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Correlation between IL-1β, IL-18 and some hematological variable in uropathogenic *E. coli* infected UTI patients.

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ABSTRACT

Urinary tract infection (UTI) is one of the most common types of infection acquired from society and hospitals, so the current study aimed to know the extent of this infection and to detect the causative bacteria, as well as to know the effect of urinary tract infections on some blood and immune indicators represented by interleukin 1 beta (IL-1β) and interleukin 18 (IL-18). The relationship of some hematological and immunological indicators with urinary tract infection and the study of the relationship of IL-1β and IL-18 with each other on the one hand and with some hematological indicators on the other hand was studied, which is a first study of its kind. In this study, 77 blood and urine samples were collected from patients with urinary tract infection, while 10 samples were taken from healthy patients without UTI and who do not suffer from chronic diseases and they were used as a control group. All urine samples were examined and cultured, in addition to conducting laboratory blood tests to identify blood variables, and the level of some immune variables, which included IL-1β and IL-18, was measured using the Enzyme Linked Immune Sorbent Assay (ELISA) technique. The results obtained from urine culture, 61 out of 77 samples recorded bacterial growth (positive result) and 16 samples did not record bacterial growth (negative result), while all healthy samples were culture negative, 4 types of bacteria causing urinary tract infection were isolated. In our study, *S. aureus* bacteria constituted the largest percentage, 57.4%, followed by *E. coli* bacteria, 24.6%. The current study also showed that the most affected age group of adults is the group (15-29 years), with a rate of 37.7%. When evaluating the level of IL-1β in patients and comparing it with the healthy group, a significant increase of 0.0957 pg./ml was found in the patients compared with the healthy controls. And when evaluating the level of IL-18, a significant increase was found at a rate of 0.1107 pg./ml in patients compared to healthy controls with a lower level. With regard to blood variables, an increase in the level of white blood cells was observed (7.82 ±3.32) in the affected patients when compared with the control group (6.71 ±1.01). As for the other variables, non-significant differences were noted when comparing patients with the healthy ones. The interrelationships between the variables were studied. Hematological and immune variables in patients and comparing them with the control group, it was found that there is a positive correlation between IL-1β and IL-18, as well as a positive relationship between white blood cells with the two types of immune variables.

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1. INTRODUCTION

Urinary tract infections are one of the most common types of infections worldwide that occur as a result of the entry and multiplication of microorganisms inside the urinary system consisting of the kidneys, ureters, urinary bladder and urethra, which is one of the important organs in the human body due to the nature of the function it performs in purifying the blood from substances The harmful and excess of the body's need and disposal in the form of urine. The contents and characteristics of urine are good indicators that reflect the normal and pathological physiological state [20]. Urinary tract infections affect both sexes and at different ages, and usually females are more susceptible to infection than males. The reason is due to some physiological and anatomical differences in the composition of the urinary system for both sexes [26]. These infections are either accompanied by symptoms or without symptoms [15].

The majority of UTIs are caused by bacteria. Gram-negative Enterobacteriaceae, particularly *E. coli*, have the highest incidence compared to other bacterial pathogens, and the pathogenicity of these bacteria is associated with many of their virulence factors [3], followed by other bacterial species of the same family such as *Proteus spp* and *Klebsiella spp*, while Gram-positive bacteria such as *Staphylococcus spp* and *Streptococcus spp* play a lesser role in infection rate [24]. Urinary tract infection is accompanied by many changes in the immune system and in the blood cells, which are one of the most important elements and mechanisms of the immune system because of their effective defensive role against pathogens and foreign bodies that enter the body. Any change in the proportions and numbers of these cells is an indication of an infection Inflammation in the body [11]. The immune system performs its protective function through two types of immune response: the innate immune response and the specific (innate) immune response [1]. The pathogen is identified by the binding and activation of PAMPs on pathogen surfaces with PRRs on host cell surfaces followed by recruitment of immune cells such as neutrophils, mononuclear cells and macrophages to perform their immune functions such as phagocytosis and ingestion of pathogens, causing apoptosis in addition to stimulating the production of cytokines. Such as interleukines, interferons, tumor necrosis factor and chemokines, especially the production of pro-inflammatory cytokines [16]. Interleukins are small protein molecules produced by immune cells in response to microbes and other antigens that regulate the local and systemic inflammatory response belong to the interleukin-1 (IL-1) family and Interleukin-18 (IL-18) are among the most important pro-inflammatory cytokines [18]. They are produced by many cells in the form of inactive proteins that are activated by activating the inflammasome, which in turn binds with Caspase-1 and activates it to cleave the inactive interleukin, converting it to the active form and releasing it (Dinarello, 2018). IL-1β exerts its protective role against infection by activating several immune responses including rapid recruitment of neutrophils to inflammatory sites and activation of endothelial adhesion molecules, induction of other cytokines (such as IL-2) and chemokines, and induction of a specific type of adaptive immune response (specifically). From the T_h17 response, has a role in mediating auto-inflammatory diseases, and despite the protective and defensive role played by IL-1β, when its production and activity is not controlled, it is harmful, it is considered one of the most causes of tissue damage [16].

