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Mycoplasma pneumoniae Respiratory Tract Infections in Nablus District

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Dedication

I dedicate this modest success to all who love the science and knowledge.

To the one who taught me to give without waiting .. to whom I bear his name proudly and fondly .. to my father.

To the meaning of love, compassion, dedication and the secret of my success.. to my mother.

To my husband thank you for your financial and psychological support offered to me to prove myself and realize my dream.

To gifts of God, my children Elias and Ahmed, I love you.

To all who supported me and look after my children to give me time to study, I love you and will not forget you

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الإقرار

أنا الموقع أدناه، مقدم الرسالة التي تحمل العنوان:

Mycoplasma pneumoniae Respiratory Tract Infections in Nablus District

أقر بأن ما شملته هذه الرسالة إنّما هو نتاج جهدي الخاص، باستثناء ما تمّت الإشارة إليه حيثما ورد، وأنّ هذه الرسالة ككل، أو أيّ جزء منها لم يقدّم من قبل لنيل أيّ درجة أو لقب علميّ لدى أيّ مؤسسة تعليمية أو بحثية أخرى.

Declaration

The work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

Student's Name: **اسم الطالب:**

Signature: **التوقيع:**

Date: **التاريخ**

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List of Abbreviations

M. pneumoniae	*Mycoplasma pneumoniae*
mm	Millimeter
DNA	Deoxyribonucleic acid
PCR	Polymerase chain reaction
CFT	Complement fixation test
IgM	Immunoglobulin M
IgG	Immunoglobulin G
CAP	Community-acquired pneumonia
MLs	Macrolides
TCs	Tetracyclines
FQs	Fluoroquinolones
ELISA	Enzyme Linked Immunosorbent Assay
CBC	Complete Blood Count
WBCs	White Blood Cell Count
ESR	Erythrocyte sedimentation rate
CRP	C-reactive protein
dNTP	Deoxynucleoside triphosphate
PBS	Phosphate buffer saline
TMB	Tetramethylbenzidine
IRB	Institutional Review Board
NTU	Nova Tec Unit

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Abstract

M. pneumoniae is known to be a common cause of respiratory tract infections in different age groups. Worldwide, the lack of rapid and standardized tests for the diagnosis of *M. pneumoniae* infection is a common problem that encounters researchers in this field. This prospective study was conducted to determine the prevalence of *M. pneumoniae* using classical ELISA and nested PCR techniques among patients with respiratory tract infections in Nablus Districts. The study included 129 inpatients and out patients visited or admitted to the involved clinics during September 2015 to April 2016. At the time of first visit to clinical settings, a throat swab was collected from all participants. Serum specimens were collected from 103 patients. A second serum specimen was available for 16 patients 7 to 15 days later. Throat swab specimens were tested by nested PCR for the detection of *M. pneumoniae*. Serum samples were tested for the presence of IgG and IgM antibodies by ELISA. Out of 129 examined throat swabs specimens, DNA of *M. pneumoniae* was detected by PCR in 15(11.6%) samples. *M. pneumonia*e specific IgM was detected in 4(3.9%) out of the 103 first serum sample. A total of 47(39%) patients possessed IgG at different titers in the first and/or second

serum specimens. According to the applied diagnostic criteria (positive IgM with PCR or/and IgG serology [high titer, seroconversion or twofold increase in titer] or/and PCR confirmed by IgG serology), 10(7.8%) patients were diagnosed with current *M. pneumoniae* infection. Among these diagnosed cases, PCR detected 10 cases, while IgM in first serum specimen detected 4 cases. Most of *M. pneumoniae* infections were diagnosed during winter (10.4%). No difference in the prevalence of *M. pneumoniae* infection was found with respect to gender. The highest percentage of *M. pneumoniae* respiratory infection (11.3%) was found in patients with age range 25-64 years, followed by patients age range 0-9 (9.1%) and 10-24 (7%).

No significant differences in the frequency of signs and symptoms in patients with *M. pneumonia*e infection compared to those with other infectious agents. Laboratory parameters showed significantly higher frequency of increase in lymphocytes count (***P***=0.001) in patients with *M. pneumonia*e infection compared to other infectious agents. On the contrary, the frequency of increase in WBCs and granulocytes counts were significantly lower in patients with *M. pneumonia*e infection (***P***= 0.006 and 0.000, respectively).

In conclusion, *M. pneumonia*e seems to be an important etiological agent of respiratory tract infections in the area, thus more attention is required in adopting health policy for diagnosis and used medication policies.

Chapter One

Introduction and Literature Review

Introduction

Mycoplasma pneumoniae, *Chlamydophila pneumoniae* and *Legionella pneumophila* are atypical bacterial pathogens, which are involved in respiratory tract infections. The prevalence of respiratory tract infections caused by these atypical bacterial agents is probably underestimated due to the difficulty in isolating and identifying them (Ginevra *et al*, 2005). *Mycoplasma pneumoniae* (*M. pneumoniae*) is responsible for a large number of upper and lower respiratory tract infections in children and adults (Jacobs, 1993). It's an important etiological agent of pneumonia (3-10%) (Clyde, 1993; Jain S *et al*., 2015; Williams *et al*., 2015) and one of the leading causes of pneumonia (0-30%) in children (Principi *et al*., 2001; Korppi, 2004). Pneumonia caused by *M. pneumoniae* is commonly described by the expression ‘walking pneumonia to distinguish it from classical pneumococcal pneumonia (Krause and Taylor-Robinson, 1992). This term can be misleading, as it indicates that mycoplasmal pneumonia cases are not dangerous. On the contrary, *M. pneumoniae* is a serious respiratory tract pathogen (Foy, 1993).

1.2 General Characteristics of *Mycoplasma pneumoniae*

M. pneumoniae belongs to the family Mycoplasmataceae, order Mycoplasmatales and class Mollicutes. *M. pneumoniae* was first isolated in 1961 from sputum of a patient using tissue culture method. All members in the class Mollicutes including *M. pneumoniae* lack cell wall (Waites and Talkington, 2004; Eaton *et al*., 1944), which makes bacteria

resistant to β-lactam antibiotics, not stained with Gram stain (Eaton *et al*., 1944; Principi and Esposito, 2001) and make these organisms pleomorphic (Krunkosky *et al*., 2007).

M. pneumoniae is the smallest and simplest bacterium, which is capable of self –replication. It possess small genome compared with *Escherichia coli* (Himmelreich *et al*., 1996; Roca., 2006). The unique small size of *M. pneumoniae* allow it to pass through 0.45μm pore size filters, make them undetectable by light microscopy (Waites, 2004; Principi and Esposito, 2001) and do not produce visible turbidity in broth growth media (Andreu *et al*., 2006). *M. pneumoniae* possesses extremely small genome and consequently limited biosynthetic capabilities. This explains their parasitic or saprophytic existence and fastidious growth requirements (Waites, 2003). Culture of *M. pneumoniae* from clinical specimens is rarely performed in routine context because it requires specialized media and it's time consuming, where it takes to 21 day to obtain a visible colony (Waites and Taylor-Robinson, 1999; Souliou, 2007; Kashyap and Sarkar., 2010).

1.3 *Mycoplasma pneumoniae* and respiratory tract infection

Considerable interest arose in elucidation and characterization of *M. pneumoniae* prevalence, mode of spread and spectrum of disease after the identification of *M. pneumoniae* as an etiological agent of primary atypical pneumonia, (Golubjatnikov *et al*., 1975; Huong ple *et al*.,2014; He *et al*., 2013; Joosting *et al*., 1976). *M. pneumoniae* infections can involve both the upper and lower respiratory tract. It's respiratory

infections occur both endemically and epidemically worldwide (Ansarin *et al*., 2011). Infection occurs by respiratory droplets and direct contact with an infected person. *M. pneumoniae* respiratory tract infections occurs in persons of all ages, but is most common among children aged 7–16 years and young adults (Feizi *et al*., 1967; Clyde, 1993;Ozerol *et al*.,2006; Bulletin, 2014).

