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**Ministry of Higher Education and Scientific Research**

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**College of Science**

**Department of Biology**

**Some immunological parameters in patients with Mycoplasmal urogenital  
infection**

**A research**

**Submitted to the council of the college of science / university of Babylon in partial  
fulfillment of the bachelor's in biology / microbiology**

**By**

**Saba Rahman Sihud**

**Supervised by**

**Prof. Dr. Azhar Omran Lateef Abood Al-Thahab**

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

﴿ وَمَا يَعْلَمُ تَأْوِيلَهُ إِلَّا اللَّهُ وَالرَّاسِخُونَ فِيهِ ﴾

﴿ الْعِلْمِ يَقُولُونَ آمَنَّا بِهِ كُلٌّ مِنْ عِنْدِ رَبِّنَا ﴾

صدق الله العظيم

سورة - آل عمران الآية (٧)

## الاهراء و الشكر

الحمد لله على فضله ومنه الاعظم والصلاة والسلام على نبي الهدي الامي الاكرم والى بيته النجباء

مشاعل العلم وقادة الامم

وتقدم بالشكر والامتنان لى الدكتوراه الفاضله ازهار عمران

والى الاستاذة الموقرة نور عباس على ما بذلت من جهد ووقت من اجل ان نكمل مشوارنا

والى لجنة الموقرة لكم كل الشكر والتقدير

لكل بداية نهاية، مهما طالت وها انا الان ادخل حروف نهاية كلمتي

و نلتمس منكم العذر ان ورد منا بعض التقصير

فالكمال لله وحده و النقصان من شيم الانسان

واليوم ها قد اكملنا ان شاء الله الدررسة الجامعية

وننتظر بشاره التخرج ونسيان التعب

فى الختام نسال الله تعالى ان يرزقنا وياكم العلم النافع والعمل به

وان نلتقى على خير ان شاء الله

## Abstract

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Mycoplasmas are fastidious slow growing organisms lacking a cell wall and mostly isolated from genitourinary tracts. Although many species of mycoplasmas regard as normal flora, but some species causes serious genital disease .

The present study was aimed to determine the relationships between the fastidious bacterial infection (*Mycoplasma* species and *Ureaplasma* species) and some of immunological parameters for urinary genital tract infections (UGTIs) for married women only (pregnant). For purpose, and to obtain reliable results, the following technique was used: RADIAL IMMUNODIFFUSION (concentration IgG ,IgM , IgA,C3,C4).

The study includes (30) patients were distributed into three age groups (20-29) year 10 patients , (30 – 39) year 10 patients , (40 – 49) year 10 patients and (15) samples as control samples which were apparently (Health women) pregnant (married).

The result show Concentration of IgA mg/dl in Patients and Control significant difference at p value (**0.018\***) and it was found that **Mean ± SD** in patient (**370.28 ± 136.82**) less than control (**456.28 ± 60.88**) .

Concentration of IgG mg/dl in Patients and Control significant difference at p value (**0.000\*\***) and it was found that **Mean ± SD** in patient (**2595.49 ± 708.37**) higher than control (**2000.67 ± 266.82**).

Concentration of IgM mg/dl in Patients and Control significant difference at p value (**0.024\***) and it was found that **Mean ± SD** in patient (**285.0 ± 80.72**) less than control (**327.0 ± 38.53**).

Concentration of **C3 mg/dl** in Patients and Control not significant difference at p value (**0.441**) and it was found that **Mean ± SD** in patient (**220.95 ± 38.77**) higher than control (**186.62 ± 38.96**).

## Abstract

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Concentration of **C4 mg/dl** in Patients and Control significant difference at p value ((**0.001\*\***)) and it was found that **Mean ± SD** in patient (**59.63 ± 23.74**) higher than control (**44.36 ± 6.76**).

In this study, it was found positive correlation in IgG (**0.385\***) with IgA and negative correlation in IgM (- 0.441)with IgA. And show positive correlation in IgM with IgG (0.191). Correlation C3 with IgA and IgG is negative correlation (- 0.233 , - 0.286 ) and positive correlation with IgM (0.262).Correlation C4 with IgA and IgG is negative correlation (- 0.260 , - 0.156 ) and positive correlation with IgM (0.348).Correlation C4with C3 show positive correlation (**0.370\***).

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***Chapter one***  
***Introduction***  
***and***  
***Literature***  
***Review***