One of the primary functions of IL-18 includes activating immune-important cells by binding to the IL-18R receptor composed of two polypeptide chains, IL-18Rα and IL-18Rβ; The IL-18R chain is responsible for binding, however, it binds with IL-18 with low affinity, the second IL-18Rβ chain promotes binding where it acts as a co-receptor enhancing the strength with which the receptor binds to IL-18 and its signal transduction

into the cell [7], [25].

2. Materials and methods

2.1 Sample collection

A total of 77 urine and blood samples were collected from patients with urinary tract infection, based on the initial diagnosis by a specialist doctor based on clinical symptoms, who attended KIRKUK General Hospital and TOZ General Hospital, and 10 samples were collected as a control group from healthy people without urinary tract infection and not suffering from any chronic diseases. The samples included the age group ranging from 15-60 years.

2.2 Urine samples collection

All urine samples were collected in sterile plastic bottles, after instructing the patient in the correct way to collect the sample, by collecting the sterile middle part of the urine and neglecting the rest to avoid contamination. The sample was transferred to the laboratory for diagnostic tests [5].

2.3 Blood sample collection

77 blood samples were collected from the same people from whom urine samples were taken and 10 blood samples were taken from the same healthy ones, 5 ml of blood was withdrawn using a sterile medical syringe and divided into two parts: the first section 3 ml of blood was placed inside sterile gel tubes free of any blocking substance Then it was placed in a centrifuge at 3000 rpm for 15 minutes to separate the serum from the rest of the blood components. - It was later used in the ELISA technique to measure the level of IL- 1β and IL-18 [22]. As for the second section of blood, 2 ml, 2 ml was placed in EDTA (Ethylene Diamine Tetra Acetic Acid) tubes, which contain an anticoagulant, and it was used in the CBC blood count test to examine some blood variables.

2.4 Culture of urine samples and bacterial isolation

Urine samples were cultured on blood agar medium and MacConkey agar medium using the Streaking method on the surface of the agar in the Petri dishes. Then the growth of bacteria was observed and the bacterial colonies growing on the plate were counted, and the culture result was positive if the number of bacteria was greater or equal to 104 colony- forming units per ml of urine (104≤ CFU/ml).

2.5 Diagnosis of isolated bacteria

Bacterial isolates were diagnosed by studying the phenotypic and cultural characteristics of bacterial colonies growing on different culture media. The study included: the shape, size, color, texture and height of the colonies, as well as a study of the type of hemolysis present on the medium of the blood agar [23] and a Gram-stain test to differentiate positive bacteria for dye-negative bacteria. Also, studying the chemical properties of each type by conducting biochemical tests for each type of positive and negative bacteria separately, based on what was stated by [8], [17], [14], in addition to that. Using API 20E strip to diagnose Enterobacter species.

2.6 Measurement of the level of IL-1β and IL-18 in serum

Quantification of IL-1β and IL-18 in serum was performed using Sandwich enzyme-linked immunosorbent assay technology (ELISA) according to the kit manufacturer's instructions. The IL-18 Human kit is provided with all materials and reagents needed for the examination.

3. Results and discussion

After the urine samples were transplanted, the results of our study showed that 61 urine samples i.e., 79.2%, out of 77 samples gave bacterial growth and confirmed infection, while 16 urine samples 20.8% did not give any bacterial growth. The results of our study differ with the results of [9]. The infection rate in his study was 38.09%, and the reason for the difference in results may be due to the difference in the number of samples, methods of isolation, and the difference in age group between our study. While the result of culturing healthy samples that were used in our study as a control group was negative, it did not show bacterial growth.