In colonization of *M. pneumoniae*, there is an interaction between pathogen adhesion proteins (e.g. P1, P30, P116) (Waldo and Krause., 2006) and accessory protein (HMW1, HMW3) and the host respiratory epithelium cells (Razin, 1999; Page and Krause, 2013). *M. pneumoniae* is usually associated with mild acute respiratory infections such as sore throat, pharyngitis, rhinitis and tracheobronchitis, however it can also cause more critical infections including pneumonia or lung abscess (Waites and Talkington, 2004; Nour *et al*., 2005). The most common clinical manifestations resulting from *M. pneumoniae* respiratory infection include sore throat, hoarseness, fever, dry cough, headache, chills, coryza, myalgia and general malaise (Waites, 2004; Principi and Esposito, 2001).

Pulmonary complications caused by *M. pneumoniae* that may take place include pleuritis, pneumothorax, respiratory distress syndrome, lung abscess (Waites and Talkington, 2004; Nour *et al*., 2005; Foy, 1993). Other complications are acute exacerbations of asthma and chronic obstructive pulmonary disease (Lieberman *et al*., 1996; Varma-Basil *et al*., 2009).

Para-pneumonic complications of *M. pneumonia* infection are rare and are generally limited to patients at the extremes of age such as 6 months or 65 years and those with comorbid medical illness (Thompson *et al*., 2003). For example, severe infections due to *M. pneumoniae,* with involvement of the joints often develop in patients with humoral immunodeficiency. Extrapulmonary manifestations, involving almost all organs of the human body and including neurological (e.g. encephalitis), renal failure, hepatic, hemolytic anemia, cardiac diseases, polyarthritis, erythrema multiforme, Guillain-Barre syndrome, transverse myelitis or Stevens-Johnson syndrome (Foy, 1993; Annemarie *et al*., 2001; Nelson, 2002; Waites, 2003;Mulholland *et al*.,2012).

1. 4 Epidemiology of *Mycoplasma pneumoniae* infection

M. pneumoniae infections were reported as endemic infections in most regions of the world (Annemarie *et al*., 2001). It was proposed that climate could affect the rate of *M. pneumoniae* infection. Although, some study reported that *M. pneumoniae* activity increased as the average temperature and relative humidity increased (Onozuka *et al*.,2009), *M. pneumoniae* infection rate peaks in winter or spring. (Sidal et al., 2007). Similar to influenza, *M. pneumoniae* causes community outbreaks (Gomez *et al*., 1996). Cyclic outbreaks and global pandemics of *M. pneumoniae* respiratory tract infections can be expected to occur on average every 3–7 years (Kumar and Hammerschlag, 2007). In the United States, it was

found that most outbreaks tend to occur in the late summer and early fall (Foy, 1993; Lind *et al*., 1997). It was estimated that *M. pneumoniae* caused more than 100,000 adults hospitalizations each year in the USA (waits *et al*., 2004), it affect both upper and lower respiratory tract infection and caused 5–20% of all cases of community acquired pneumonia, and 40% of cases among children. Furthermore, it was reported that 30% or more of *M. pneumoniae* infections occurred in children between 5–15 years and 18% of them required hospitalization (Korppi, 2004; Hammerschlag *et al*., 2001).

In an Indian study, *M. pneumoniae* infection has been diagnosed in 35% of patients with community acquired pneumonia in both children and adults (Kumar and Hammerschlag, 2007). In addition to *M. pneumoniae* infection was diagnosed in 22% of infants and young children suffering from respiratory tract infection in Northern Greece (Almasri *et al*., 2013).

Another study found that *M. pneumoniae* respiratory infection was strongly associated with smoking; lower pre-existing immunoglobulin G levels, and lower lymphocyte counts (Eyal *et al*., 2006).

1.5 Diagnosis of respiratory infections caused by *M. pneumoniae*

Patients with *M. pneumoniae* respiratory infection show clinical presentations not significantly different from that of patients with respiratory infections caused by other respiratory pathogens such as *Chlamydia pneumoniae* (Waites and Taylor-Robinson, 1999; Almasri *et al*., 2013). Diagnosis of *M. pneumoniae* respiratory infection relies

primarily on laboratory diagnosis including serodiagnosis and detection of pathogen in respiratory tract.

1.5.1. Detection of antibodies against M. pneumoniae in patient's serum

Serological diagnostic techniques for *M. pneumoniae* in respiratory tract infections are well known for long time. These techniques were used for several epidemiological studies because of the relative lack of sensitivity and time-consuming nature of culture of *M. pneumoniae* (Ken *et al*., 2004). Serodiagnosis of this pathogen became popular in part due to the ease of specimen collection and the widespread availability of these tests. However, also with regard to the definite proof of a causative role of *M. pneumoniae* in a given respiratory tract infection, serology is far from being replaced by direct pathogen detection in respiratory secretions.

Detection of cold agglutinins was considered a valuable tool for diagnosis of *M. pneumoniae* infection. It was found that the formation of cold agglutinins is the first humoral immune response to *M. pneumoniae* infection (Jacobs,1993; Swiecicki ,2013).

Complement fixation assay was the first serological diagnostic method developed for detecting *M. pneumoniae* infection and was used as a standard method for diagnosis of respiratory infections caused by this pathogen. The lack of both sensitivity and specificity of the complement fixation test has been described extensively as a back draw for its use as this assay is based on detection of immune response against glycolipid

antigen mixture, which may be found in other microorganisms (Atkinson *et al*., 2008; Ruuskanen *el al*., 1992; Pönka *et al*., 1981). Other disadvantages of complement fixation test is that it's procedure is technically demanding and time consuming (Waites, 2001).

1.5.1.1 Enzyme Linked Immunosorbent Assay (ELISA)

ELISA is a widely used commercial method for detecting *M. pneumoniae* infection (Atkinson *et al*., 2008). It is more sensitive in the detection of acute infections than cultures, and reported to have sensitivity comparable to that of PCR. Furthermore, an important advantage of ELISA is that it can be used to determine specific IgG, IgM and IgA antibodies class expressed at different intervals and thus, can give clue to elapsed time of infection, distinguishing between current and previous infections (Jacobs *et al*.,1986; Beersma *et al*., 2005; Talkington *et al*., 2004). During the first week of illness, IgM antibodies appears in the blood of the patient. This class of antibodies reach peak titer during third week and seems to decline towards low levels (below the cut-off value of commercial assays) within few months. Although, presence of *M. pneumoniae*-specific IgM in human serum is a reliable indicator of recent primary infection, these antibodies in particular may be absent in adults (Uldum *et al*., 1992). IgA antibodies could be an indicator of recent infection but have been reported to possess low sensitivity and specificity (Wreghitt and Sillis,1985; Hirschberg *et al*.,1990; Souliou, 2007).

In the course of *M. pneumoniae* illness, the level of specific IgG antibodies increases slowly reaching peak titers 5 weeks after the onset of clinical symptoms. One important diagnostic limitation if IgG class is that usually no measurable IgG response is observed during the first week of the illness (Jacobs *et al*.,1986). In a study from National Taiwan University Hospital, 290 children were clinically diagnosed to have mycoplasma pneumonia. Testing their respiratory swap samples for *M. pneumoniae* by RT-PCR showed that fifty-four children (19%) were positive. Serological testing for 63% (182/290) of these children showed that 44 (24%) were IgM positive (Hsin-Yu *et al*., 2014).