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## 1.1. Introduction

*Mycoplasmas* are fastidious slow growing organisms lacking a cell wall, which are not detected on routinely used media and mostly isolated from genitourinary tract (Metwally *et al.*, 2014). They are constructed of only three organelles: plasma membranes, ribosomes, and prokaryotic chromosome (Distelhorst *et al.*, 2017; Razin, 2018). *Mycoplasmas* constitute a large group of microorganisms, but only some, *Mycoplasmaspp.* and *Ureaplasmaspp.* are pathogenic for humans. They mainly inhabit the mucous membranes of the respiratory tract and genitourinary system (Jafar *et al.*, 2010). *Mycoplasmas* harbor a variety of virulence factors that enable them to overcome numerous barriers of entry into the host; using accessory proteins, *Mycoplasma* adhesions can bind to the receptors or extracellular matrix of the host cell. During proliferation, successfully surviving *Mycoplasmas* generate numerous metabolites, including hydrogen peroxide, ammonia and hydrogen sulfide; or secrete various exotoxins, such as community-acquired respiratory distress syndrome toxin, and hemolysins; and express various pathogenic enzymes, all of which have potent toxic effects on host cells. Furthermore, some inherent components of *Mycoplasmas*, such as lipids, membrane lipoproteins, and even *Mycoplasma*-generated super antigens, can exert a significant pathogenic impact on the host cells or the immune system (Yiwen *et al.*, 2021). *Mycoplasmas* may also be a component of the commensal flora of the genitourinary tract mucosa and may be found in the majority of sexually active humans. The adverse effects of genital *Mycoplasmas* on outcomes of pregnancy include ectopic pregnancy, preterm birth and chorioamnionitis, postpartum endometritis, salpingitis, low birth weight and late miscarriage (Taylor-Robinson and Lamont, 2011).

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Three species have been isolated from the mucosal surfaces of the genitourinary tract: *Mycoplasma hominis*(*M. hominis*), *Ureaplasma urealyticum*(*U. urealyticum*) and the recently discovered *Mycoplasma genitalium*(*M. genitalium*) . They are commonly referred to as “genital mycoplasmas”, as the infection occurs via sexual contact (Moridiet *al.*, 2020) . Different detection techniques of mycoplasmal infections have been developed, each one of them has its advantages and limitations with respect to cost, time, reliability, specificity, and sensitivity. According to the laboratory’s infrastructure, the most common methods include : (I-culture-based test for isolation, detection, identification, antimicrobial susceptibility profile ; II-antigen detection, Mycoplasmal-specific serologic responses ; and III-Polymerase Chain Reaction)(Flores-Medina *et al.*, 2012).Cytokines mediate inflammatory responses, are important in intercellular communication, and play a multifaceted role in the reproductive physiology of men and women. These potent polypeptides are released from inflammatory cells in response to a wide variety of signals, frequently initiated by infection or injury, and usually act, in a network of other cytokines, locally in an autocrine or paracrine fashion but also have systemic effects. Excessive production or actions of cytokines can lead to pathologic consequences(Eggert-Kruse*et al.*, 2007).

Innate Immune cells, such as neutrophils, Macrophages, and natural killer cells, not only have the capacity to recognize pathogen-related molecular patterns (PAMPs) of *Mycoplasma* via toll-like receptors , but also can kill these microorganisms (Qin *et al.*, 2019).

### **Aim of study :**

Due to the existence of high abortion, infertility and difficulty to identify the causative agent of UGTIs the real, so this study was planned. The present study was aim to determine the relationships

between the fastidious microbial infection (*Mycoplasma* spp. and *Ureaplasmaspp.*) and some of immunological parameters for chronic UGTIs and according to our knowledge's clinical studies on the organisms and their role in colonization of human urogenital *Mycoplasma* in Iraqi population.

The achievement of this aim by the following objectives :-

Concentration measurement for some immunological parameters such as IgG,IgM,C3,C4 in patients and control.

## **1.2.Literature Review**

### **1.2.1.Background of *Mycoplasma***

*Mycoplasmas* are unique types of bacteria. They are the smallest free living organism known on the planet able to multiply autonomously (Naheret *al.*, 2014). Since mycoplasmas lack a cell wall, they can assume a variety of shapes and are therefore challenging to distinguish. The tip of the filamentous has an attachment organelle. On agar, there are colonies that resemble fried eggs. They are so challenging to grow in the lab and frequently ignored as disease-causing pathogens. (Pascual *et al.*, 2010;Mavedzengeret *al.*, 2012). *Mycoplasma* refers to a genus of bacteria that lack a cell wall, consequently, they are unaffected by many common antibiotics such as penicillin or other beta-lactam antibiotics that target cell wall synthesis. They can be parasitic or saprotrophic. Several species are pathogenic in humans, including *M.pneumoniae*, which is an important cause of atypical pneumonia and other respiratory disorders, and *M.genitalium* is believed to be involved in pelvic inflammatory diseases.

Mycoplasmas are the smallest living cells yet discovered can survive (Ryan and Ray, 2004). *Mycoplasma genitalium* show in Fig 1-1

**Figure 1-1: *Mycoplasma genitalium***



### 1.2.2. Pathogenesis

Although it is believed that mycoplasmas remain attached to the surface of epithelial cells as extracellular organisms (Kornspanet *et al.*, 2015), some *Mycoplasmas* have evolved mechanisms for entering host cells. These organisms have invasive properties enabling them to localize in the cytoplasm and perinuclear regions (Qin *et al.*, 2019). Intracellular localization has been reported for several species including *M. genitalium*, *M. hominis*, *M. penetrans* as well as *M. pneumoniae*. This localization may protect the *Mycoplasma* against host defense and contribute to disease chronicity (Chenoget *et al.*, 2011). Toxins are rarely found in *Mycoplasmas*. The end products of *Mycoplasma* metabolism were responsible for tissue damage. Hydrogen peroxide ( $H_2O_2$ ) the end product of respiration in *Mycoplasmas*, has been implicated as a major pathogenic factor ever since it responsible for the lysis of erythrocytes by mycoplasmas. The *Mycoplasmas* must adhere closely enough to the host cell surface to maintain a toxic, concentration of  $H_2O_2$  sufficient to cause direct

