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Studying the relationship between IL-18, INF- β and mycoplasmas among urinary-genital tract infections for females in Babylon Province, Iraq

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Abstract---Mycoplasma is causing many reproductive diseases and genital disorders, as they are associated with other bacteria such as Ureaplasma urealyticum. The aim of the study is to determine the relationships between the fastidious microbial infection (Mycoplasma spp. and Ureaplasma spp.) and some immunological parameters for urinary-genital tract infections (UGTIs). The study includes a collection of (223) samples (urine and blood) from UGTIs patients (female only), (according to the initial laboratory diagnosis by the Parasitological unit for all the patients under study), who arrived at the Hospital in Babylon (from January to June 2021). The results of the immunological examination of some immunological parameters were done using ELISA for the measurement of IL-18 and INF- β concentration. The mean of IL-18 urine concentration in patients (13.39 ± 3.3 pg/ml) was significantly increased at a p-value ≤ 0.05 compared with the control (10.63 ± 2.1 pg/ml), while the mean of IL-18 serum concentration in patients (12.01 ± 9.8 pg/ml) has no significant difference compared with the control group (9.22 ± 2.4 pg/ml). The mean INF- β urine concentration in patients (269.89 ± 66.1 pg/ml) was significantly increased compared with the control (210.22 ± 69.3 pg/ml), also the mean INF- β serum concentration in patients (230.56 ± 91.5 pg/ml) was significantly increased compared with the control group (174.38 ± 36.2 pg/ml). We concluded that the present study indicates that the patients with UGTIs due to Mycoplasma s had significantly higher IL-18 than the control group in urine only, while INF- β was significantly higher in urine and serum.

Keywords---Urinary-genital tract infections (UGTIs), Mycoplasmas, IL-18, INF- β

1 Introduction

Mycoplasma is one of the bacteria associated with the genitourinary system infection, and there are other types, was previously belong to *Mycoplasma* called *Ureaplasma urealyticum*, these bacteria have the ability to lysis urea. This bacterium causes urinary tract infection and is considered a difficult case to diagnose because it shares many other pathogens in addition it can cause infection without pathological symptoms and cannot be diagnosed by traditional laboratory techniques like grown on solid culture media only by using live cells [1]. *Mycoplasma* is characterized by its ability to cause chronic inflammatory diseases such as sterile leukopenia, and there is little evidence of the mechanism of infection and the occurrence of the disease because it may share with other infections in the same place [2,3]. These bacteria have the ability to infect mucous tissues, and it is also possible to invade the lower layers of tissues due to forming bacterial colonies that cause necrosis because have a great ability to grow in anaerobic environments under high tolerance conditions. So, the places where it is infected It makes the diagnosis more difficult compared to other pathogens [5]. Th1 T cells are characterized by their ability to secrete inflammatory immune proteins, including cytokines such as IL-18, and interferons, which help the immune system to kill or inhibited pathogens. These immune proteins are produced by connective tissues and other cells. The effect of IL-18 initiates by binding to receptors on the surface of target cells and thus initiates the neutralization of intracellular antigenic peptides such as lipopolysaccharides (LPS), after being stimulated by IL-18, there is another immune protein released by T cells and natural killer (NK) cells called type II interferon (IFN- γ) that plays an important role in activating the inflammatory response [6]. Interferons are immune proteins, which include many types that have an integrated immune function in the inflammatory response, including IFN- α & IFN- β which have an important role in virus infection and (IFN- γ) that have a role in resistance to bacterial infection in addition to another type of interferon like (IFN- λ), that belong to cytokine family [7,8].

