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# Studying the relationship between tlr-6 and mycoplasmas among urinary-genital tract infections for females in al-hillah city, Iraq

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***Abstract*---**Mycoplasma is fastidious, a slow-growing organism that lacks a cell wall and is often isolated from the genitourinary tract. The aim of the study determines the relationships between the fastidious microbial infection (Mycoplasma spp. and Ureaplasma spp.) and toll-like receptor 6 concentration for urinary-genital tract infections (UGTIs). The study includes a collection (of 223) samples (females' urine and blood) from UGTIs patients, who arrived Hospital in Babylon (from January to June 2021). The isolates were identified according to appropriate culture media by urine culture, Biochemical analyses have been used to verify the authenticity. The results of the immunological examination of TLR-6 concentration were done using ELISA for measurement. The mean TLR-6 urine concentration in patients (2.08±0.3 pg/ml) was significant increase p< 0.05. compared1with the control (1.70±0.4 pg/ml) while showing the mean TLR-6 serum concentration in patients (2.21±1.4 pg/ml) was no significant difference compared with the control group (1.80±0.5 pg/ml). we concluded that the risk factors associated with Mycoplasma ssp. are numerous. Therefore, it is necessary to use accurate and rapid diagnostic methods. The present study indicates that the patients with UGTIs due to mycoplasmas had significantly higher Toll-like receptor-6 than the control group in urine only.

***Keywords*---**Urinary-Genital Tract Infections (UGTIs), Mycoplasmas, Toll-Like Receptor 6 (TLR-6).

**Introduction**

The tiniest self-replicating organisms in the Mollicutes class are Mycoplasma and Ureaplasma geniuses [1]. Mycoplasmas are a vast family of prokaryotic cells that are thought to have evolved from gram-positive bacteria through the degeneration evolutionary process and are primarily able to cause a wide range of human and animal illnesses. Mycoplasma species can be found both within and outside of the cell as membrane surface parasites and as "silent parasites "because mollicutes lack traditional bacterial PAMs (e.g., lipoteichoic acid, flagellin, and some lipopolysaccharides), the precise molecular ways of Mycoplasma identification for immune system cells are being studied for their pathogenic implications. Mycoplasmas are the smallest and most basic self-replicating organisms. This bacterium is solely kept together by an external structure consisting of the capsule, an adhesive structure, and adhesion-related proteins, as well as a unit membrane because it lacks a strong cell wall. Despite having a simpler basic structure, than ordinary

Gram-negative bacteria, Mycoplasma and the host immune system have a complex cross-talk that includes Mycoplasma-induced non-specific and specific immune responses. In comparison to other bacteria, the innate immune response, which consists mostly of innate immune molecules and innate immune cells at the beginning of sickness, is non-specific yet plays a significant role in the resistance against such a germ [2]. Using toll-like receptors, innate immune cells such as neutrophils, macrophages, and natural killer cells can recognize pathogen-related molecular patterns (PAMPs) of Mycoplasma but also destroy them. To begin with, phagocytizing neutrophils and monocytes/macrophages invariably result in oxidant bursts provoked by bacterial infections, resulting in the production of reactive oxygen species (ROS). ROS, comprising oxidative radicals such as superoxide, hydroxyl radicals, H2O2, and organic hydroperoxides, are crucial players in numerous innate immune cell responses to pathogens [3].

Cytokines are crucial responsive and modulatory molecules in host defense, including immune cell recruitment and activation and the stimulation of early cell differentiation. The complement system is not only a component of the innate immune system, but it is also one of the primary mechanisms through which antigens exert their immunological effects. The adaptive immunity, whose distinct participant cells and molecules are various types of T/B lymphocytes and antigens, supports further elimination of the invading pathogen in the late stage of infection[4]. Mycoplasmas may proliferate and persist within the host for a long period after entering a suitable host, despite a robust immune system and limited metabolic capacity. Mycoplasmas have virtually all gradually evolved extremely complex methods to resist coercion by the human immune system to maintain their survival and infection. In addition to molecular mimicry and antigenic variation, which were originally found and frequently employed by Mycoplasma, we will explore several innovative Mycoplasma methods such as oxidative stress defence, NET degradation, immunoglobulin capture and cleavage, cell fusion, and cell fusion[5].