The results of the current study also showed that the infection rate in females reached 76.6%, which is higher than that of males, 23.4%. Rate the ratios between one study and another [27]. As for the relationship of urinary tract infection with age, our results showed that the highest infected group is the age group 15-29 years with a rate of 37.7%. Studies indicate that adults are at risk of infection, especially (15-65 years) due to the most active sexual relations and the low level of urinary tract functions in the age of more than 50 years.

Figure (1) shows the types of bacteria isolated in this study. *Staphylococcus aureus* bacteria constituted 57.4% of the largest percentage, followed by *E. coli* bacteria, and this result is consistent with the result of [19] study, in which *S. aureus* bacteria also constituted the highest percentage among the isolates taken from Children with urinary tract infection and *E. coli* ranked second. In recent years *S. aureus* has been documented as a common cause of hospital and community acquired infections that range from dermatitis, endocarditis, pneumonia and urinary tract infection. Most bacteria are found on the skin in various areas of the body, including the vagina and urethra, and are opportunistic. Entering the urinary tract, it can cross the urinary opening and the urethra and reach the bladder and kidneys when conditions are created for it, causing a urinary tract infection. The same applies to *E.coli*, which is the main cause of urinary tract infection, especially in women, and ranked first among the bacteria isolated in many other studies related to urinary tract infection, due to the fact that it is a natural flora that lives in the intestine and infection often occurs through the outlet. , either because of contamination of the hands with this bacteria or because of the anatomical structure of the female and the proximity of the urinary system opening to the outlet opening, which leads to contamination and colonization of bacteria present in this area and their ascent to the bladder and other parts of the urinary system [10].

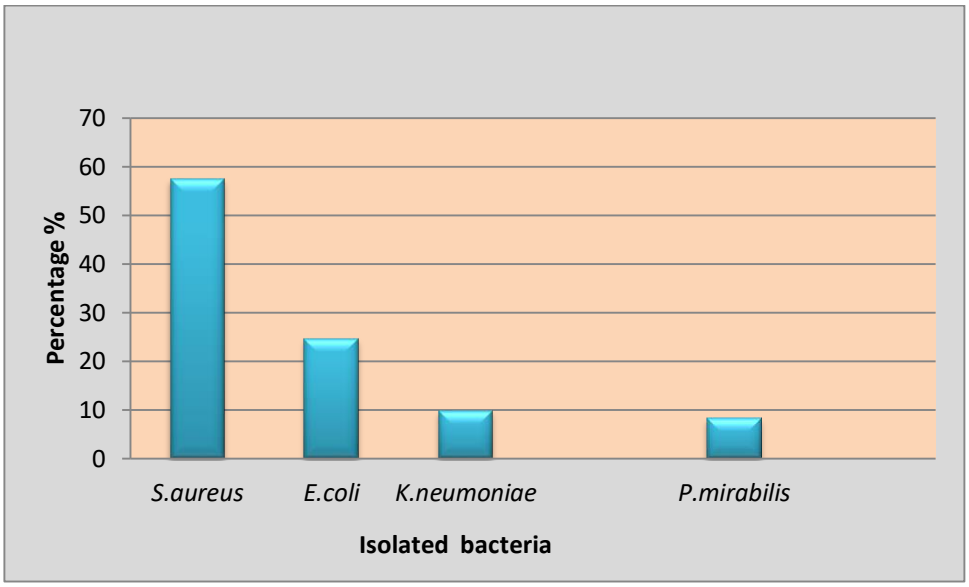

Figure (1): Percentages of the bacteria species isolated.

Among the objectives of the current study is to measure the serum level of IL-1β and IL-18 in patients with

UTI, to confirm that they are an important diagnostic marker for UTI. In general, the study included 77 patients and 10 healthy subjects (as a control group), as shown in Table (1). It was found that there was a significant difference, as it was noticed that the level of IL-1β in the infected was higher compared to the healthy.

Table (1): shows the level of IL-1β in healthy and UTI patients.