damage, such as lipid peroxidation of cell membrane. The accumulation of malonyldialdehyde, an oxidation product of membrane lipids. Moreover, *Mycoplasma* inhibits host cell catalase by excreting superoxide radicals ( $O_2^-$ ). This would be expected to further increase the accumulation of  $H_2O_2$  at the site of parasite-host cell contact (Lloyd *et al.*, 2021). *Mycoplasma genitalium* increasingly appreciated as a common cause of sexually transmitted disease syndromes (STDS), including urethritis in men and cervicitis, endometritis, pelvic inflammatory disease, and possibly preterm birth, tubal factor infertility and ectopic pregnancy in women (McGowin *et al.*, 2017). *Mycoplasma genitalium* infections show in Fig 1-2

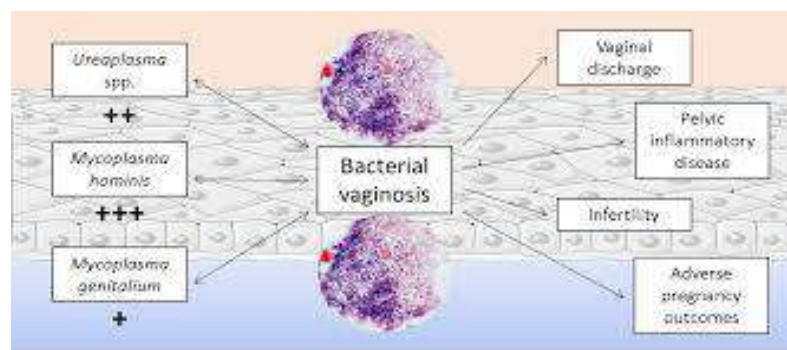


Figure 1-2 :*Mycoplasma genitalium* infection

### 1.2.3. Serological test in Mycoplasmas genital tract infections

Several techniques have been proposed for detection of antibodies against bacterial antigens. These techniques are: Growth inhibition test (GIT), Indirect haemagglutination test (IHA), Metabolic inhibition test (MIT), Mycoplasmacidal test (MT), Immunofluorescence test (IFT), Enzyme-linked immunoassay sorbent assay (ELISA), and Complement fixation test (CFT)

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(Clausen *et al.*, 2001).The most common test, used for diagnosis of genital mycoplasmas is the complement fixation test (Kumar *et al.*, 2014).

Mycoplasma test in the presence of antibody and complement, *Mycoplasma.spp* are rapidly killed by lysis (Afriat *et al.*,2013).The use of ELISA for detection of antibodies is one of the most sensitive and convenient methods for measuring antigens and antibodies, it can be performed in short time and without using radiolabelled materials, multiwell microtitre plates used as the solid phase are easy to handle and wash, and when used with automated readers and multiple well washers allow a large number of samples to be assayed (Meager *et al.*, 1987).

### 1.2.3.1.Immunoglobulin(IgA,IgM,IgG)

An immunoglobulins blood test measures the amounts of IgM, IgG, and IgA in your blood to help diagnose different types of health conditions that may affect your immune system .An immunoglobulins test usually measures three main types of immunoglobulin (Ig) antibodies that do different jobs to protect your health :

- **IgM** antibodies are the first immunoglobulins your body makes after you're exposed to germs. They provide short-term protection while your body makes other antibodies. IgM antibodies are in your blood and lymph fluid (a watery fluid that carries the cells that fight infections and diseases to all parts of your body).
- **IgG** antibodies are very important for fighting infections from bacteria and viruses. Most of the immunoglobulins in your blood are IgG. You also have some IgG antibodies in all your body fluids. Your body keeps a

"blueprint" of all the IgG antibodies you have made. That way, if you're exposed to the same germs again, your immune system can quickly make more antibodies.

- **IgA** antibodies protect your respiratory tract (the organs you use to breathe) and your digestive system (the organs you use to eat and digest food) from infections. You have IgA antibodies in your blood, saliva, and gastric "juices." ( Bradley *et al* .,2005).

### **1.2.3.2.Complement system**

The serum complement system, which represents a chief component of innate immunity, not only participates in inflammation but also acts to enhance the adaptive immune response. Specific activation of complement via innate recognition proteins or secreted antibody releases cleavage products that interact with a wide range of cell surface receptors found on myeloid, lymphoid and stromal cells. This intricate interaction among complement activation products and cell surface receptors provides a basis for the regulation of both B and T cell responses.

(Carroll.,2004).



# *Chapter Two*

## *Materials and Methods*

## 2. Materials and Methods

### 2.1. Materials

#### 2.1.1. Laboratory equipment and instruments

The laboratory equipment and instruments used in the present study are listed in Table (2-1).