A classic study published in 2010 stated that most Mycoplasma species might produce antigenic variation, including Mycoplasma genitalium, M. penetrans, M. hominis, M. hyorhinis, M. gallisepticum, M. capricolum subsp. capricolum, M. pulmonis, M. Bovis, M. agalactiae, M. synoviae, Ureaplasma parvum, and U. urealyticum, This mechanism allows these viruses to evade the host immune response and alter surface accessibility by masking and unmasking stably expressed components required for host contact and survival [6]. Mycoplasma spp. need host cells to carry out their biological functions. Hence, Mycoplasmas have a strong capacity to acclimate to stay alive on exposed surfaces of host tissues, evading host defences through a variety of mechanisms. Some Mycoplasmas interact with and affect biological processes of the host cell, both at the regulatory and/or functional levels, whether sticking to the surface of eukaryotic cells or after the invasion. To protect it from specific negative consequences, the host organism initiates a series of responses involving multiple signalling pathways, ultimately leading to the activation of both innate and acquired immunity, which elicits processes that stimulate acute and chronic inflammation, respectively. Mycoplasmas, in turn, evolved mechanisms to circumvent immune control, allowing them to colonize mucosal surfaces and infiltrate various bodily areas [7].

## 2 Materials and Methods

### 2.1 Samples collection

This study includes a collection (223) samples (urine and blood) from UGTIs patients for married women only ( according to the initial laboratory diagnosis by the Parasitological unit for all the patients under study), who arrived at the Maternity and Children Hospital in Babylon ( from January to June 2021 ), their age of patients ranged from (20-49) years old, to investigate the fastidious microbial pathogen.

### 2.2 Isolation, identification, and biochemical testing

Mycoplasma spp. was found in all of the specimens. Using agar and modified arginine–urea broth ( pleuropneumonia-like organism broth and agar added to it Mycoplasma enrichment supplement). the specimens under test were inoculated in MAU- broth medium for (M.hominis, M. genitalium and U.urealyticum ) and incubated at 37°C aerobically or facultative anaerobically for 24-72 hours, with daily checking for growth of U. urealyicum and 3-5 days for M. hominins and M. genitalium [8; 9]. Since Mycoplasma colonies develop within the agar, a block of agar-containing colonies was cut and rotated on the face of a fresh Mycoplasma agar plate (this method was repeated 2-3 times) to obtain pure colonies. Of each primary positive culture, a single colony was chosen and transmitted for identification based on diagnostic features indicated by morphological factors (colony size, shape, and colour). The colonies were examined under a dissecting microscope because the colonies of M. hominins and M. genitalium had a fried egg appearance, but the colonies of U. urealyticum exhibited granular colonies and a dark brown tint due to manganese oxide deposition within the colony. Glucose fermentation, arginine deamination, and other biochemical approaches were used to identify Mycoplasma spp.

### 2.3 Determination of urine and serum levels of TLR-6

TLR-6 levels in urine and serum were measured using the Enzyme-Linked Immunosorbent Assay (ELISA kits). The TLR-6 Human ELISA kit (Bioassay Technology Laboratory Shanghai, China) employs an antibody suitable for human TLR-6 coated on a 96-well plate. The results were calculated and interpreted using the unique standard curve of each interleukin.

### 2.4 Statistical method

Excel 2010 and SPSS version 23 were used to examine the data. The results were shown as mean SD. The Student's T-test was used to compare two subgroups. Pearson coefficient was judged significant at $P<0.05$ when evaluating correlations between immunological parameters and causal agents using one-way ANOVA.

## 3 Results and Discussion

A total of (223) urine and blood samples were collected from patients. Twenty patients as control samples. Of 223 samples (70) (31.39%) were given positive genital Mycoplasma's infection culture, whereas (153) (68.61%) cases showed negative genital Mycoplasma's culture even after 5 days. The positive genital Mycoplasmas infection was divided into M. genitalium the most common 44 (19.73%) followed by U. urealyticum 13 (5.83%) and M. hominis 5 (2.24%) , while mixed growth (M. homins + U. urealyticum) 5 (2.24%), (M. genitalium + U. urealyticum) 2 (0.90%) and finally (M. homins + M. genitalium) 1 (0.45%) as shown in Table 1.