Samples	Numbers	IL-1β (Pg/ml)	P-value
		Mean ± SD	
Patients	77	0.0957 ± 0.0245	0.0002**
Healthy (controls)	10	0.0825 ± 0.0064	
** It means that there is a significant difference from the level **0.01**			

And depending on the result of intrauterine transplantation, the samples taken were distributed among 3 totals. The first group included 61 samples representing samples with a positive result of transplantation, and the second group included samples with a negative transplant result, which is 16 samples, and the third group represented the control group that included 10 samples. The results obtained showed a significant increase in the level of IL-1β in the first group that was culture-positive samples (0.09959 ± 0.02599 Pg./ml), while the other two groups recorded a lower level. This study showed that the serum level (IL-1β) It was higher in patients with urinary tract infection, especially the group with positive urine culture results than in the healthy group, as in the study of AL-Tikrit and his group (2019) which recorded the level of IL-1β (10,948 ± 2.982 Pg./ml) in patients with urinary tract infection. Urinary tract infection is higher than the level of the healthy group (0.794 Pg./ml ±0.945) in agreement with our study in that the level of IL-1β in patients is higher than the healthy group, but in contrast with our results in terms of IL-1β level values, the reason for this difference may be the difference The quality of the equipment used and its manufacturer, as well as the different quality of the device used in the ELISA technique. It should be mentioned that the results of our study were within the limits of the IL-1β standers curve supplied by the manufacturer of the kit. When comparing the level of IL-1β between the isolated Gram-positive and Gram-negative bacteria, it was found that its level in Gram-negative bacteria was higher (Pg./ml 0.1028 ± 0.0241) compared to In Gram-positive bacteria (0.0972 ± 0.0274 Pg./ml), in a study supporting our results, AL-Tikrit and his group (2019) mentioned in his study that the concentration of IL-1β in infected with Gram-negative bacteria is higher than that in patients with positive bacteria may be the reason for this difference in the level of Gram-positive bacteria. IL-1β between the two types of bacteria is due to antigenic factors and PAMPs, especially LPS, which negative bacteria possess have a greater effect than positive bacteria in activating the inflammasome that activates Cspase-1, which in turn converts the inactive Pro-IL-1β to IL-1β Active and assists in its editing [21]. In addition, some studies indicate that phagocytosis of peptidoglycan from the cell wall of S. aureus bacteria leads to the activation of NLRP3 including the release of active IL-1β, but it is interesting that these bacteria can modify their cell wall peptidoglycan to avoid recognition by NLRP3 and this may be from Among the reasons that lead to a lower level of IL-1β in infected with these bacteria [18].

As well as for the level of IL-18 in the serum, we also found that its level in infected patients is higher than in healthy ones, as shown in Table (2).

Table (2) shows the level of IL-18.

Samples	Numbers	IL-18 (Pg/ml)	P-value
		Mean ± SD	

Patients	77	0.1107± 0.04580	(0.0003) **
Healthy (controls)	10	0.0817± 0.00663	
** It means that there is a significant difference from the level 0.01			

And when we compared the level of IL-18 between patients according to the result of implantation of urine, we found that the level of IL-18 in the blood serum of patients whose urine samples showed bacterial growth was higher than the level of patients whose samples did not show bacterial growth. This result does not contradict the result of the study of [12], [13] which stated that the serum IL-18 level in patients with glomerulonephritis (which is a type of urinary tract infection) is higher compared to healthy controls.

IL-1β and IL-18 are produced by a variety of cells, the most important of which are mononuclear cells, dendritic cells, and macrophages, and are secreted as a response by host cells upon recognition of the pathogen in the form of inactive primitive proteins called Pro-IL-1β and Pro-IL 18- Cytosol accumulates in the cytosol until it is processed by activating NLRP3, which in turn binds to another protein complex called the Inflammasome to activate it, then binds them with the enzyme Caspse-1 (cysteine protease-1) to cleave and convert pro-IL-1β and Pro- IL-18 to IL-1β and IL-18 are allowed to be released from cells [4], [6], [7]. Despite the results of all the studies mentioned, research and studies are still insufficient to analyze and interrogate many questions related to how IL-1β and IL-18 are activated and work and their relationship to bacterial infections. Those studied on them differ in the severity of their infection, in addition to their different geographical areas, and also the different nature of the samples taken. They may be blood samples, tissue samples (it is mentioned that the level of interleukinin is higher in tissues) or urine, or the reason may be technical such as the difference in the kit and devices used for detection.

