$	0.733 0.382 $\pm$	0.506 0.212 $\pm$	* 0.047	0.326
60 – 40	0.596 0.115 $\pm$	0.463 $\pm$ 0.787	$\pm$ 1.115 0.432	$\pm$ 0.801 0.290	0.076	NS
70 – 60	$\pm$ 0.462 0.105	0.039 $\pm$ 0.606	$\pm$ 0.629 0.295	$\pm$ 0.599 0.106	0.360	NS
P value	** 0.002	0.392	* 0.050	* 0.048		
LSD	0.146	NS	0.423	0.247		

Many studies have indicated the role of the TLR4 receptor in distinguishing the presence of microbial infection and achieving the initial antibacterial inflammatory response as well as its effective role in the phagocytosis process. Therefore, an increase in the level of TLR4 can be considered one of the important and potential indicators for distinguishing between patients and healthy people. This receptor in the serum of patients during infection confirms the ability of the receptor to stimulate inflammatory reactions in the patient (Molteni *et al.*, 2016).

On the other hand, in this study, the studied groups were classified into three age groups. With reference to the results shown in Table 1, it is also clear that the age group between (40-60) years showed the highest immune response, as the concentration of the immune receptor TLR4 reached 1.115 ng / ml. The results of the statistical analysis confirmed the presence of significant differences $p > 0.05$ in the concentration of this receptor in the age group between (20-40) years, while the remaining two age groups did not show any significant differences. These results indicate the basic role played by these receptors in regulating the primary immune response without being significantly affected by age, although older age groups are often exposed to a condition known as immune tolerance with age, which affects the effectiveness of the immune system directly with age (Chessa *et al.*, 2014; Boehmer *et al.*, 2004).

3-3-Estimation of the activity of the superoxide dismutase (SOD) enzyme:

The activity of the SOD enzyme was studied in patients with renal failure and patients with renal failure who had urinary tract infections as well as those with urinary tract infections and compared it with the activity of the enzyme in healthy people. It is clear from Figure 4 that the highest activity of the SOD enzyme was recorded in healthy people, which amounted to 15.35 units / ml, while the activity of approximately 14,221 units / ml was recorded in

people with urinary tract infections, while the lowest activity of the enzyme was recorded in patients with renal failure, which amounted to 1.645 units / ml.

The results of the statistical analysis came to confirm the results above, as the effectiveness of the SOD enzyme activity decreased significantly in patients with both renal failure and renal failure accompanied by urinary tract infection. The enzyme activity reached (1.649 and 2.608) units / ml, respectively, while it was not observed There were no significant differences in enzyme activity between healthy subjects and patients with urinary tract. While no significant differences were achieved between patients with renal failure and patients with renal failure accompanied by urinary tract infection.