Table 1
Culture Isolation Percentage of UGTIs for Patients.

| Culture Mycoplasmas | No. | (%) | p-value |
|---|---|---|---|
| Negative Mycoplasmas | 153 | 68.61% | |
| M. genitalium | 44 | 19.73% | |
| U. urealyticum | 13 | 5.83% | |
| M. hominis | 5 | 2.24% | |
| Mix (M. homins+U. urealyticum) | 5 | 2.24% | 0.0001** |
| Mix (M. genitalium+ U. urealyticum) | 2 | 0.90% | |
| Mix (M. homins +M. genitalium) | 1 | 0.45% | |

Mycoplasma has become essential in the study of chronic illnesses. As an external and intracellular pathogen, a greater knowledge of Mycoplasma spp. virulence pathways will give a new insight of how to detect and battle this disease. Mycoplasma was detected and identified using various techniques. Primary isolation, bacteriological inspection, biochemical identification, and immune research were all part of the culture procedure. Mycoplasma was discovered through microscopic inspection and biochemical analysis. In this study, 70 out of 223 people (31.39 percent) tested positive for Mycoplasmas, which included Mycoplasma spp. and Ureaplasma spp. in our study M. genitalium accounted for 44/70 (19.73%) of the case, which is consistent with prior research that indicated M. genitalium to be the most prevalent Mycoplasma isolale acquired from sexually transmitted illness[10].

Kerman [11] also believed Mycoplasma genitalium to be the most significant bacterial agent recovered from Individuals with genital illness. Ureaplasma urealyticum was found in 13/70 (5.83 percent ) of positive Ureaplasma spp. In our investigation, which was previously published in a study in which Ureaplasma urealyticum was found Ureaplasmas can be found in the cervix or vagina of up 40% to 80% of heathy adult women [12]. M. hominis was detected in 5/70 (2.24 percent) of positive Mycoplasma spp. Isolates taken from clin ically sick noncontrol individuals [13].

Similar to the findings of Kasprzykowska [14], According to the same study, the prevalence of Ureaplasma spp. in women (14.4 percent) is higher than M. hominis in women (0.2 percent) with urogenital tract infection in Poland. Similarly to the Moosavian [15] finding, PCR revealed U.uealyticum (28%) and M.hominis (10%) in infertile men's sperm collections, and culture extracted 22 percent of U. urealyicum and 2 percent M. hominis from the same samples. Positive frequencies of M. genitalium, M. hominis, and U.urealyicum vary widely over the world [16]. The urban percentage was different from the rural percentage in UGTIs, with patients distributed as 83 urban percentage (37.22 percent) and 140 rural percentage (62.78 percent). Among women living in urban settings, 26 (11.66 percent) were found positive Mycoplasma, while 44(19.73 percent) were found positive for Mycoplasma, with found significant in the settlement type (p=0.0001), as illustrated in table 2.

Table 2
Percentage of Residency Groups and Culture Positive Mycoplasmas.

| Residency Groups | No. | (%) | No. Positive Mycoplasma | (%) | No. Negative Mycoplasma | (%) | p-value |
|---|---|---|---|---|---|---|---|
| Urban | 83 | 37.22% | 26 | 11.66% | 57 | 25.56% | 0.0001** |
| Rural | 140 | 62.78% | 44 | 19.73% | 96 | 43.05% | |

This study differs from the findings of Peretz [17], who discovered that 91 (42.7 percent) of women lived in urban environments, while 122 (57.3 percent) resided in rural settings.

Ten (11%) of women residing in cities were found to be positive. There was no significant connection between carriage rates and settlement type (p=0.25). especially among rural women 8 (6.6 percent). Our findings are consistent with those of Zhang [18], who discovered that women with an undergraduate degree or higher had a lower risk of Mycoplasma isolation, and that a history of gynecological diseases was an independent risk factor for Mycoplasma isolation, whereas a bachelor's, master's, or greater was a protective measure against Mycoplasma isolation. A total of (223) patients married women only were distributed into three groups according to age, 20-29 years 103 (46.19%) patients, 30 – 39 years 72 (32.29%) patients, 40 – 49 years 48 (32.29%) patients, among women aged in (20-29) settings, 36 (16.14%%) were found positive Mycoplasmas, and women aged in (30-39) setting, 21 (9.42%) were found positive Mycoplasmas,

while women aged in (40-49) setting, 13 (5.83%) were found positive Mycoplasmas, as shown in Table 3.

Table 3
Characteristic of Subjects According to Age and Culture Positive Mycoplasmas.

| Age (years) | No. | (%) | No. Positive Mycoplasma | (%) | No. Negative Mycoplasma | (%) | p-value |
|---|---|---|---|---|---|---|---|
| 20-29 | 103 | 46.19% | 36 | 16.14% | 67 | 30.04% | |
| 30-39 | 72 | 32.29% | 21 | 9.42% | 51 | 22.87% | 0.0001 |
| 40-49 | 48 | 32.29% | 13 | 5.83% | 35 | 15.70% | ** |

In this study appear that the age group (20-29) 36 (16.14%), showed the most infection with UGTIs . this study similar with the results of Peretz et al., [17] who found the age group (18-29) 12 (10.5%) more than the age group (30-39) 7 (7.5%) . Our study agree with the results of the Zhang [18],they discovered that women aged 30-39 years or with a history of pregnancy or gynecological disorders were at a greater risk of Mycoplasma isolation, whereas postmenopausal women were at a lower risk of Mycoplasma isolation.