1.5.2 Detection of *M. pneumoniae* in respiratory secretions

Culture of *M. pneumoniae* is carried out on an artificial expensive media, which is supplied by fastidious requirements for growth such as horse serum. Furthermore, antibiotics are also added to prevent growth of other types of bacteria and fungi. However, culture of *M. pneumoniae* requires a laborious effort and take relatively long incubation time for growth to occur, where the incubation is up to 5 weeks or more. Another important limitation of culture is the insensitivity of this method for detecting *M. pneumoniae* infection in comparison to serological assays (Jacobs,1993). In a study carried out in Iran, among 149 patients included, *M. pneumoniae* infection was diagnosed in 9 (6.15%), 8 (5.3%), and 3 (2.01%) patients by PCR, ELISA, and culture methods, respectively (Reza *et a.l*, 2013).

Rapid methods for direct antigenic detection of *M. pneumoniae* in specimens obtained from respiratory tract have included direct immunofluorescence and antigen capture enzyme immunoassay (Gerstenecker and Jacobs, 1993; Kleemola *et al*., 1993). However, such detection assays were with low sensitivity and cross-reactivity with other mycoplasmas (Hirschberg, 1991; Hirai, *et al*. 1991, Souliou, 2007). DNA hybridization techniques through the application of DNA probes were also used for the diagnosis of *M. pneumoniae* respiratory tract infections (Razin, 1994). Cross-reactivity between *M. pneumoniae* and *Mycoplasma genitalium,* and the lack of probes sensitivity as antigen detection techniques are major problems associated with such technique (Jacobs, 1993; Daxboeck *et al*., 2003).

1.5.2.1 Detection of M. pneumoniae in respiratory secretions by Polymerase chain reaction (PCR)

PCR is a rapid diagnostic technique and is commonly used in medical and biological research laboratories for a variety of applications (Saiki *et al*., 1985). Several primer sequences were constructed to detect *M. pneumoniae* in respiratory secretions such primers that target ATPase, operon, P1 protein gene and the 16S rRNA (Van Kuppeveld *et al*., 1994; Abele-Horn *et al*.,1989; Ieven *et al*.,1996; Buck *et al*.,1992).

The sensitivity of PCR technique is very high, corresponding to the presence of a single organism when purified DNA is used. Other advantages of this technique is the ability to complete the procedure in 1 day, thus allow to obtain results more quickly after onset of illness

compared to time required using serology tests. The need for only one specimen containing organisms that do not have to be viable and the ability to detect nucleic acid in preserved tissues are also advantages in favor of this technique (Waites and Talkington, 2004). The need of specialized equipment, and false-positive results from cross-contamination of samples were considered as major problems associated with this technique (Razin, 1994; van Kuppeveld *et al*., 1994).

Nested PCR was applied for diagnosis of *M. pneumoniae* respiratory infection in a number of researches. This technique is a two-step procedure in which the products of first PCR run using outer primers are re-amplified using a second set of inner primers located within the previously amplified sequence. Nested PCR possesses improved sensitivity and specificity compared to conventional PCR. Reverse transcriptase PCR have also been used to detect *M. pneumoniae* in respiratory secretions (Picken *et al*., 1996; Kumar *et al*., 2008).

It seems to be logical to combine more than one method for optimal diagnosis of *M. pneumoniae* infections (Waites, 2003; Dowell, 2001; Razin, 2002; Souliou *et al*., 2007; Sun *et al*., 2013).

1.6 Treatment of *M. pneumoniae* infections

B-Lactam antibiotics target the cell wall and are active against most respiratory bacterial pathogens; however, it is ineffective against *M. pneumoniae* because *M. pneumoniae* lacks cell wall. In contrast macrolides, tetracyclines act as protein synthesis inhibitors, and fluoroquinolones act against topoisomerases (inhibit DNA synthesis and replication). Macrolides, tetracyclines and fluoroquinolones are highly

effective against *M. pneumoniae*. Macrolides are the drug of choice for the treatment of all infections due to atypical bacteria, including *M. pneumoniae* (Principi and Esposito, 2001). The resistance rates to Macrolide were repored to be 3% in Germany (Dumkeat *et al.*, 2009). However, higher resistance rates were found in France (9.8%) and Japan (13.2%) (Morozumi *et al.*, 2008; Peuchant *et al.*, 2009)

1.7 Prevention

M. pneumoniae is a respiratory pathogen and consequently it's transmission occurs via respiratory droplets, requiring close contact with an infected individual (Denny, 1971). Individuals with active Mycoplasma infection carry organisms in the nose, throat, trachea and sputum with transmission facilitated by coughing.(Waites *et al.*,2005; Lin *et al.*,2002). Control of *M. pneumoniae* infection outbreaks requires effective measures and substantial costs. Control practices include mainly prevention of the exchange of respiratory droplets between patients and health workers and antibiotic prophylaxis of members of the community (Hyde *et al.*, 2001; Gray *et al.*, 1998; Klausner *et al.*, 1998).

1.8 Aim of this Study

There's limited information about the incidence of *M. pneumoniae* in respiratory tract infections in Palestine. The present study was conducted to determine the prevalence of *M. pneumoniae* infections among patients with respiratory tract infection in Nablus Districts using both ELISA and PCR.

Chapter Two

Materials and Methods

2.1 Patients

The study included patients with respiratory tract infections admitted to or visited the participated clinical settings during September 2015 to April 2016. Participated clinical settings included: two Governmental hospitals (Al-Watani and Rafidya), Palestinian Red Crescent Center in town of Asira,and An Najah National University clinic. All of these clinical settings are at Nablus district. The study protocol was approved by IRB at An-Najah University.

Patients with evidence of cancer, disorders of the pulmonary or cardiovascular system, and those who had received antibiotics active against *M. pneumoniae,* were excluded. All patients included in the study were clinically diagnosed by physicians to have respiratory tract infection.

A specially designed questionnaire was used for data collection of the concerned patients. The questionnaire was filled by the researcher with the help of the treating physician or assistant nurse.

The questionnaire included information related to disease diagnosis, onset of disease and related symptoms in a set of four sections. The questionnaire included a set of questions concerning the following information:

A. Personal data (age, gender, place of residence, number of family members and smoking status).
B. Date of onset of the disease and time of admission to hospital.
C. Clinical data (reported symptoms of cough, sputum production, vomiting, abdominal pain, headache, difficulty of breathing, shortness of breath and fever ≥ 38 °C).

D. Available laboratory findings (laboratory parameters) such as:

1. CBC: WBC (lymphocytes and granulocytes percentages) and platelets.
2. Erythrocyte sedimentation rate (ESR).
3. Titer of C-reactive protein (CRP).

2.2 Sample collection

At time of hospital admission or first visit to the clinic, from each patient a throat swab specimen was obtained with the help of a doctor or a nurse. Medical stuff were asked to provide the first serum specimen. Second serum specimens were taken after 7-15 days after initial sample collection when possible. Throat swab specimens were placed in 2ml normal saline and immediately placed in ice. Samples were then transported to laboratory and placed in sterile Eppendorf tubes and stored at -20°C until time of examination.

In case of blood specimens, the blood was allowed to clot and then centrifuged to obtained serum. However, in most cases serum was obtained from the laboratory of participating medical settings. The serum was placed in Eppendorf tube and kept at -20°C until examination. In case of presence of 2 serum specimens, first and second serum specimen were examined together.