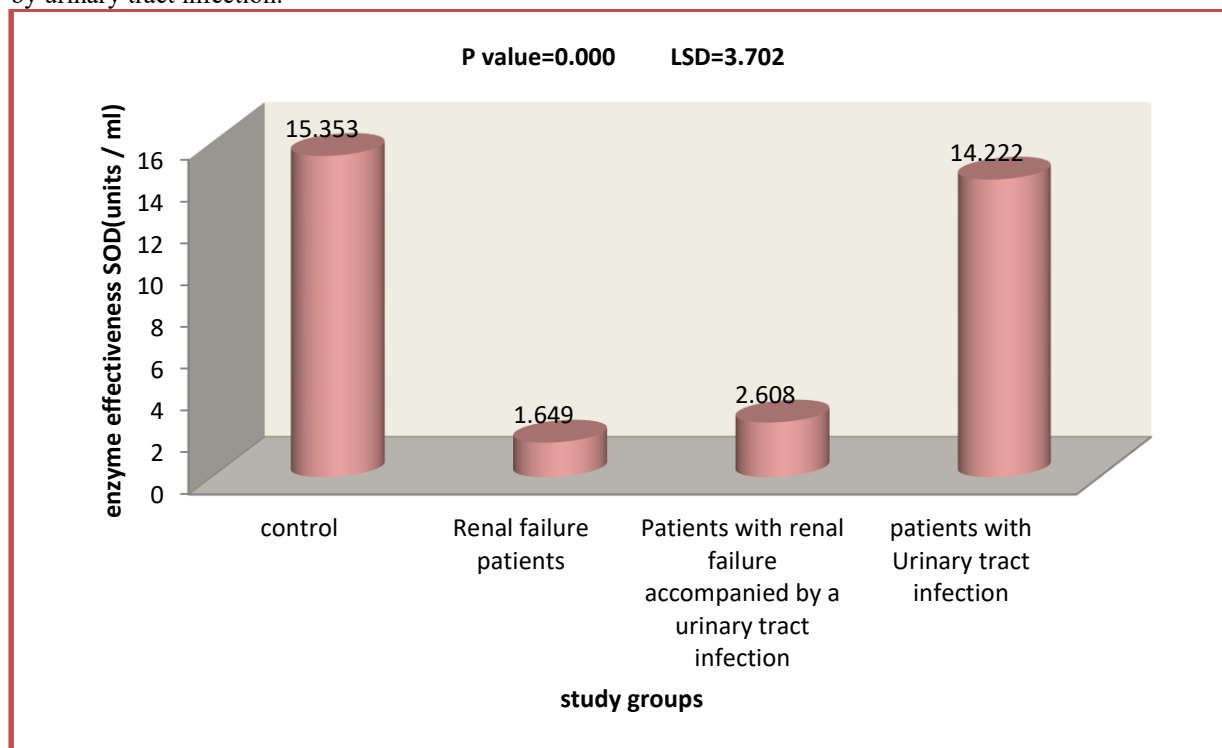


Figure 4 : SOD enzyme activity (unit/ml) in healthy subjects, renal failure patients, and urinary tract infection patients.

3-4-Estimation of Glutathione Concentration

The concentration of glutathione was estimated in patients with renal failure and patients with renal failure who have urinary tract infections as well as those with urinary tract infections and compared it to the concentration of glutathione in healthy people. It is clear from Figure 5 that the concentration of this compound in healthy subjects increased to 6.7 micromoles / ml, while its concentration reached 6.1 micromoles / ml in patients with urinary tract infections, while the lowest concentration was 2.41 micromoles / ml in patients with kidney failure who had urinary tract infections.

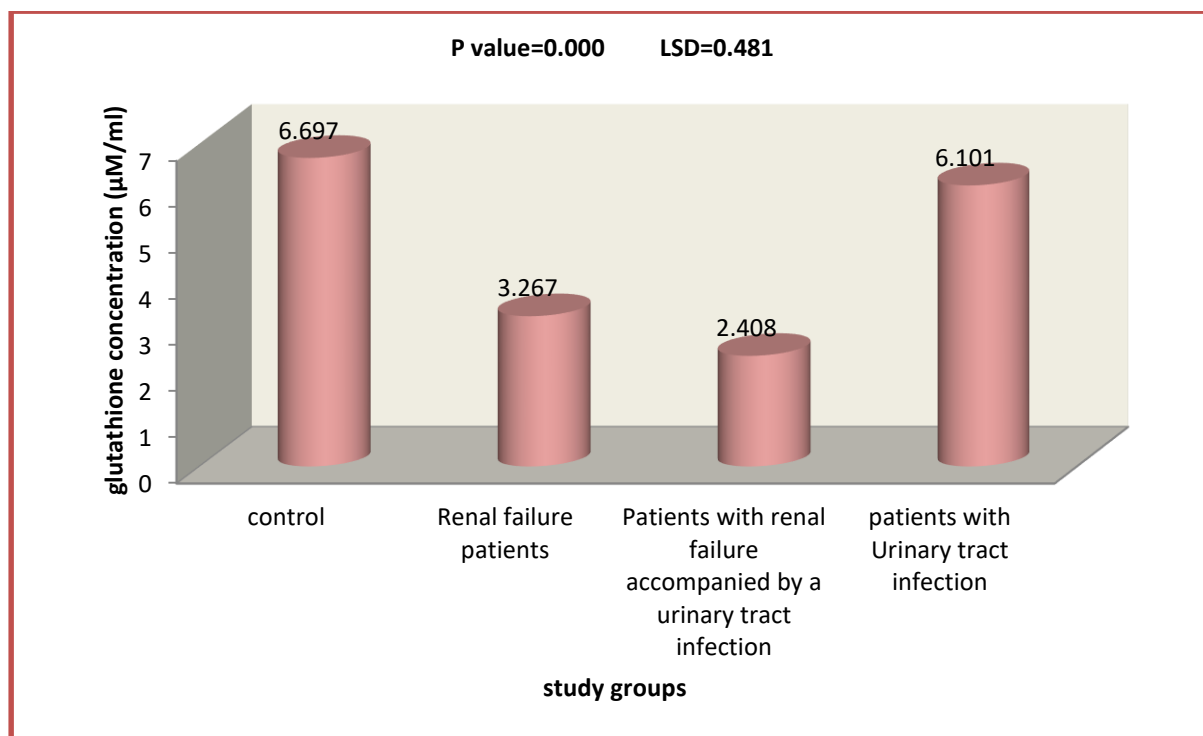


Figure 5 : Glutathione concentration in healthy subjects, renal failure patients and urinary tract infection patients.