In this investigation, M. hominis, M. genitalium, and U. urealyticum isolates were identified using colonial morphology on MAU-medium, where colonies that appeared like fried eggs were conclusive and suggestive of the presence of U.urealyticum, as shown in Figure (1), under an X40 dissecting microscope.

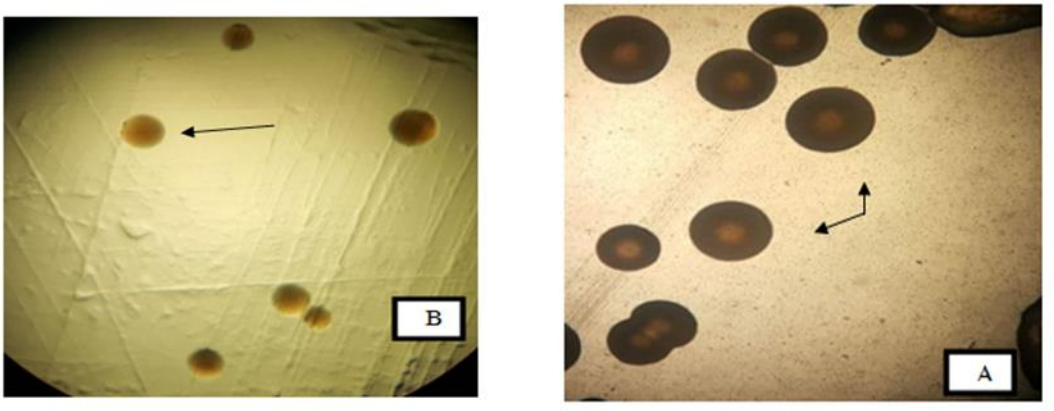


Figure 1. Colonies morphology of genital Mycoplasmas grow on under 4X dissecting microscope in culture agar (MAU – medium) A- fried egg colony of Mycoplasma spp. ,B- tiny brown colony of Ureaplasma urealyticum.

All M.hominis isolates tested positive for arginine hydrolysis but negative for glucose fermentation, tetrazolium reduction, and urea hydrolysis, according to the biochemical tests utilized in this investigation. M. genitalium isolates, on the other hand, were positive for glucose fermentation but negative for arginine hydrolysis, tetrazolium reduction, and urea hydrolysis. Positive urea hydrolysis and negative glucose fermentation were seen in U.urealyticum isolates, as were arginine hydrolysis and tetrazolium reduction., as shown in Table 4.

Table 4
Morphological and Biochemical characteristics of M.hominis, M. genitalium and U.urealyticum Isolates.

| Mycoplasma spp. | Colony morphology | Arginine hydrolysis | Glucose fermentation | Tetrazolium reduction | Urea hydrolysis |
|---|---|---|---|---|---|
| M. hominis | Fried-egg | + | - | - | - |
| M. genitalium | Fried-egg | - | + | - | - |
| U. urealyticum | Granular | - | - | - | + |

These characteristics were compared with standard characteristics of Mycoplasma spp. recommended by [19] [20] .
The concentration of TLR-6 in urine and serum of UGTIs patients for Mycoplasmas and control. as illustrated in Table 5 that showed the mean TLR-6 urine concentration in patients (2.08±0.3 pg/ml) was significantly increased (0.000) compared with the control (1.70±0.4 pg/ml), while the mean of TLR-6 serum concentration in patients (2.21±1.4 pg/ml) was not significantly increase compared with the control group (1.80±0.5 pg/ml).