2.3 DNA extraction

DNA was extracted by simple boiling method as described by Waring *et al* (Waring *et al*., 2001). A total of 500µl of the original specimen was placed

in to an Eppendorf tube and was concentrated by centrifugation at 13,000xg for 10min at room temperature. The supernatant was removed keeping about 20µl of original specimen. Then 30µl of sterile distilled water was added to the remaining supernatant and the pellet. The sample was then vortexed and heated to 95°C for 15min. Aliquot of the sample lysate was then used directly for PCR amplification or kept at -20°C till use.

2.4 Polymerase chain reaction for detection of *M. pneumoniae* in throat swabs

DNA extracted from throat swab samples were tested for *M. pneumoniae* by nested PCR. The DNA sequence of the used primers targeting the P1 adhesion were obtained from (Lam *et al.*, 2007) and are shown in Table 2.1. PCR Ready mix (SIGMA-ALDRICH, USA) was used and PCR was performed with a total reaction mixture volume of 25µl. The mixture contained 1x PCR buffer [1.5 units Taq DNA polymerase, 10mM Tris-HCl, 50 mM KCl, 1.5 $MgCl_2$, 0.001% gelatin, 0.2mM deoxynucleoside triphosphate (dNTP)]. A concentration of 0.8pmole/µl was used for each of the used primers.

PCR reaction was performed using AmpliTaq Gold (Tprofessionla Biometra thermocycler, Germany). First and second rounds of nested PCR were the same and carried out as follows: three minutes at 94°C (for denaturation), 35 cycles of amplification (one minute at 94°C [for denaturation] and one minutes at 64°C [for annealing] and one minutes at

64°C [for extension]), and final extension of 10 minutes at 72°C. A negative control with no template was included and specimens known to be positive was used as positive control in each PCR run. PCR products were detected by 2% agarose gel electrophoresis with ethidium bromide staining and the bands were visualized under UV light.

Table(2. 1):primers Used for detection of *M. pneumoniae* bacteria

References	Size(bp)	Primer sequence(5´ to 3´)
First round of nested PCR		
(Lam *et al*., 2007)	343	F, GACCATTCCACCCAGCCCCAGC R, GTTCAGCGAGTGGGGTGCGTACAATA
Second round of nested PCR		
(Lam *et al*., 2007)	160	F, AGGGGGTTCTTCAGGCTCAGGTCAA R, CCCCACCACATCATTCCCCGTATTA

2.5 Detection of IgM and IgG antibodies against *M. pneumoniae*

Enzyme-Linked Immunosorbent Assay (NovaTec Immundiagnostica GmbH, Germany) was used for determination of IgM and IgG antibodies in serum specimens of the patients. The procedure was carried out according to manufacture instructions.

Patients sera were added to microtiter plates wells coated with *M. pneumoniae* antigens and antibodies (IgM and IgG) against *M. pneumoniae* in serum specimens were allowed to bind to coated antigens. Unbound materials were then removed by washing the wells with phosphate buffer saline (PBS). This was followed by addition of anti- IgM antibodies conjugate to detect IgM or anti-IgG conjugate to detect IgG. These anti-antibodies bind to captured *M. pneumoniae* specific antibodies. The unbound conjugate molecules were removed by washing with PBS.

Complexes formed by conjugate -*M. pneumoniae* antibodies were visualized by adding Tetramethylbenzidine (TMB) substrate, which gave blue color product. Sulphuric acid was added to stop the reaction and a yellow color was formed. Then the absorbance of each well was read at 450nm using ELISA micro well reader (Awareness Technology INC, USA).

The intensity of produced color was proportional to the amount of *M. pneumoniae* - specific antibodies in sample. Results were calculated in Nova Tec Unit (NTU) and interpreted according to manufacturer instructions. The cut-off value was first calculated, which was equal to the mean of absorbance values of 2 wells to which cut-off control was applied. Nova Tec Unit equal to the patient absorbance value × 10 ÷ cut off . The interpretation of results using this unit was:

Negative <11 NTU

Positive >11 NTU

2.6 Diagnosis of *M. pneumoniae* infection:

In the present study, laboratory diagnosis of *M. pneumoniae* infection was carried out similar to previously described by (E. Souliou,*et al*., 2007). Current or recent infection of *M. pneumoniae* was definitely diagnosed if at least **two** of the following criteria were found:

1) positive PCR in throat swab specimen
2) positive IgM antibodies (in the first and/or the second sample)

3) seroconversion or significant increase of IgG antibodies (twofold increase), or IgG titers >40 Nova Tec Unit

Probable cases of recent or current *M. pneumoniae* infections were diagnosed when only one of the three diagnostic criteria mentioned above was found.

2.7 Statistical analysis

SPSS version 21 for windows was used for comparison of the prevalence of *M. pneumoniae* infections between genders, different age ranges and seasons. In addition, frequencies of clinical and laboratory parameters in patients with *M. pneumoniae* infections were compared to those without such infection. ***P*-**value < 0.05 was considered significant.

Chapter Three

Result

3.1 Patients and specimens' collection

The study included 129 patients with respiratory tract infection symptoms (diagnosed by physicians) in the period between September 2015 to April 2016. Patients were inpatients and out patients at Al-Watani National Hospital (n=70), Rafidya Hospital (n=11), out patients in clinic of An Najah university (n=18), out patients in Red Crescent center in Asira (n=30). All of these clinical settings are at Nablus district.

Throat swabs were obtained at time of admission or first visit from all of the 129 patients, and first serum specimens were obtained from 103 patients. Second serum specimens were obtained from 16 of 103 patients 7 to 15 days later after first serum sample collection.

3.2 Results of PCR and Serology

Table 3.1 shows the results of nested PCR (throat swab specimens) and ELISA (serum specimen). Among the 129 throat swabs specimens, Positive PCR of *M. pneumoniae* was detected in 15(11.6%) patients. Figure 3.1, is a representative agarose gel of PCR amplified products. ELISA assays for the detection of IgM and IgG were carried out for 103 patients. *M. pneumoniae*-specific IgM was detected in 4(3.9%) of the first serum samples. Collected second serum (16) showed 2 additional IgM positive samples.

In the first serum specimen of 103 patients, IgG antibody was detected in 47 (45. 6%), while in the collected (16) second serum specimen, IgG antibodies were detected in 11(68.8%) cases. No seroconversion was

detected (negative IgG in first and positive IgG in second), thus the patients with positive IgG in first and/or in second serum specimen were 47 (45.6%).