Through the statistical analysis of correlation coefficient between IL-1β and IL-18, a positive correlation R = 0.089 was found between interleukinin in patients with urinary tract infection (P-value = 0.441) as shown in Figure (2). In healthy subjects, a positive correlation was also shown (R = 0.175, P-value = 0.630) as shown in Figure (3).

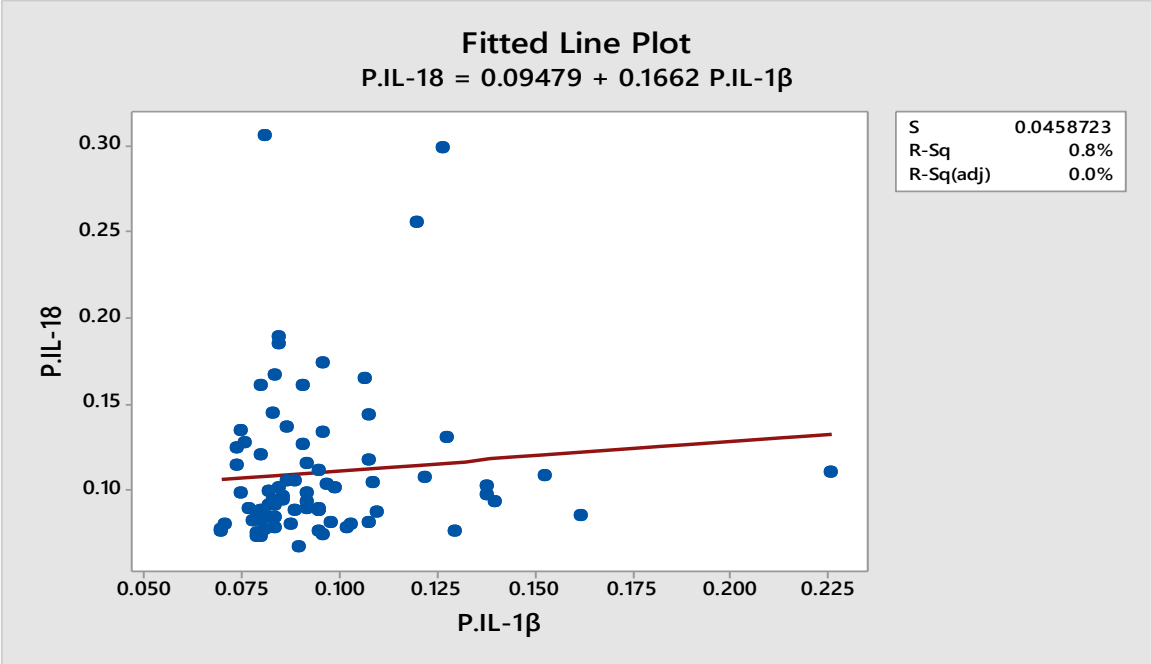

Figure (2): shows the correlative relationship between IL-1β and IL-18 in patients.