The results of the statistical analysis showed that there were significant differences between the study groups, as the concentration of glutathione decreased significantly in patients compared to healthy subjects. A significant decrease was also noted in the concentration of this antioxidant in patients with renal failure compared to patients with urinary tract, as well as a significant decrease in its concentration in patients with renal failure.

4-DISCUSSION

The results showed that the *E.coli* bacteria isolates were 100% sensitive to imipenem, as this antibiotic is classified within the carbapenem group, which is a class of beta-lactam antagonists. Its mechanism of action is summarized as causing lysis of sensitive bacterial cells through its high tendency to bind to (penicillin binding protein, PBPs) (Zhanel et al., 1998).

On the other hand, microorganisms are able to resist carbapenem antibiotics through a number of mechanisms, including the production of oxacillinase enzymes that break down the beta-lactam cycle (Turton et al., 2006) or through the loss of one of the outer membrane proteins through which the antibiotic passes, known as the *Caro* protein (Mussi et al., 2005).

Tobramycin, Gentamicin, and Amikacin belong to the group of Aminoglycosides. Bacteria resist these antibiotics through several mechanisms, including: The first: inhibition of the antibiotic through an enzyme that changes the structure of the antibiotic by transferring a functional group of the antibiotic such as acyl, ribosyl, phosphoryl, or thiol groups. By changing the composition of the antibiotic by the action of the nucleotide transferase enzyme, and the second: it is represented by modifying the target of the antibiotic by the process of methylation on the 16S rRNA DNA, and the third: reducing the permeability of the bacterial wall to antibiotics and thus preventing them from entering the bacterial cell (Kumar and Varela, 2013). TCC, CAZ and ATM antagonists are classified within the group of β -lactams, and bacterial resistance to these antibiotics can be achieved by three mechanisms. As for the third cell, it depends on changing the specific target of the antibiotic present in the cell, which makes it impossible for the

antibody to bind to the target for which it is intended (Kumar and Varela, 2013). Ciprofloxacin belongs to the group of Fluoroquinolones, and bacteria can resist it by reducing the permeability of the bacterial wall to antibiotics, and thus not killing the bacteria (Kumar and Varela, 2013).

Many studies have indicated the role of the TLR4 receptor in distinguishing the presence of microbial infection and achieving the initial antibacterial inflammatory response as well as its effective role in the phagocytosis process. Therefore, an increase in the level of TLR4 can be considered one of the important and potential indicators for distinguishing between patients and healthy people. These results indicate the basic role played by these receptors in regulating the primary immune response without being significantly affected by age, although older age groups are often exposed to a condition known as immune tolerance with age, which affects the effectiveness of the immune system directly with age (Chessa et al., 2014; Boehmer et al., 2004). Only a few studies have reported low expression of the immune receptor TLR4 on monocytes in adult patients and recurrent urinary tract infections, while several studies have indicated high expression of the immune receptor mentioned on the same cells in children (Karananou et al., 2016).

A study by Boehmer et al. (2004) showed that the levels of the TLR4 receptor did not change with age, with the participation of TLR4 in lipopolysaccharide (LPS)-stimulated inflammatory cytokine activation, and thus the decreased expression of this receptor on the cell surface of the automaton might be responsible for the associated development. by age and decreased TNF and IL-6.

Panda et al (2010) explained that there is a clear relationship between age and the decrease in the rate of mononuclear cells and macrophages, with a variation in the rate of expression of surface receptors TLRs, and thus a weakness in the immune response, which confirms the weakness of immunity in the elderly.

The result obtained from this study is consistent with what was obtained by Magalhães et al (2011), as it was noted that the activity of SOD enzyme decreased in patients with renal failure 3533 units / g hemoglobin, compared to healthy subjects 4120 units / g hemoglobin, and Tian *et al*, (2022) got the same result The activity of the SOD enzyme in patients with renal failure was 108.4 units / ml compared to healthy subjects (164.23 units / ml). (Nouri et al., 1999).