**Table (2-1): Laboratory Equipment and Instruments**

No.	Laboratory Equipment and Instruments	Manufacturer/Origin
1.	Autoclave	Hirayama (Japan)
2.	Benzen burner	Amal (Turkey)
3.	Compound light microscope	Zeiss (Germany)
4.	Digital camera	Sony (Japan)
5.	Disposable (Pteri Dish ,Syringe, Plane and gel tube) and glassware	Citro (China)
6.	ELISA reader , ELISA washer	BioTeck (USA)
7.	Eppendrof tube	Eppendrof (Germany)
8.	Gloves	Malaysia
9.	Cotton	Iraq
10.	Hood	LabTech (Korea)
11.	Incubator, Oven	Memmert (Germany)
12.	Inoculating loop	Memmert (Germany)
13.	Micropipette (0.5-10 $\mu$ l , 5-50 $\mu$ l , 100-1000 $\mu$ l)	Capp (Denmark)
14.	Refrigerator	Concord (Lebanon)
15.	Vortex mixer	Gemmy (Taiwan)

### 2.1.2. Serological kits test

The Serological kits test used in the present study are illustrated in Table (2-2).

**Table (2-2): Serological kits test**

No.	Type of Kit	Company(Origin)
1.	RADIAL IMMUNODIFFUSION PLATE IgA , IgM, IgG	ITALY (LTA)
2.	RADIAL IMMUNODIFFUSION PLATE C3	ITALY (LTA)
3.	RADIAL IMMUNODIFFUSION PLATE C4	ITALY (LTA)

## 2.2. Methods

### 2.2.1. Sample collection

A total of 45 specimens (serum)(30)patients (15)control were collected from patients laboratory diagnosed with UGTIs , who attended the Teaching Hospital of Maternity and children Hospital and Private Clinics in Babylon province. According to previous study (Al-Ezzi and Al-Thahaba, 2022). The ages of those patients ranged from 20 –49 year. The blood samples (serum) saved at – 20 C° until systemic and innate immunological study.

### 2.2.2. Estimation of Immunoglobulin (IgG, IgM, IgA) and Complement (C3,C4) Levels

ESAY RID(Radial immune diffusion) are plates with wells for quantitative determination in radial immunodiffusion of human serum

are used to estimate antibodies IgG,IgM ,IgA and complement C3,C4 were used in the study.

### **2.2.2.1. Principle of method**

When antigen diffuses from a well into agar containing suitably diluted antiserum, initially it is present in a relatively high concentration and forms soluble complexes; as the antigen diffuses further the concentration continuously falls until the point is reached at which the reactants are nearer optimal proportions and a ring of precipitate is formed. The higher the concentration of antigen, the greater the diameter of this ring.

### **2.2.2.2. Test procedure**

1- ESAY RID was removed from the envelope ,opened the plate and leave to stand for about 5 minutes at room temperature so that any condenser water in the well can evaporate.

2- The well was filled with 5  $\mu$ l of undiluted patient sample.

3- The plate was closed with the lid, after the sample has diffused into the gel for about 20,leaved to stand ,overtured into the envelope at room temperature for 96 hours.

### **2.2.2.3. Interpretation of result**

Measure the precipitating ring to the nearest 0.1 mm, after the required period according to the procedure should be followed, and the type of protein. **Fig( 2-1)** Show interpretation of result Radial immune diffusion



**Figure 2-1: interpretation of result Radial immune diffusion**

### **2.2.3. 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.

### **2.2.4. Statistical Analysis**

Data were processed and analyzed with one way ANOVA using statistical program social science ( SPSS version 23) and the results were expressed as ( Mean  $\pm$  S.D ). P-values below 0.05 were considered to be statistically significant, and differences between means.

*Chapter*

*Three*

*Results and*

*Discussion*

### 3. Results and Discussion

#### 3.1. Study Population

A total number of (45) 30 specimens were collected from all (serum) pregnant married women and checked up for colonization with (*M.hominis*, *M.genitalium* and *U.urealyticum*) were selected from patients diagnosed by laboratory with urinary genital tract infections (UGTIs), who attended the Teaching Hospital of Maternity and children Hospital and Private Clinics in Babylon province, during 3 months from January to march, 2023. (15) control samples which were apparently (Health women) pregnant (married). The specimens percentage compared with control. A total number of (30) patients were distributed into three age groups (20-29) year 10 patients, (30 – 39) year 10 patients, (40 – 49) year 10 patients.

#### 3.2. Concentration immunological parameters

##### 3.2.1. Concentration of IgA mg/dl in patients and control

The table (3-1) show concentration of IgA mg/dl in patients and control significant difference at p value (0.018\*)

In this study, it was found that **Mean ± SD** in patient (370.28 ± 136.82) less than control (456.28 ± 60.88).

**Table (3-1): Concentration of IgA mg/dl in patients and control**

IgA mg/dl	N	Mean ± SD.	P. Value of T - Patient
Patient	30	370.28 ± 136.82	(0.018*)
Control	15	456.28 ± 60.88	

(\* ) mean significant difference in comparison with control at the 0.05 level

### 3.2.2. Concentration of IgG mg/dl in patients and control

The table (3-2) show concentration of IgG mg/dl in patients and control significant difference at p value (0.000\*\*)

In this study, it was found that **Mean ± SD** in patient (2595.49 ± 708.37) higher than control (2000.67 ± 266.82).