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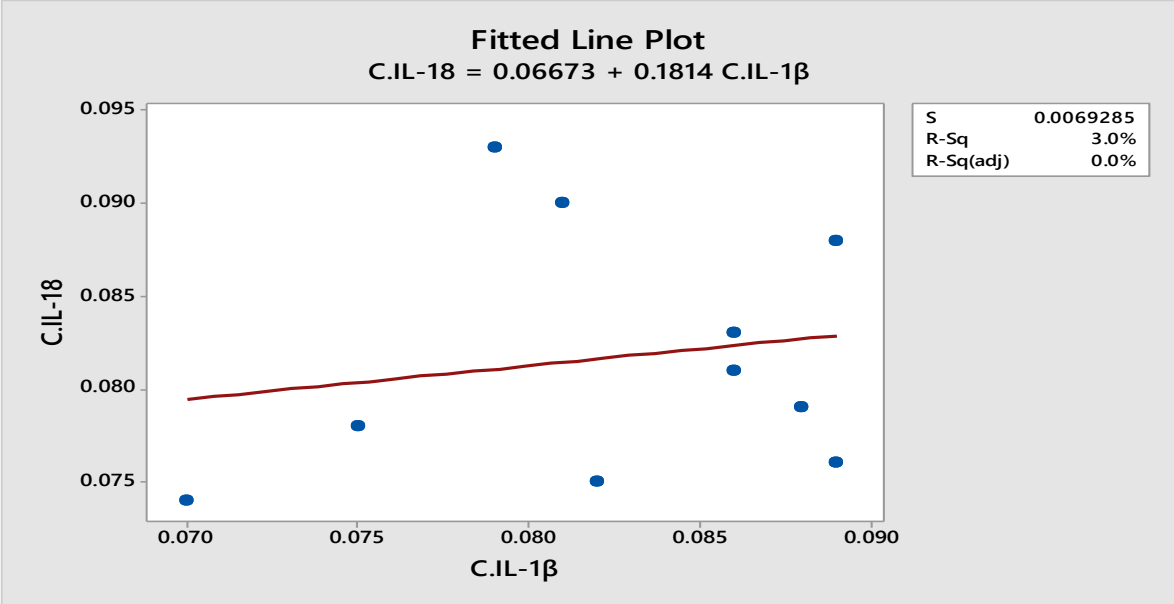

Figure (3): shows the correlative relationship between IL-1β and IL-18 in healthy.

And our aim in this study was also to know the relationship of some blood variables with urinary tract infection, so we divided the samples into three groups depending on the result of urine culture as shown in Table (3). It was found that there is a significant and clear difference in the level of white blood cells. The positive level of culture was high (8.115 ± 3.600) compared with patients who did not show any bacterial growth (6.700 ± 1.481) and with the control group (6.710 ± 1.013).

Table (3) shows the values of hematological variables for the injured and healthy people, depending on the result of the urine culture.

Patients	**Hematological variables**				
	WBC 10^9/L	**Neu 10^9/L**	**LYM 10^9/L**	**PLT 10^9/L**	**MPV Fl**
Positive culture	8.115±3.600 a	5.175±3.213 a	2.259±0.728 a	272.1±67.0 a	8.272 ±0.983 a
Negative culture	6.700±1.481 b	4.075±1.288 a	2.006±0.543 a	255.5±42.7 a	8.094 ±1.165 a
Controls	6.710±1.013 b	3.790±0.711 a	2.280±0.333 a	256.3±59.1 a	8.030 ±0.411 a
Different letters mean there is a significant difference					

White blood cells are one of the main immune mechanisms and their main function is to secure the body's defense against pathogens and other foreign bodies that invade the body, so their numbers increase when inflammatory infections occur, including urinary tract infection.

As for the correlation between hematological variables and between IL-1β and IL-18, the correlation factor between WBC and IL-1β was positive with R = 0.061 and P-value = 0.597 for the group of patients, while for the control group, the association between WBC and IL-1β was negative R = -0.181 and P-value = 0.616. Figures (4) and (5) show this.

While the correlation coefficient between WBC and IL-18 was also positive for the group of patients, R=0.227 and P-value=0.047, while their correlation coefficient in the control group was negative R=-0.734 and P-

vale=0.016. As shown in Figures (6) and (7).