Table(3. 1):PCR and ELISA (IgG and IgM) findings in tested samples

Assay	No. of examined specimen No. (%)	Positive PCR No. (%)	Positive result in [a] 1st specimen No. (%)	Positive result in 2nd specimen No. (%)	Patients with positive result in 1st &/or [a] 2nd specimen No. (%)
PCR	129	15(11.6%)	-	-	-
ELISA-IgM	103+16[b]	-	4(3.9)	2 (12.5)[c]	6(5.8)
ELISA-IgG	103+16	-	47(45.6)	11(68.8)	47(45.6)

[a] 1st , First serum sample; 2nd , second serum sample

[b] 103 first serum sample and 16 second serum sample

[c] percentage calculated using 16 second serum sample

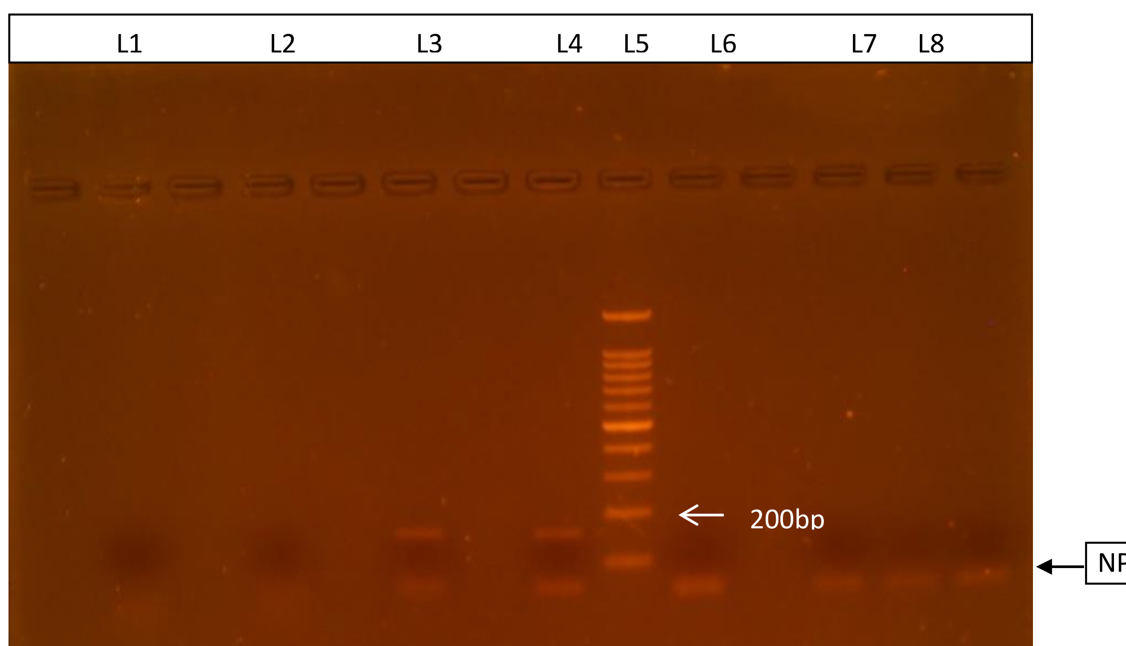

Figure (3. 1): Agarose gel electrophoresis of nested PCR products stained with Ethidium bromide. Lane (L) 1, negative control; L 2,7,8,9 negative sample; L 3, positive control (160bp); L4, positive sample; L5, 100 bp ladder. NP, not PCR product (like primer dimmers).

3.3 Patients diagnosed with current *M. pneumoniae* infection

According to diagnostic criteria of *M. pneumoniae* current infection, mentioned in materials and methods, 10 (7.8%) patients were diagnosed with the infection. Table 3.2 shows the PCR, IgM and IgG results of the 10 diagnosed cases. *M. pneumoniae* bacterium was detected by PCR in throat swabs in all of these 10 patients diagnosed with *M. pneumoniae* current infection. IgM antibodies were detected in 4 cases (3.9%) in first serum sample. Seroconversion of IgM antibody (negative IgM in first and positive IgM in second) was detected in one patient indicated by number 9 in Table3.2. Testing for IgG antibodies was with positive varied titters in all of the 10 cases in first serum sample. A significant increase (twofold increase) in IgG antibodies was detected in one case (patient number 9). Five patients possessed IgM antibodies positive in the first and/or the second serum specimen and had positive PCR for *M. pneumoniae* in throat swab specimen at the same time. However, PCR appears to be more sensitive (100%) compared to IgM in first serum specimen (40%).

3.4 Probable and possibly previous cases of *M. pneumoniae* infection

Eight (7. 7%) patients did not meet the diagnostic criteria of current *M. pneumoniae* infection and were considered as probable cases. Among these patients, 5 cases (number 11-15) had positive PCR result with no appearance of IgM in first serum specimen and no second serum sample. Seroconversion of IgM was detected in one case (patient 16) with negative PCR and no significant increase in IgG titer. In 4 cases (number 11, 12, 17,

18) IgG antibodies was detected in 4 first serum samples. Among these 4 cases, 2 patients (number 17,18) were with high IgG titer (>40 NTU).

Evidence of possibly previous *M. pneumoniae* infections was detected in 33(25.6%) patients who were negative by PCR and negative for IgM in first and in second sample, but IgG antibodies were positive in first or/ and second sample.

Table(3. 2):Patient diagnosed with current, probable, or possible previous *M. pneumoniae* infection

Sample number	PCR	First serum sample		Second serum sample	
		IgM (NTU)	IgG (NTU)	IgM (NTU)	IgG (NTU*)
Patients with current *M. pneumoniae* infection					
1	P*	N*	P(43)	NA*	NA
2	P	N	P(54)	NA	NA
3	P	P (28.3)	P(97)	NA	NA
4	P	N	P(102)	NA	NA
5	P	P(12.6)	P(15)	NA	NA
6	P	N	P(50)	NA	NA
7	P	N	P(83)	NA	NA
8	P	P (23.6)	P(12)	NA	NA
9	P	N	P (27)	P (15)	P(74)
10	P	P (35)	P (15)	NA	Na
Patients with probable *M. pneumoniae* infection					
11	P	N	P(28)	NA	NA
12	P	N	P(15)	NA	NA
13	P	N	N	NA	NA
14	P	N	N	NA	NA
15	P	N	N	NA	NA
16	N	N	P(34)	P(13)	P(31)
17	N	N	P(49)	NA	NA
18	N	N	P(40)	NA	NA
Patients with evidence of possible *M. pneumoniae* previous infection					
19-26	N	N	P(15-28)	N	P(15-30)
27-51	N	N	P(12-37)	NA	NA
Patients with no evidence of *M. pneumoniae* infection					
52-57	N	N	N	N	N
58-129	N	N	N	NA	NA

***P, positive; N, negative; NA, not available; NTU, Nova Tec Unit**

No evidence of *M. pneumoniae* infection by any of applied methods (both PCR and ELISA) were found in 78(60.5%) of the studied cases.

3.5 *M. pneumoniae* infection in relation to gender and age

Diagnosis of *M. pneumoniae* infection was found in 5 out of 71 (7.1%) females and in 5 out of 58 (8.6%) males. The infection rate in males was found to be insignificantly higher than females (P =0.753). Patients age included in the present study ranged from 1 to 91 years with a mean age of

32 years. Patients were grouped in age ranges as shown in Table 3.3 and Figure 3.2.

Table**(3. 3):**Distribution of cases with *M. pneumoniae* infection among different age ranges

Age range	Number of patients	*M. pneumoniae* infection No.(%)
0-9	11	1 (9.1)
10-24	43	3 (7)
25-64	53	6 (11.3)
≥ 65	12	0 (0%)

0-9 vs 10-24, p=0.615; 10-24 vs 25-64, P=0.383

0-9 vs 25-64, P=0.663; 10-24 vs >65, P=0.492

0-9 vs>65, P=0.50; 25-64 vs >65, P=0.315

The highest percentage of *M. pneumoniae* respiratory infection (11.3%) was found in patients with the age ranged between 25-64 years, which was insignificantly higher than that of patients ranged 0-9 years (9.1%, P=0.663) and 10-24 years (7%, P=0.383). Furthermore, *M. pneumoniae* infection was not diagnosed in 12 patients with age ≥ 65 years.