**Table (3-2): Concentration of IgG mg/dl in patients and control**

IgG mg/dl	N	Mean ± SD.	P. Value of T - Patient
Patient	30	2595.49 ± 708.37	(0.000**)
Control	15	2000.67 ± 266.82	

(\*\* ) mean significant difference in comparison with control at the 0.01 level

### 3.2.3. Concentration of IgM mg/dl in patients and control

The table (3-3) show concentration of IgM mg/dl in patients and control significant difference at p value (0.024\*)

In this study, it was found that **Mean ± SD** in patient (285.0 ± 80.72) less than control (327.0 ± 38.53).

**Table (3-3): Concentration of IgM mg/dl in patients and control**

IgM mg/dl	N	Mean ± SD.	P. Value of T - Patient
Patient	30	285.0 ± 80.72	(0.024*)
Control	15	327.0 ± 38.53	

(\* ) mean significant difference in comparison with control at the 0.05 level



This result is comparable with result of previous studies done show not resemble with result study In 2007, a Swedish study compared serum *M.genitalium* antibodies of pregnant female patients with EP and found an antibody positive rate of 18% in EP patients compared with 15% in controls, indicating no significant correlation between *M.genitalium* antibodies and EP (Jurstrand *et al.*, 2007).Interestingly, another Swedish study in 2015 used the *M. genitalium* serological patient to assess differences in *M. genitalium* antibodies between infertile and pregnant women the results showing that the rate of *M. genitalium* positive serum IgG levels in infertile women was 5.4% relative to 1.6% in the control group (Idahl *et al.*, 2015).

#### 3.2.4. Concentration of C3 mg/dl in patients and control

The table (3-4) show concentration of **C3 mg/dl** in patients and control not significant difference at p value (**0.441**). In this study, it was found that **Mean ± SD** in patients(**220.95 ± 38.77**)higher than control (**186.62 ± 38.96**).

Table (3-4):Concentration of C3 mg/dl in patients and control

C3 mg/dl	N	Mean ± SD.	P. Value of T – Patient
Patient	30	220.95 ± 38.77	(0.441)
Control	15	186.62 ± 38.96	

#### 3.2.5. Concentration of C4 mg/dl in patients and control

The table (3-5) show concentration of **C4 mg/dl** in patients and control significant difference at p value (**0.001\*\***)

In this study, it was found that **Mean  $\pm$  SD** in patients (**59.63  $\pm$  23.74**) higher than control (**44.36  $\pm$  6.76**).

**Table(3-5): Concentration of C4 mg/dl in patients and control**

<b>C4 mg/dl</b>	<b>N</b>	<b>Mean <math>\pm</math> SD.</b>	<b>P. Value of T – Patient</b>
<b>Patient</b>	<b>30</b>	<b>59.63 <math>\pm</math> 23.74</b>	<b>(0.001**)</b>
<b>Control</b>	<b>15</b>	<b>44.36 <math>\pm</math> 6.76</b>	

(\*\* ) mean significant difference in comparison with control at the 0.01 level

This result is comparable with result of previous studies done show not resemble with result study immunological findings included the determination of serum levels of IgG, IgM, IgA and complement protein C3 and C4. Results showed that IgG and IgA concentrations significantly increased ( $1311.13 \pm 72.54$  and  $279 \pm 21.31$ ) respectively in UTI patients in comparison to the healthy control group which was  $1089.88 \pm 37.33$  and  $117.611 \pm 4.19$  respectively, While IgM concentrations were increased non significantly in UTI patients ( $153.331 \pm 6.45$ ) in comparison to healthy control ( $145.2 \pm 13.49$ ). Complement components C3 showed a significant increase in UTI patients with mean values of  $125.95 \pm 6.22$  compared to the control group with mean values of  $55.191 \pm 9.64$ , while C4 showed statically non-significant among UTI patients in comparison with the control group ( $35.195 \pm 2.34$  and  $34.371 \pm 1.22$ ) respectively.(Al Otrachiet *al .*,2021).

The levels of serum IgM, IgG, complement C3 and C4 in the disease group were notably higher than those in the control group, and IgA levels were notably lower than those in the control group, and the above indexes showed statistical difference between severe and mild to

moderate *Mycoplasma pneumonia* ( $P < 0.05$ ). Compared with mild to moderate *Mycoplasma pneumonia* group, the predicted values of FEV1/FVC and FEV1% in severe mycoplasma pneumonia group were notably reduced ( $P < 0.05$ ). Correlation between FEV1/FVC and the detection indexes was analyzed by Spearson method. Pulmonary function of patients with *Mycoplasma pneumonia* was negatively correlated with serum IgM, IgG, C3 and C4 levels, and positively correlated with IgA levels. ROC curve showed that detection of IgM, IgA, IgG and complement C3 and C4 and their combination could reveal the severity of *Mycoplasma pneumonia*. (Zhu *et al.*., 2020). Studies on the pathogenesis of *Mycoplasma pneumonia* have revealed that Ig is an important active substance involved in humoral immune response. After *Mycoplasma pneumonia* infection occurs in the body, IgM, IgA and IgG are secreted and released into the blood by cells due to the induction effect, which changes the antigen structure of the body, leading to damage of the immune function of the body and multiple organs of the body (Kumar *et al.*., 2018). Complement C3 and C4 are the most common immunologically active substances in the complement activation pathway. *Mycoplasma pneumonia* infection can activate the body to promote the infiltration and activation of immune cells, and finally trigger autoimmune injury to the body (Miklaszewska *et al.*., 2016). The comparative study of the changes of detection indexes in this experiment between normal subjects and patients with *Mycoplasma pneumonia* showed that the serum IgM, IgG, complement C3 and C4 levels were higher and IgA levels were lower in patients with mycoplasma pneumonia. *Mycoplasma pneumonia* infection leads to the formation of immune complexes and a series of immune response reactions. It activates complement alteration and lysis (bacteria and cells) and other