Epithelial cells are the main producers of interferons and other immune proteins (cytokines) and this is done by activating the JAK-STAT signalling pathway, which results in sending a signal to the nucleus that stimulates the genes responsible for the production of interferons, which are called IFNAR. Thus, the cells secrete these immune proteins that have the immune role that was mentioned previously [9,10]. The first and second types of interferons (IFN- α and IFN- β) play an important and key role in resistance to pathogenic viruses, being the first line of immune defence. They also contribute to the connection between innate immunity with acquired immunity. Interferons are mainly produced by many cells such as fibroblasts and macrophages. And lymphocytes, white blood cells and dendritic cells have a major role in eliminating bacterial and viral infections that affect humans [11]. *Mycoplasma* is considered one of the parasites that can grow on the surfaces of cell membranes and also inside cells, where they are called silent parasites. In addition, considered equivalent to living organisms and have the

ability to cellular metabolism and physiology [12]. Mycoplasma bacteria have many mechanisms to evade the immune system, such as the expression of two types of antigenic proteins (MgpB and MgpC) that prevent the attachment of immune proteins to target cells, in addition, could be interfering with the cellular pathways of the host cells. That makes it have the ability to invasion many cells in the body like epithelial cells and white cells to establish chronic urogenital infection [13,14].

Chronic UTI causes the activation of many signalling pathways that lead to the activation of innate and acquired immunity within the host body, but these immune mechanisms are met with strong resistance by Mycoplasma. It has highly virulent factors that invade the mucous membranes lining the urinary system and make it able to escape from the immune system, which leads to acute and then chronic inflammation, these events that occur between the immune system and Mycoplasma usually take more time because these bacteria can adapt with different conditions [15,16]. Several studies indicated the ability of Mycoplasma bacteria to produce LPS antigenic lipoproteins that stick to the membranes of target cells such as white blood cells, which enter into the cells and cause infection [15,17].

2 Materials and Methods

Determination of urine and serum levels of IL-18 and INF- β

We evaluated the concentration of immune proteins (IL-18 and INF- β) in the serum of patients with UTI by using the ELISA technique to determine their concentration according to the manual protocol (Bioassay Technology Laboratory /Shanghai-China) then Calculation the result and interpretation were done according to a specific standard curve of each interleukin, such IL-18 and INF- β .

Statistical Analysis

The data were statistically analyzed using SPSS version 23 as well as using the Excel system, where the mean $\pm$ SD was found and the comparison between two different groups of samples using the T-test, while the correlation coefficient between the immunological parameters and the pathogen was found using the ANOVA test at a probability level of $P < 0.05$.

3 Results and Discussion

A total of (223) urine and blood samples were collected from patients (UGTIs) were only (70) samples positive for culture to Mycoplasma spp. (Mycoplasma hominis and Mycoplasma genitalium) and Ureaplasma spp. (Ureaplasma urealyticum) (Based on the results of our current study, it was mentioned in previous research under publication), measurement of the concentration of IL-18 and INF- β . Twenty patients as control samples. Aged 20–49 years, married women only.

The concentration of IL-18 in urine and serum of UGTIs patients for Mycoplasmas and control

As illustrated in Table (3-12) that showed the mean IL-18 urine concentration in patients (13.39 ± 3.3 pg/ml) which was significant increase by $p < 0.05$ compared with the control (10.63 ± 2.1 pg/ml), while the mean of IL-18 serum concentration in patients (12.01 ± 9.8 pg/ml) has no significant difference compared with the control group (9.22 ± 2.4 pg/ml).

Table 1
IL-18 Concentration in Urine and Serum of UGTIs Patients and Control

IL-18 pg/ml	No.	Mean $\pm$ S.D.	P.Value
Patients (urine)	70	13.39 ± 3.3	0.000**
Control	20	10.63 ± 2.1	
Patients (serum)	70	12.01 ± 9.8	0.215
Control	20	9.22 ± 2.4	