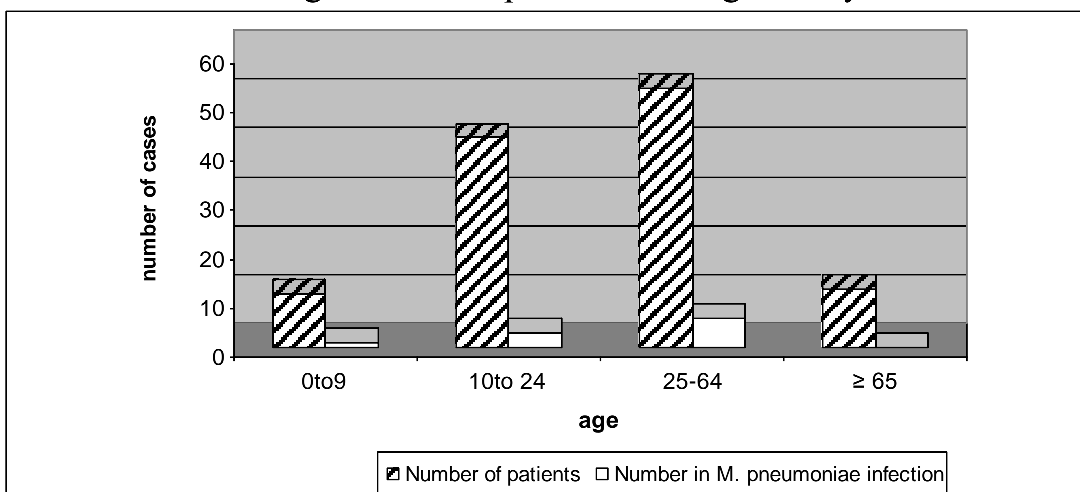

Figure (3. 2): Age distribution of *M. pneumoniae* infection

Patients positive for *M. pneumoniae* infection had a mean age of 36.3±18.51 years, which was insignificantly (P=0.516) higher than the mean age of patients without *M. pneumoniae* infection (32.1±19.8 years).

3.6 Clinical data

Table 3.4 shows the clinical signs and symptoms in patients with or without *M. pneumonia*e infection. No significant difference was found in the comparison of signs and symptoms between patients with *M. pneumonia*e infection and those with other causatives of respiratory infections, however, some variations were reported. All of the 10 patients with *M. pneumonia*e infection reported to have cough, thus cough seems to be the most common symptom. Cough frequency in patients with *M. pneumonia*e infection was insignificantly higher than that with non-mycoplasmal infections. In addition, sputum production, fever and abdominal pain were insignificant higher in cases diagnosed to have *M. pneumonia*e infection. On the contrary, headache, difficulty of breathing, shortness of breath, vomiting and average of days onset the disease were insignificantly lower in patients with *M. pneumonia*e infection.

Table(3. 4):Signs and symptoms among patients with or without *M. pneumoniae* infection

Sings and symptoms	*M .pneumoniae* positive (n= 10) No. (%)	*M. pneumoniae* negative (n=119) No. (%)	***P***-value
Cough	10 (100)	110 (92.4)	0.287
Sputum production	8(80)	93 (78.2)	0.212
fever ≥ 38 °C	6(60)	54 (45.4)	0.367
Abdominal pain	4(40)	28 (23.5%)	0.627
Headache	4(40%)	76(63.9%)	0.563
Difficulty of breathing	8(80%)	98(82.4%)	0.602
Shortness of breath	8(80%)	101(84.9%)	0.653
Vomiting	2(1.6%)	27(20.9%)	0.125
Average of days onset the disease	6.11± 2.83	6.7± 2.45	0.526

Data presented in Table 3.5 compares means of laboratory parameters (CBC) among patients with *M. pneumoniae* infection and among those without this infection. No significant differences were observed, however small variations were found.

Table(3. 5):Mean value of various laboratory parameters in patients with *M. pneumoniae* infection and non *M. pneumoniae* infection

Parameters	*M. pneumoniae* *N=10* mean ± SD	*Non M. pneumoniae* *N=119* mean ± SD	***P***-value
WBC count	7± 3.3	7.4± 3.2	0.695
Lymphocytes%	28.5± 13.5	28.9± 13.7	0.913
% Granulocytes	68.2±14.3	67.4±14.2	0.866
Platelets count	278.3±88.9	280.1±70.3	0.942

In order to detect elevation in count of WBCs, lymphocytes, granulocytes, and/or Platelets count, results were compared to normal ranges in different ages using three references (Principle of Hematology and Fundamentals of Homeostasis, 2009; Williams Manual of Hematology, 2009; Oxford Hand Book of Clinical Hematology, 2003). Frequencies of increase of different components of CBC in *M. pneumoniae* patients and those with other

respiratory tract infection are shown in Table 3.6. In patients with infection by respiratory pathogens other than *M. pneumoniae*, the rate of increase of WBC (8.4%) and of granulocytes (40.3%) were significantly higher (***P***= 0.006 and 0.000, respectively) than patients with *M. pneumonia*e infection. On the contrary, percentage of increase in lymphocytes among patients diagnosed to with *M. pneumonia*e infection (20%) was significantly higher than that in patients infected by other respiratory pathogens (15.9%).

Table**(3. 6):**Frequency of increase in CBC parameters in patients with *M. pneumoniae* infection and among those with other infectious agents.

Parameters	*M. pneumoniae positive N=10* No. (%)	*M. pneumoniae* negative *N=119* No. (%)	***P***-value
Increase WBC count	0(0)	10 (8.4)	0.006
Increase lymphocyte	2(20)	19(15.9)	0.001
Increase granulocytes	3(30)	48(40.3)	0.00
Increase platletes count	1(10)	6(5.1)	0.133

Table 3.7 shows clinical diagnosis of participated patients. 70% of patients with *M. pneumoniae* infection were diagnosed with upper respiratory tract infection and the rest were diagnosed as bronchitis cases. The frequency of upper respiratory tract infection caused by *M. pneumoniae* was close to that caused by other pathogens. However, bronchitis rate (30%) was insignificantly higher in patients infected by *M. pneumoniae* (***P***=0.334). On the contrary frequency of pneumonia caused by *M. pneumoniae* was insignificantly lower (***P***=0.605) than other infectious agents.

Table(3. 7):Clinical diagnosis of patients with and without *M. pneumoniae* infection

Diagnosis	*M. pneumoniae* infection N= 10 No. (%)	Non-*M. pneumoniae* infection N= 119 No. (%)	***P***-value
URTI	7 (70)	86 (72.3)	0.536
Bronchitis	3 (30)	22 (18.5)	0.344
Pneumonia	0 (0)	11(9.3)	0.605

URTI vs pneumoniae, p=0.633

URTI vs bronchitis, p=0.208

Bronchitis vs pneumoniae, p=0.485

M. pneumoniae infection frequency was highest (10.4%) during winter followed by spring (4.5%) and no cases were detected during autumn. These variations were insignificant (Table 3.8 and Figure 3.3).