effects. Cell-mediated antibodies can exhibit cytotoxic effects and have the effect of killing and destroying infected target cells (Gaoetal .,2018).

### 3.2.6. Correlation among systemic immunity parameters for patients

The table (3-6) show Correlation among in systemic immunity parameters for patients. In this study, it was found positive correlation in IgG (0.385\*) and negative correlation in IgM (- 0.441) with IgA. And show positive correlation in IgM with IgG (0.191). Correlation C3 with IgA and IgG is negative correlation (- 0.233 , - 0.286 ) and positive correlation with IgM (0.262). Correlation C4 with IgA and IgG is negative correlation (- 0.260 , - 0.156 ) and positive correlation with IgM (0.348). Correlation C4 with C3 show positive correlation (0.370\*).

**Table (3-6): Correlation Among in Systemic Immunity Parameters for Patients**

Correlation All Results	IgA	IgG	IgM	C3	C4
IgA	1				
IgG	0.385*	1			
IgM	- 0.441	0.191	1		
C3	- 0.233	- 0.286	0.262	1	
C4	- 0.260	- 0.156	0.348	0.370*	1
*Significant at 0.05					

***Conclusions***  
***and***  
***Recommendations***

### **Conclusions:**

- 1- The present study according to immunological parameters indicated that the patients with UGTIs due to mycoplasmas infection had significantly increasing IgA, IgM, IgG ,C4
- 2- Not significant C3increasing.

### **Recommendations :**

- 1- Studying other immunological parameters for UGTIs and its role in the diagnosis of infection with mycoplasmas is recommended.
- 2- It is necessary to use different methods to diagnosis infection.

# *References*

## ***Reference***

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- Afriat, R., Horowitz, S., and Priel, E. (2013). *Mycoplasma fermentans* inhibits the activity of cellular DNA topoisomerase I by activation of PARP1 and alters the efficacy of its anti-cancer inhibitor. *PloS one*. 8(8), e72377.
- Al Otrachi, K. I. B., Darogha, S. N., & Ali, B. A. (2021). Serum levels of immunoglobulin and complement in UTI of patients caused by *Proteus mirabilis* and using AgNPs as antiswarming. *Cellular and Molecular Biology*, 67(3), 11-23
- Al-Ezzi, N. J., & Al-Thahaba, A. (2022). Studying the relationship between IL-18, INF- $\beta$  and mycoplasmas among urinary-genital tract infections for females in Babylon Province, Iraq. *International Journal of Health Sciences*, 6(S5), 2619–2627.
- Bradley, S. V., Oravec-Wilson, K. I., Bougeard, G., Mizukami, I., Li, L., Munaco, A. J., ... & Ross, T. S. (2005). Serum antibodies to huntingtin interacting protein-1: a new blood test for prostate cancer. *Cancer research*, 65(10), 4126-4133.
- Carroll, M. C. (2004). The complement system in regulation of adaptive immunity. *Nature immunology*, 5(10), 981-986.
- Cheong, K. A., Agrawal, S. R., and Lee, A. Y. (2011). Validation of nested PCR and a selective biochemical method as alternatives for *Mycoplasma* detection. *Journal of Basic Microbiology*, 51(2), 215-219.
- Clausen, H. F., Fedder, J., Drasbek, M., Nielsen, P. K., Toft, B., Ingerslev, H. J., ... and Christiansen, G. (2001). Serological investigation of *Mycoplasma genitalium* in infertile women. *Human Reproduction*. 16(9), 1866-1874.
- Distelhorst, S. L., Jurkovic, D. A., Shi, J., Jensen, G. J., and Balish, M. F. (2017). The variable internal structure of the *Mycoplasma penetrans* attachment organelle revealed by biochemical and microscopic analyses: implications for attachment organelle mechanism and evolution. *Journal of Bacteriology*. 199(12), e00069-17.