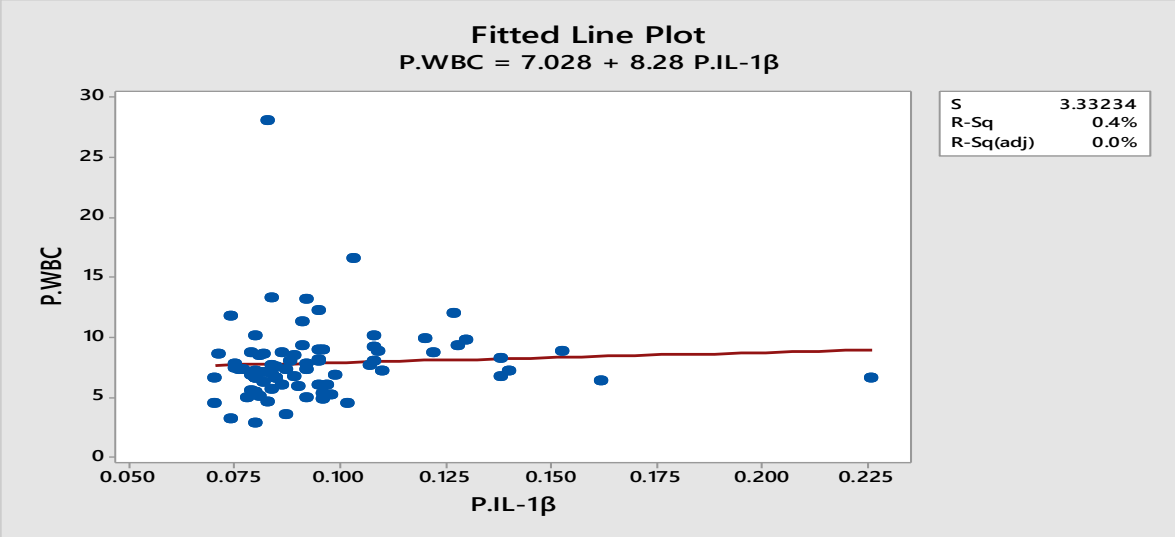

Figure (4): shows the correlation coefficient between IL-1β and WBC in patients.

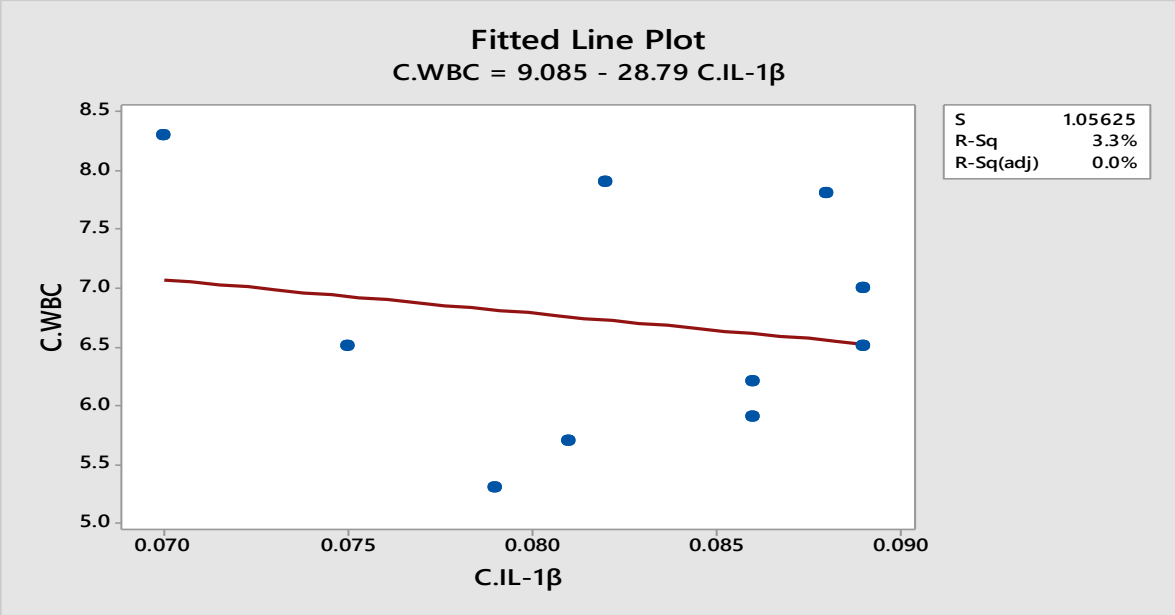

Figure (5): shows the correlation coefficient relationship between IL-1β and WBC in healthy.