Table(3. 8):Seasonal distribution of *M. pneumoniae* infection

Season	**Total number**	**Non- *M. pneumonia* No. (%)**	***M. pneumoniae* infection No. (%)**
Winter	**77**	**69(89.6)**	**8 (10.4)**
Spring	**44**	**42(95.5)**	**2(4.5)**
Autumn	**8**	**8(100)**	**0 (0)**

M. pneumoniae infection in:

Winter vs spring, P= 0.222

Winter vs autumn, P= 0.437

Spring vs autumn, P= 0.713

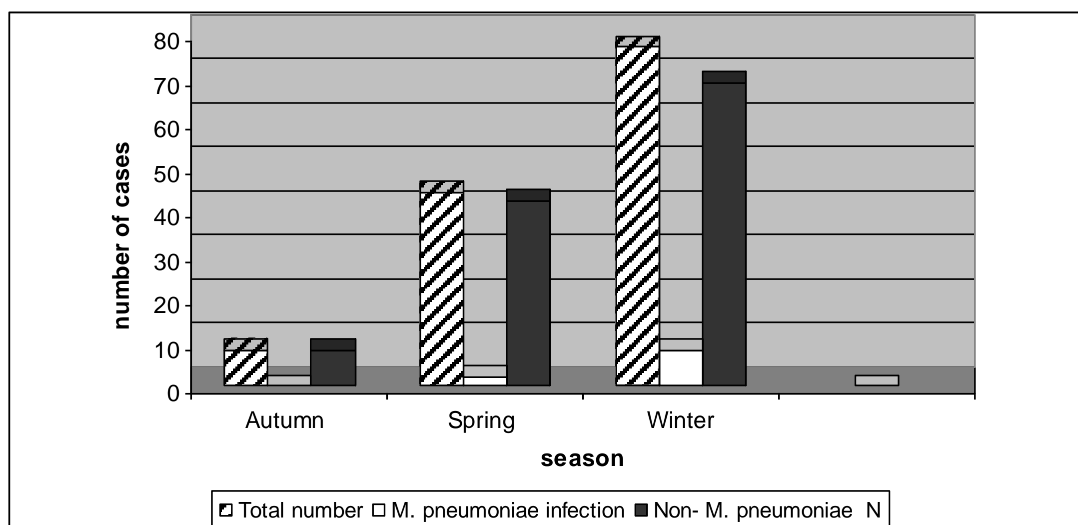

Figure (3. 3): Seasonal distribution of *M. pneumoniae* infection.

3.7 Risk factors for *M. pneumoniae* respiratory infections

Among the included 129 patients, 15 had diabetes mellitus, 2 had anemia, 20 had hypertension and 22 were smokers as shown in Table 3.9 No significant associations between *M. pneumoniae* infection and smoking (***P***=0.230), anemia (***P*** =0.85), hypertension (***P*** =0.520), diabetes mellitus (***P*** = 0.762) or number of siblings (***P*** = 0.879) were found. The finding of limited number of patients diagnosed with *M. pneumonie* makes it difficult to link such risk factors to this infection; however it is worth noting that smoking status (13.6%) is more pronounced risk factor in *M. pneumoniae* infections compared to other studied risk factors.

Table(3. 9):Risk factors for acquiring *M. pneumonie* infection

Risk factor	No. positive	*M. pneumoniae No.(%)*	*Non M. pneumoniae No.(%)*	***P*** Value
Smoking	22	3(13.6)	19(86.4)	.230
Hypertension	20	1(5)	19(95)	.520
Diabetes mellitus	15	1(6.7)	14(93)	.672
Mean No. siblings	129	4.5±1.1	4.7±1.4	0.762
Anemia	2	0(0)	2(100)	.850

Chapter Four

Discussion

Discussion

Difficulty and high costs of diagnosis of *M. pneumonia*e infection by laboratory methods hinder determination of it's prevalence in most Third World countries. To our knowledge the present study represents the first determination of the prevalence of *M. pneumonia*e in respiratory tract infections in adults and children in Nablus district. For more accurate diagnosis of *M. pneumonia*e infections a combination of PCR and serological testing using ELISA assay for the detection of IgM and IgG specific antibodies were used.

In the present study, 10 patients (7.8%) were diagnosed with current *M. pneumoniae* respiratory tract infection and 8 patient were diagnosed to be probably infected with *M. pneumoniae*. In a study carried out in Pahang in Malaysia during 2011, close percentage of *M. pneumoniae* infection rate (6.5%) determined by serology was reported among 17–80 years old patients with community acquired pneumonia (Mustafa *et al.*, 2011). Similar findings were also reported from Iran in a study conducted during 2009 to 2010, where 6.15% of patients were diagnosed with *M. pneumoniae* respiratory tract infections based on PCR, culture and serology findings (Reza *et al.*, 2013). A higher frequency (21.6%) of *M. pneumoniae* in respiratory tract infections was reported by a study carried out in 12 teaching hospitals in Beijing during 2010 to 2011 for adults and adolescent patients (≥ 14 years of age) with radiographically confirmed CAP. The findings of this study were based on IgM antibody testing, florescent quantitative PCR and culture (Jiuxin *et al.*, 2013). In a previous

research carried out in Greece, *M. pneumoniae* infection was diagnosed as the only pathogen in 11.1% of cases of children with respiratory tract infection (Almasri *et al*., 2011). Variations in the frequency of *M. pneumoniae* infection rate in respiratory tract infections in different geographical regions are expected to occur due to seasonal climate differences in temperature, geographic features, applied diagnostic methods, types of collected specimens, population spread on area, and antibiotics application policy (AtKinson *et al*., 2008).

In the present study all the diagnosed cases of *M. pneumoniae* infection were PCR positive and only 4(40%) were positive by IgM ELISA in the first serum specimen. In another study *M. pneumoniae* IgM assay determined by capture ELISA was found to show a sensitivity of 66.7% in first serum sample and conventional PCR 75% (Souliou *et al*., 2007). Variations in sensitivity of various testing techniques could be due variations in sensitivity of used ELISA kits, sample type, and patients age.

In the present study, the highest percentage of *M. pneumoniae* respiratory infection (11.3%) was found in patients within the age range of 25-64 years. This was followed by age range 0-9 (9.1%) and 10-24 (7%). No cases of *M. pneumoniae* respiratory infection were diagnosed in patients ≥ 65 years this may be due to limited number of patients at this age range . In 2015, Keping Chen et al. (reported that *M. pneumoniae* infection was most predominant (40. 8%) in school aged children 7-14 years (Keping *et al*., 2015). However, very low prevalence of *M. pneumoniae* infection rate (1%) based on RT-PCR was found among Iranian children with acute

respiratory infections during 2003 to 2004(Zer et al., 2010). During 2010-2015, a study in England found the *M. pneumoniae* infectionis predominantly found in children and adults <44 years of age (Brown *et al*., 2016). Variations in the frequency of this infectious bacterial agent among different age groups may be due to frequency of close contact with infected individuals as well as the development or decline of immune status in relation to age.

In the current study, the infection rate was highest during winter (10.4%) followed by spring (4.5%). It was reported that *M. pneumoniae* infection takes place most commonly during fall and winter (Atkinson, *et al*., 2008). On the contrary a study in China reported that *M. pneumoniae* infection was relatively high in summer and autumn (45.08% and 47.14%, respectively) and relatively low in spring (38.3%) and winter (35.5 %)(Keping *et al*., 2015).

In our study all of the 10 patients with *M. pneumonia*e infection had cough, thus coughing was the most common symptom. No significant difference was found in signs and symptoms between patients with *M. pneumonia*e infection and with other causatives of respiratory tract infections. Consistent with our findings on associated symptoms a previous study reported fever and cough to be the most common symptoms (both 84%) in patients with *M. pneumoniae* infection (Almasri *et al*., 2011).

In the present study, patients with infection by respiratory pathogens other than *M. pneumoniae* possessed rates of increase of WBC (8.4%) and of granulocytes (40.3%) significantly higher (P= 0.006 and 0.000,

respectively) than those in patients with *M. pneumoniae* infection. On the other hand, the percentage of increase in lymphocytes among patients diagnosed to have *M. pneumoniae* infection (20%) was significantly higher than that in patients infected by other respiratory pathogens. It was reported in Beijing-China (Jiuxin *et al*., 2013) that mean of lymphocyte was 19.6±10.64 in patients with *M. pneumoniae* infections, which is insignificantly (P=0.614) higher than in patients without *M. pneumoniae* infection (18.5 ± 7.14). The lower frequency of increase of WBCs among patients infected by *M. pneumoniae* may be due to lower antigenic stimulation caused by *M. pneumoniae* as this bacterium is the smallest free live one and possesses very low division rate.