## ***Reference***

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- Eggert-Kruse, W., Kiefer, I., Beck, C., Demirakca, T., and Strowitzki, T. (2007). Role for tumor necrosis factor alpha (TNF- $\alpha$ ) and interleukin 1-beta (IL-1 $\beta$ ) determination in seminal plasma during infertility investigation. *Fertility and Sterility*. 87(4), 810-823.
- Eggert-Kruse, W., Kiefer, I., Beck, C., Demirakca, T., and Strowitzki, T. (2007). Role for tumor necrosis factor alpha (TNF- $\alpha$ ) and interleukin 1-beta (IL-1 $\beta$ ) determination in seminal plasma during infertility investigation. *Fertility and Sterility*. 87(4), 810-823.
- Flores-Medina, S., Soriano-Becerril, D. M., and Díaz-García, F. J. (2012). Molecular diagnostics of mycoplasmas: Perspectives from the microbiology standpoint. *Polymerase Chain Reaction*. 119-142.
- Gao, M., Wang, K., Yang, M., Meng, F., Lu, R., Zhuang, H., ... & Wang, X. (2018). Transcriptome analysis of bronchoalveolar lavage fluid from children with *Mycoplasma pneumoniae* pneumonia reveals natural killer and T cell-proliferation responses. *Frontiers in immunology*, 9, 1403.
- Idahl, A., Jurstrand, M., Olofsson, J. I., & Fredlund, H. (2015). *Mycoplasma genitalium* serum antibodies in infertile couples and fertile women. *Sexually Transmitted Infections*, 91(8), 589-591.
- Jafar, K., Robeena, F., and Pir, B. G. (2010). Antibiotic sensitivity of human genital mycoplasmas. *African Journal of Microbiology Research*. 4(9), 704-707.
- Jurstrand, M., Jensen, J. S., Magnuson, A., Kamwendo, F., & Fredlund, H. (2007). A serological study of the role of *Mycoplasma genitalium* in pelvic inflammatory disease and ectopic pregnancy. *Sexually Transmitted Infections*, 83(4), 319-323.
- Kornspan, J. D., Tsur, M., Tarshis, M., Rottem, S., and Brenner, T. (2015). *Mycoplasma hyorhinis* induces proinflammatory responses in mice lymphocytes. *Journal of Basic Microbiology*. 55(5), 679-684.

## ***Reference***

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- Kumar, A., Rahal, A., Chakraborty, S., Verma, A. K., and Dhama, K. (2014). *Mycoplasma agalactiae*, an etiological agent of contagious agalactia in small ruminants: a review. *Veterinary medicine international*.
- Kumar, S., Garg, I. B., Sethi, G. R., Kumar, S., & Saigal, S. R. (2018). Detection of immunoglobulin M and immunoglobulin G antibodies to *Mycoplasma pneumoniae* in children with community-acquired lower respiratory tract infections. *Indian Journal of Pathology and Microbiology*, 61(2), 214.
- Lloyd, D., Chapman, A., Ellis, J. E., Hillman, K., Paget, T. A., Yarlett, N., and Williams, A. G. (2021). Oxygen levels are key to understanding “Anaerobic” protozoan pathogens with micro-aerophilic lifestyles. *Advances in Microbial Physiology*. 79, 163-240.
- Mavedzenge, S. N., Van Der Pol, B., Weiss, H. A., Kwok, C., Mambo, F., Chipato, T., ... and Morrison, C. (2012). The association between *Mycoplasma genitalium* and HIV-1 acquisition in African women. *Aids*. 26(5), 617-624.
- McGowin, C. L., and Totten, P. A. (2017). The unique microbiology and molecular pathogenesis of *Mycoplasma genitalium*. *The Journal of Infectious Diseases*. 216(suppl\_2): S382-S388 .
- Meager, A., Parti, S., Leung, H., Peil, E., and Mahon, B. (1987). Preparation and characterization of monoclonal antibodies directed against antigenic determinants of recombinant human tumour necrosis factor (rTNF). *Hybridoma*, 6(3), 305-311.
- Metwally, M. A., Yassin, A. S., Essam, T. M., Hamouda, H. M., and Amin, M. A. (2014). Detection, characterization, and molecular typing of human *Mycoplasma* spp. from major hospitals in Cairo, Egypt. *The Scientific World Journal*.
- Miklaszewska, M., Zachwieja, K., Drożdż, D., Pallinger, E., Takács, B., & Csuka, D. (2016). Hemolytic uremic syndrome with *Mycoplasma*

## ***Reference***

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- pneumoniae infection and membrane cofactor protein mutation. *Przeglad Lekarski*, 74(11), 862-864.
- Moridi, K., Hemmaty, M., Azimian, A., Fallah, M. H., Khaneghahi Abyaneh, H., and Ghazvini, K. (2020). Epidemiology of genital infections caused by *Mycoplasma hominis*, *M. genitalium* and *Ureaplasma urealyticum* in Iran; a systematic review and meta-analysis study (2000–2019). *BMC Public Health*. 20(1), 1-13
- Naher, H. S., Al-Hamadini, A. H., and Said, I. H. (2014). Pre valence of Genital Mycoplasma, *Mycoplasma hominis* and *Ureaplasma urealyticum* in Women in Al-Qadisiya Province. A New Record. *Al-Qadisiyah Medical Journal*, 10(17), 59-66.
- Pascual, A., Jatón, K., Ninet, B., Bille, J., and Greub, G. (2010). New diagnostic real-time PCR for specific detection of *Mycoplasma hominis* DNA. *International Journal of Microbiology*.
- Qin, L., Chen, Y., and You, X. (2019). Subversion of the immune response by human pathogenic mycoplasmas. *Frontiers in Microbiology*.1934.
- Qin, L., Chen, Y., and You, X. (2019). Subversion of the immune response by human pathogenic mycoplasmas. *Frontiers in Microbiology*.1934.
- Razin, S. (2018). The *Mycoplasma* Membrane. In *Organization of Prokaryotic Cell Membranes* (pp. 165-250). CRC Press.
- Ryan, K. J., and Ray, C. G. (2004). *Medical microbiology*. McGraw Hill. 4(370).
- Taylor-Robinson, D., and Lamont, R. F. (2011). Mycoplasmas in pregnancy. *BJOG: An International Journal of Obstetrics and Gynaecology*, 118(2), 164-174
- Yiwen, C., Yueyue, W., Lianmei, Q., Cuiming, Z., and Xiaoxing, Y. (2021). Infection strategies of mycoplasmas: Unraveling the panoply of virulence factors. *Virulence*. 12(1), 788-817.