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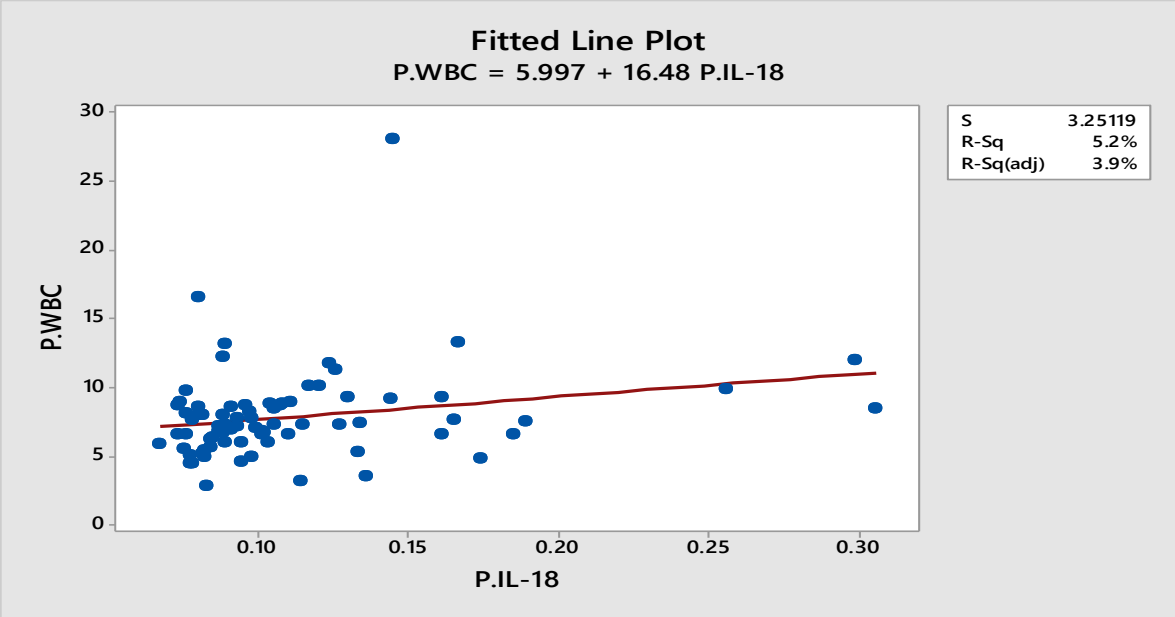

Figure (6): shows the correlation coefficient between IL-18 and WBC in patients.

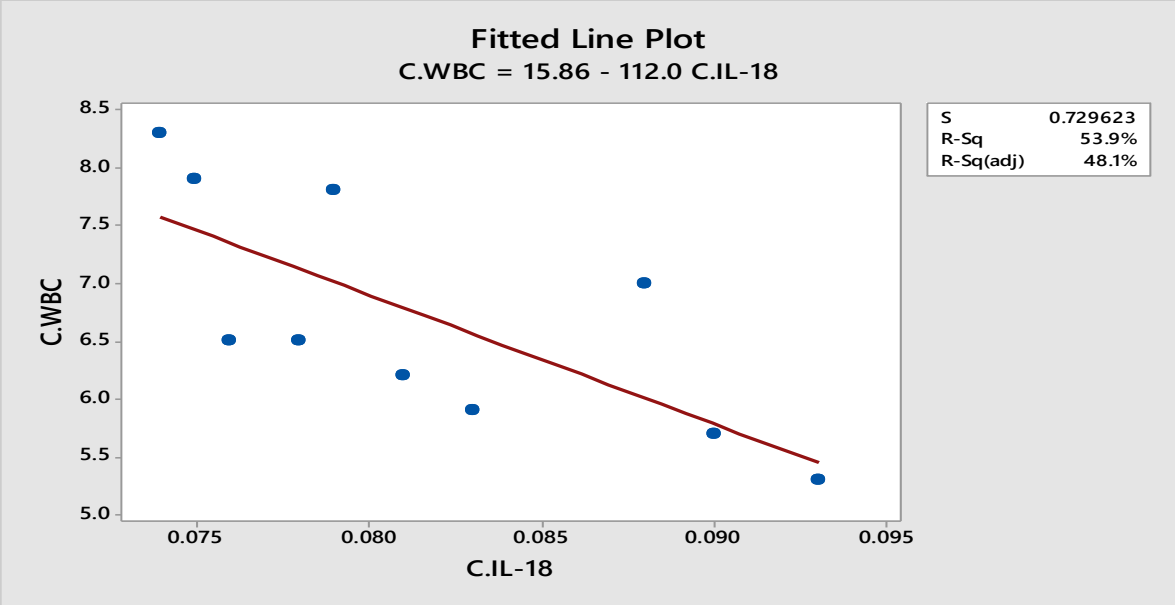

Figure (7): shows the correlation coefficient between IL-18 and WBC in healthy.

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