Some studies mentioned that interleukin-18 has a role in increasing the production of interferon-gamma, which has an important role in the host's defence against microbial infection [18,19]. The IL-18 in urine is elevated in patients with genital Mycoplasmas infection, which may be due to the humoral immune activity which induces secretion of IL-18 as a defence response of the immune system against pathogens, the IL-18 in serum not elevated in patients may be cause local inflammation cytokine rises locally and need time until it turns into a systemic, this depends on the strength and type of the microbe, these findings correspond with preceding studies [20], who stated that interferon (IFN)- γ -inducing factor was previously termed interleukin IL-18, (Interferon-gamma production by splenic, but not liver cells), The low production of the immunoglobulin protein interleukin-18 in the serum of patients, which in turn affects the production of interferon-gamma, is due to its weak production in the responsible cells of specific sites of infection due to the negative effect of bacteria. In addition, the cytokines IL-1 and IL-18 are secreted after the inflammatory response. Its secretion is regulated by both the innate and adaptive immune systems, and it was found that the production of interferon-gamma in $\alpha\beta$ T cells contributes to an increase in the protective effect of interleukin-18.

The level of IL-18 urine was in *M. genitalium* (14.14 ± 3.4 pg/ml), *U. urealyticum* (13.40 ± 3.3 pg/ml), *M. hominis* (14.91 ± 3.4 pg/ml) and mix growth (13.04 ± 2.8 pg/ml) has a significant increased $P < 0.05$ compared with control (10.63 ± 2.1 pg/ml). However, level IL-18 serum concentration was in *M. genitalium* (13.24 ± 2.2 pg/ml), *U. urealyticum* (10.10 ± 2.0 pg/ml), *M. hominis* (10.24 ± 2.1 pg/ml) and mix growth (9.47 ± 1.3 pg/ml) compared with control (9.22 ± 2.4 pg/ml) was no significant increase in patients with UTIs genital Mycoplasmas, can be observed in Table (3-13).

Table 2
The concentration of IL-18 in urine and serum of UGTI patients and control according to *Mycoplasma* spp. and *Ureaplasma* spp.

Diagnosis	IL-18 urine Mean±S.D.	IL-18 serum Mean±S.D.	<i>IL-18 urine</i> P.Value	IL-18 serum P.Value
<i>M. genitalium</i>	14.14±3.4	13.24±2.2	0.002**	0.430
<i>U. urealyticum</i>	13.40±3.3	10.10±2.0		
<i>M. hominins</i>	14.91±3.4	10.24±2.1		
Mix growth	13.04±2.8	9.47±1.3		
Control	10.63±2.1	9.22±2.4		

These results might show that the level of IL-18 urine concentration was elevated to various degrees in genital *Mycoplasmas*, and there is no significant increase in the level of IL-18 serum concentration may be due to pro-inflammation cytokine increase locally and requires time to turn into a systemic, these results agreed with Dinarello et al [21], who mentioned that the expression of adhesion molecules VCAM-1 and ICAM-1 increasing with elevated of IL-18 production [22,23], these molecules facilitated the emigration of immune cells (neutrophils and lymphocytes) to the site of infection. Also, the migration of these cells through the blood vessels to the affected tissue is an essential process that occurs during the response to inflammatory diseases. In this regard, interferon-gamma plays an important role in the control of infection, the production of which is stimulated by IL-18 [24].

The concentration of INF-β in urine and serum of UGTIs patients for Mycoplasmas and control

As illustrated in Table (3-16) that showed the mean INF-β urine concentration in patients (269.89±66.1 pg/ml) was significantly increased (0.001) compared with the control (210.22±69.3 pg/ml), also the mean INF-β serum concentration in patients (230.56±91.5 pg/ml) was significantly increase (0.009) compared with the control group (174.38±36.2 pg/ml).

Table 3
The concentration of INF-β in urine and serum of UGTIs patients and control

INF-β pg/ml	No.	Mean ± S.D.	P.Value
Patients (urine)	70	269.89±66.1	0.001**
Control	20	210.22±69.3	
Patients (serum)	70	230.56±91.5	0.009**
Control	20	174.38±36.2	