Table 5
TLR-6 Concentration in Urine and Serum of UGTIs Patients and Control.

| TLR-6 pg/ml | No. | Mean ± S.D. | P.Value |
|---|---|---|---|
| Patients (urine) | 70 | 2.08±0.3 | |
| Control | 20 | 1.70±0.4 | 0.000** |
| Patients (serum) | 70 | 2.21±1.4 | |
| Control | 20 | .80±0.5 | 0.208 |

This stagreesgree with [9], Mycoplasma spp. and Ureaplasma spp. were able to induce innate immune responses via multiple types of TLRs, including TLR2/6 and TLR1/10 (neutrophil activation and release of mucosal antibody of IgA). Our study discovered a significant difference in urine TLR-6 content between patients infected with Mycoplasmas and the control group when P-value (0.000**), which might suggest the activation of a signaling system that will help in the eradication of germs from UGTIs such as Mycoplasmas.. This study could TLRs may have a role in the genesis of UGTIs in female , according to an increasing body of evidence, also agree with studies [21] [22], Given that TLR2-TLR6 heterodimers may recognize 2 kDa Mycoplasmal activating lipoproteins (MALP), The identification of Mycoplasmas target ligands may result in the release of proinflammatory cytokines. TLR2-TLR6 heterodimers are critical in recognizing significant microbiological causal agents of UTIs, such as Mycoplasma spp., Ureaplasma spp., Staphylococcus spp., and Streptococcus spp. TLRs 1, 2, 4, and 6 are binded by bacterial LPs, and the first lipopeptide produced in Mycoplasmas to be shown to bound TLRs was Mycoplasma spp. macrophage-activating .'s lipopeptide-2 (MALP-2). Following that, triacylated or diacylated lipopeptides were shown to bind TLR 1/2 or TLR 2/6 heterodimers, respectively [23] [24].TLR-2 and TLR-6 heterodimers were important in recognizing key microbiological causative agents of urinary tract infections such as Candida albicans, Staphylococcus spp., Streptococcus spp., Mycoplasma spp., and Ureaplasma spp. TLRs identify and interact with a diverse spectrum of microorganisms in Gram-positive bacteria,

including lipopeptides, peptidoglycan, and lipoteichoic acid. Lipoproteins are also present in Mycoplasmas and Mycobacteria. TLR4, on the other hand, is a signaling receptor for lipopolysaccharide (LPS) [25].

The level of TLR-6 urine concentration with diagnosis in M. genitalium (2.12±0.4 pg/ml), U. urealyticum (2.00±0.2 pg/ml), M. hominis (1.14±0.4 pg/ml) and mix growth (1.92±0.2 pg/ml) has a significant increased (0.004) compared with control (1.70±0.4 pg/ml) . However level TLR-6 serum concentration was in M. genitalium (2.20±1.5 pg/ml), U. urealyticum (2.39±1.5 pg/ml), M. hominis (1.83±0.3 pg/ml) and mix growth (2.21±1.2 pg/ml) compared with control (1.80±0.5 pg/ml) was no significant increase in patients with UTIs genital Mycoplasmas, can be observed in Table 6.

Table 6
The concentration of TLR-6 in Urine and Serum of UGTIs Patients and Control According to Mycoplasma spp. and Ureaplasma spp.

| Diagnosis | TLR-6 urine Mean±S.D. | TLR-6 serum Mean±S.D. | TLR-6 urine P.Value | TLR-6 serum P.Value |
|---|---|---|---|---|
| M. genitalium | 2.12±0.4 | 2.20±1.5 | | |
| U. urealyticum | 2.00±0.2 | 2.39±1.5 | | |
| M. hominis | 2.14±0.4 | 1.83±0.3 | 0.004** | 0.692 |
| Mix growth | 1.92±0.2 | 2.21±1.2 | | |
| Control | 1.70±0.4 | 1.80±0.5 | | |

These results might showed that the level TLR-6 urine concentration were elevated to various degrees in genital mycoplasmas , and there is no significant increase at level TLR-6 serum concentration may be due to local inflammation increase locally and requires time to turn into a systemic, these results were similar to the study of [25] , who mentioned that TLR-6 were elevated in urine of the patients with UTIs genital mycoplasmas , is the characteristic of T-helper 2 responses, whose mentioned that elevation of pro-inflammatory TLR-6 marker of UTIs genital mycoplasmas and may be useful in differentiating Mycoplasma spp. and Ureaplasma spp.

**Conclusion**

1- Two species of Mycoplasma and U. urealyticum existed in the samples studied.
2- Mycoplasma spp. clinical presentation varies, and diagnostic confirmation is difficult for even the most skilled clinicians.
3- Although time-consuming, culture techniques and biochemical assays were shown to be specific for detecting live Mycoplasma spp. and U. urealyticum. Bacterial culture was widely recognized as the gold standard Mycoplasma diagnosis technique. Culture needs specialized media, which takes time (up to 5 days).
4- The present study indicates that the patients with UGTIs due to mycoplasmas had significantly higher Toll-like receptor-6 than the control group in urine only.

**Ethical Statements**

Every volunteer has given written informed permission. This research received ethical approval (MHS-S-1258) 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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