The above results can be explained by the fact that some factors play an important role in changing the activity of the SOD enzyme in patients, including the lack of trace elements necessary to activate the activity of the enzyme, such as zinc (Zn) (Magalhães *et al.*, 2011) and on the other hand, the decrease in the activity of the enzyme can be attributed To an increase in the concentration of hydrogen peroxide H₂O₂ caused by a decrease in the activity of the enzyme Glutathione peroxidase (Nouri *et al.*, 1999), the SOD enzyme plays an important role in offsetting the increase in free radicals in many pathological conditions (Rosa et al., 2021).

The results obtained from this study agree with what was found by (Rico et al., 2006) that the value of glutathione decreased to 14.23 nmol / mg protein in patients with renal failure compared to healthy subjects, which amounted to 26.19 nmol / mg protein.

Glutathione performs several physiological functions, including protecting cells from the destructive effect of active oxygen species, and removing the toxicity of some substances such as drugs and environmental pollutants, and maintaining the stability of the red cell membrane, as well as enhancing immune function through its effect on lymphocytes (Julius et al., 1994). Glutathione is present in two forms in cells: the first is the oxidative form (GSSG), which is highly toxic, so the organism converts it into the second form (reduced .GSH), so the ratio between these two forms is important to determine the degree of oxidative stress in the cell (Rico et al., 2006).

5-CONCLUSION

1- Despite the diversity of bacterial species associated with renal failure, E.coli bacteria prevailed.

2- The high incidence of kidney failure in the holy city of Karbala, which reflects the deterioration of the health situation that the Iraqi citizen suffers from.

3- The possibility of using the concentration of the Toll-like immune receptor TLR4 as an indicator to detect the presence of *E. coli* and its association with cases of renal failure.

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الخلاصة

مرض الكلى المزمن (CKD) هو اضطراب شائع يتزايد في جميع أنحاء العالم. يتعلق الأمر بتطور المرض الكلوي في نهاية المرحلة (الداء الكلوي بمراحله الأخيرة) ، وقد تضمنت هذه الدراسة فحص حساسية عزلات *E.coli* للمضادات الحيوية باستخدام نظام Vitec وجمع عينات الدم من المرضى الذين يعانون من الفشل الكلوي والتهابات المسالك البولية. اللذين يرتادون مدينة الإمام الحسين الطبية / وحدة غسيل الكلى واستشاري المسالك البولية للفئات العمرية من (20-70) سنة لكلا الجنسين لتحديد تركيز المستقبل المناعي الشبيهة بالتول (TLR₄). بالإضافة إلى تقدير فعالية انزيم Superoxide dismutase (SOD) وتركيز الجلوتاثيون، ومن خلال نتائج تقدير المستقبل المناعي (TLR₄) أتضح أن الفئة العمرية بين (40-60) سنة أظهرت أعلى استجابة مناعية حيث بلغ تركيز هذا المستقبل المناعي 1.115 نانوغرام / مل. وأكدت نتائج التحليل الإحصائي وجود فروق معنوية $P > 0.05$ في تركيز هذا المستقبل في الفئة العمرية بين (20-40) سنة ، بينما لم تظهر أي فروق معنوية في المجموعتين العمريتين المتبقيتين. من خلال تقدير نشاط مضادات الأكسدة الإنزيمية في المجموعات قيد الدراسة ومقارنتها مع نشاط الإنزيم لدى الأشخاص الأصحاء ، اتضح من النتائج أن أعلى نشاط لإنزيم SOD تم تسجيله في الأشخاص الأصحاء والذي بلغ 15.35 وحدة. / مل بينما لوحظ انخفاض معنوي في نشاط هذا الإنزيم لدى جميع مرضى الفشل الكلوي والمرضى المصابين بالفشل الكلوي المصحوب بالتهابات المسالك البولية. وأظهرت نتائج تقدير تركيز الجلوتاثيون (GSH) في المجموعات قيد الدراسة تركيزاً عالياً لهذا المركب في الأشخاص الأصحاء 9.7 ميكرومول / مل ، بينما لوحظ انخفاض كبير في تركيزه في كل من مرضى الفشل الكلوي ومرضى الفشل الكلوي المصحوبين بالتهابات المسالك البولية.