## ***Reference***

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Zhu, F., Yang, J., & Li, J. (2020). Diagnostic value of immunoglobulin combined with complement C3 and C4 in mycoplasma pneumonia. *INTERNATIONAL JOURNAL OF CLINICAL AND EXPERIMENTAL MEDICINE*, 13(11), 8660-8667.

الميكوبلازما هي كائنات حية سريعة النمو بطيئة النمو تفتقر إلى جدار خلوي وتعزل في الغالب من المسالك البولية التناسلية. على الرغم من أن العديد من أنواعها تعتبر نبيتات طبيعية ، إلا أن بعض الأنواع تسبب أمراضاً تناسلية خطيرة.

هدفت الدراسة الحالية إلى تحديد العلاقة بين العدوى البكتيرية شديدة الحساسية (نوع *Mycoplasma* و *Ureaplasma*) وبعض المؤشرات المناعية لعدوى الجهاز التناسلي البولي (UGTIs) للنساء المتزوجات فقط (الحوامل). لغرض الحصول على نتائج موثوقة ، تم استخدام التقنية التالية: RADIAL IMMUNODIFFUSION لقياس تركيز المعايير المناعية لـ IgG ، IgM ، IgA ، C3 ، C4.

اشتملت الدراسة على (30) مريضاً تم توزيعهم على ثلاث فئات عمرية (20-29) سنة 10 مرضى ، (30 - 39) سنة 10 مرضى ، (40-49) سنة 10 مرضى و (15) كعينة ضابطة (سيطرة) .

أظهرت النتيجة تركيز IgA mg / dl في المرضى والتحكم بفرق كبير عند قيمة (p 0.018) \* ووجد أن المتوسط  $\pm$  الانحراف المعياري في المريض (136.82  $\pm$  370.28) أقل من المجموعة الضابطة (60.88  $\pm$  456.28).

و تركيز IgG mg / dl في المرضى والتحكم بفرق كبير عند قيمة (p 0.000)\*\* ووجد أن المتوسط  $\pm$  الانحراف المعياري في المريض (708.37  $\pm$  2595.49) أعلى من المجموعة الضابطة (266.82  $\pm$  2000.67).

و تركيز IgM mg / dl في المرضى والتحكم بفرق كبير عند قيمة (p 0.024) \* ووجد أن المتوسط  $\pm$  الانحراف المعياري في المريض (80.72  $\pm$  285.0) أقل من السيطرة (327.0  $\pm$  38.53).

لم يكن تركيز C3 مجم / ديسيلتر في المرضى والتحكم فرق معنوي عند قيمة (p 0.441) ووجد أن المتوسط  $\pm$  الانحراف المعياري في المريض (38.77  $\pm$  220.95) أعلى من المجموعة الضابطة (38.96  $\pm$  186.62).

و تركيز C4 مجم / ديسيلتر في المرضى والتحكم فرق كبير عند قيمة (p 0.001)\*\* ووجد أن المتوسط  $\pm$  الانحراف المعياري في المريض (23.  $\pm$  59.63) أعلى من السيطرة (44.36  $\pm$  6.76).

في هذه الدراسة وجد ارتباط موجب في 0.385(IgG\*) وارتباط سلبي في IgM (-0.441) مع IgA. وتظهر علاقة ارتباط موجبة (0.191) في IgM مع IgG. الارتباط C3 مع IgA و IgG هو ارتباط سلبي (-0.233، - 0.286) وارتباط موجب مع IgM 0.262، ارتباط C4 مع IgA و IgG هو ارتباط سلبي (- 0.260، - 0.156) وعلاقة موجبة مع IgM (0.348). يظهر الارتباط C4 مع C3 ارتباط إيجابي (0.370\*).



جمهورية العراق  
وزارة التعليم العالي و البحث العلمي  
جامعة بابل  
كلية العلوم  
قسم علوم الحياة

## بعض المعايير المناعية في مرضى مايكوبلازما المسالك البولية التناسلية

بحث

مقدم الى مجلس كلية العلوم / جامعة بابل تحصيلاً لمرحلة البكالوريوس في علوم الحياة /  
فرع الاحياء المجهرية / الدراسة المسائية

من قبل

صبا رحمن صهيود

بإشراف

أ.د. أزهار عمران لطيف عبود الذهب

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