The production of interferon-β (IFN-β) during a bacterial or viral infection is stimulated after the receptors of host cells (PRR) recognize the antigens of the pathogen (PAMP), Which has an important role in ending the infection, but the increase in its production may have a negative and harmful effect on the cells of the host. In addition, other types of interferons, including IFN-α and IFN-β, have many positive effects depending on their activities during bacterial infection, as

their production contributes to killing pathogens, stimulating the apoptosis response, or enhancing or inhibiting the immune response to cells of T helper cell type Th1 depending on the kinetics of the immune response or the location of the tissues, and in general, the immune response dependent on interferon's represents the first line of immune defence against pathogens [25]. Our study shows the concentration of IFN- β in urine and serum for patients' genital Mycoplasmas is higher than that in the control. This study agrees with Solis et al [26] and Sen et al [27] who noticed that many metabolic pathways that occur inside host cells contribute to the increase in the production of interferon type I after viral/bacterial infection. Also, the presence of many receptors for these immune proteins in many cells of the body confirms the important role they play in resisting pathogens and recovery from infection.

The level of IFN- β urine concentration with diagnosis in *M. genitalium* (277.98 $\pm$ 64.2 pg/ml), *U. urealyticum* (253.37 $\pm$ 74.9 pg/ml), *M. hominis* (292.25 $\pm$ 81.6 pg/ml) and mix growth (238.28 $\pm$ 44.4 pg/ml) has a significant increased (0.005) compared with control (210.22 $\pm$ 69.3 pg/ml). Also, level IFN- β serum concentration was in *M. genitalium* (221.86 $\pm$ 16.5 pg/ml), *U. urealyticum* (237.79 $\pm$ 61.2 pg/ml), *M. hominis* (228.04 $\pm$ 10.6 pg/ml) and mix growth (268.21 $\pm$ 64.2 pg/ml) compared with control (174.38 $\pm$ 36.2 pg/ml) was no significant increased (0.06) in patients with UTIs genital Mycoplasmas, can be observed in Table (3-17).

Table 4

The concentration of IFN- β in urine and serum of UGTI patients and control according to Mycoplasma spp. and Ureaplasma spp.

Types of bacteria	INF- β serum Mean $\pm$ S.D.	INF- β urine	INF- β urine	INF- β serum
			P.Value	
<i>M. genitalium</i>	277.98 $\pm$ 64.2	221.86 $\pm$ 16.5		
<i>U. urealyticum</i>	253.37 $\pm$ 74.9	237.79 $\pm$ 61.2	0.005**	0.06
<i>M. hominis</i>	292.25 $\pm$ 81.6	228.04 $\pm$ 10.6		
Mix growth	238.28 $\pm$ 44.4	268.21 $\pm$ 64.2		
Control	210.22 $\pm$ 69.3	174.38 $\pm$ 36.2		

These results might show that the level of IFN- β urine concentration was elevated to various degrees in genital Mycoplasmas, and there is no significant increase in the level of IFN- β serum concentration. these results agree with Guiton et al [28] Who reported that there are many cells such as neutrophils, macrophages and mononuclear cells that are important for the innate immune response of the host against urinary tract infection. Schiwon et al [29] and Duell et al [30] also mentioned that these cells contribute to the regulation of antibacterial defences and produce inflammatory immune proteins (cytokines). And chemokines) such as tumor necrosis factor-alpha, interferons alpha, beta, gamma and interleukin-17, which attract immune cells to the site of infection during UTI and contribute to the termination of the disease. In addition, interferons contribute to inhibiting bacterial infection that causes infection inside or outside cells [31].

4 Conclusions

We concluded that there is more than one type of Mycoplasmas that causes UGTIs in women, it has been isolated *Mycoplasma dentalium*, *Mycoplasma hominis* and *Ureaplasma urealyticum*, in addition, indicated that the patients with UGTIs due to Mycoplasmas had significantly higher IL-18 than the control group in urine only, while INF- β significantly higher in urine and serum.

Ethical Statements

Every volunteer has given written informed permission. This research received ethical approval (MHS-S-1264) for scientific research from the Ministry of Health MOH and Ministry of Higher Education and Scientific Research MOHESR ethics committees in Research Ethics Committee, University of Babylon.

Conflict Of Interests

The authors have no conflict of interest to be declared.

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