Among the investigated risk factor in the present study, the highest frequency in association with was *M. pneumonie* respiratory infection was smoking (13.6%). Similar findings were reported by (Eyal *et al*.,2006) as smoking was reported to be strongly associated with *M. pneumonie* respiratory infections.

One limitation of the present study should be mentioned. It's the limited number of second serum specimen and time of first serum sample collection . However, this problem is not expected to affect the results of the study to considerable level because the results also depends on PCR. Nested PCR technique is very sensitive (Lam *et al*., 2007) in detecting the pathogen in acute phase before the development of antibodies. In our results, only 5 cases out of 129 (3.9%) were PCR positive without

detecting diagnostic immune response and these were called probable cases of infection.

In summary, *M. pneumoniae* plays an important role as an etiological agent of respiratory tract infection in Nablus district. Although detected in almost all age ranges, *M. pneumoniae* respiratory infection rate was highest in the age range 25-64 years. Most cases of *M. pneumoniae* respiratory infection were diagnosed during winter followed by spring. With exception of increase in lymphocytes percentage, CBC values, symptoms and signs appear to have limited diagnostic value of *M. pneumoniae* infection.

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Appendix

Appendix A

بسم الله الرحمن الرحيم

An - Najah National University
Faculty of Medicine & Health Sciences
Department of Graduate Studies

جامعة النجاح الوطنية
كليـة الطب وعلـوم الصحة
دائرة الدراسات العليا

IRB Approval letter

Study title:
***Mycoplasma pneumoniae* Respiratory Tract Infections in Nablus District**

Submitted by:
Aseel Thaher

Date Reviewed:
Dec 21 ,2014

Date approved:
Mar 18, 2015

Your study titled: "***Mycoplasma pneumoniae* Respiratory Tract Infections in Nablus District**" with archived number 239/Dec/2014 , Was reviewed by An-Najah National University IRB committee & approved on Mar 18, 2015 .

Hasan Fitian , MD

IRB Committee Chairman,
An-Najah National University

نابلس – ص.ب 7 او 707 هاتف 2342902/4/7/8/14(09)(970) ، فاكسميل 2342910 (09) (970)
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Appendix B

Questionnaire

Master Research program

Student: Aseel Thaher

This study is a research aiming to determine the frequency of *Mycoplasma pneumoniae* respiratory tract infections in Nablus district. Throat swab and serum specimen will be obtained from each participant at initial meeting. A second throat swab and blood specimen will be collected 2 weeks latter. The swabs and blood specimens will be used to detection *Mycoplasma pneumoniae* infection. No risk or discomfort will be caused to participant. If *Mycoplasma pneumoniae* infection is detected, the participant will be informed in order to take the suitable treatment prescribed by a doctor. The information provided by the participant will be kept confidential. In case the participant have any question or have research-related injury he can call Aseel Thaher (0598133931). Subject participation is only obtaining swabs and blood specimen. The participant will be asked to come again to obtain a second serum specimen.

A: Personal Information

Name	
Telephone	
Sex	Male Female
age of the patient	
Job	
addresses (city or village)	
Number of family members	
Smoker	
Use tooth brushing every day	
Have diabetes	
Have hypertension	
Have anemia	

B.

date of onset of the disease	
time of admition to hospital	
Diagnosis	
Upper respiratory tract infection____ Tracheobronchitis ____ Pneumonia____	
X-ray (if made):	

C. Available laboratory findings (laboratory parameters) such as:

CBC	
Platletes count	
hemoglobin	
WBC count	
%Lymphocytes	
% Neutrophils	
% granulocytes	
Erythrocyte sedimentation rate (ESR)	
C-reactive protein (CRP)	

D. Clinical information such as;

Sputum production	
cough	
vomiting	
abdominal pain	
headache	
difficulty of breathing,	
shorthness of breath	
fever $\geq$ 38 °C	

جامعة النجاح الوطنية
كلية الدراسات العليا

إصابات الجهاز التنفسي بالبكتريا من نوع مايكوبلازما الرئوية في منطقة نابلس

إعداد
أصيل فازع حمد ظاهر

إشراف
د. معتصم المصري
د. نائل ابو الحسن

قدمت هذه الأطروحة استكمالاً لمتطلبات الحصول على درجة الماجستير في العلوم الحياتية، بكلية الدراسات العليا، في جامعة النجاح الوطنية، نابلس، فلسطين.

2017

إصابات الجهاز التنفسي بالبكتريا من نوع مايكوبلازما الرئوية في منطقة نابلس فلسطين

إعداد

أصيل فازع حمد ظاهر

إشراف

د. معتصم المصري

د. نائل ابو الحسن

الملخص

في جميع أنحاء العالم، من المعروف ان البكتريا من نوع مايكوبلازما الرئوية سبب شائع في التهابات الجهاز التنفسي في مختلف الفئات العمرية. عدم وجود اختبارات سريعة وموحدة لتشخيص العدوى في المايكوبلازما الرئوية هي مشكلة مشتركة واجهت الباحثين في هذا المجال.

وقد أجريت هذه الدراسة البحثية لتحديد مدى انتشار بكتريا المايكوبلازما الرئوية بين المرضى الذين يعانون من التهابات الجهاز التنفسي في مناطق نابلس.وقد استخدم لهذا الغرض تقنيات ELISA الكلاسيكية و PCR معا. وشملت الدراسة 129 مريضا من العيادات الخارجية المعنية و من المرضى النائمين في المستشفى خلال الفترة مابين سبتمبر 2015 إلى أبريل 2016.

في وقت الزيارة الأولى للمرضى تم جمع مسحات الحلق واخذ الاعراض السريرية لكل مريض من جميع المشاركين بينما تم جمع 103عينة دم اولى. وكذلك تم جمع16 عينة دم ثانية بعد سبعة إلى خمسة عشر يوما من اخذ العينة الاولى .

ولقد تم اختبار عينات مسحة الحلق عن طريق ل PCR للكشف عن المايكوبلازما الرئوية واختبار عينات الدم لوجود الاجسام المضادة المناعية (IgG و IgM) بواسطة ELISA.

من أصل 129 فحص عينات مسحات الحلق، تم الكشف عن الحمض النووي للمايكوبلازما الرئوية بواسطة PCR في 15 (11.6٪) عينة. وتم الكشف عن الجسم المناعي المضاد IgM في 4 (3.9٪) من العينة الدم الأولى. اما بالنسبة الى الجسم المضاد المناعيIgG كانت النسبة تساوي 47 (45.6٪) من المرضى في عينة الدم الأولى و / أو عينة الدم الثانية.

في هذه الدراسة لقد تم وضع معاييرلتشخيص الاصابة بالمايكوبلازما الرئوية وهي IgM الإيجابي مع PCR الايجابية أوالتاكيد بواسطة نتائج IgG (IgG ذو عيار عالي، التحول من السالب في العينة الاولى الى الموجب في العينة الثانيه أو زيادة بمقدار الضعف في وحدةIgG) و PCR الايجابي مع التاكيد بواسطة نتائج IgG. ووفقا لمعاييرتشخيص الاصابة بالمايكوبلازما الرئوية المستخدمة في هذه الدراسة كانت نسبة الاصابة 10 (7.8٪).

ومن بين المرضى تم تشخيص 10 حالات بواسطة تقنيه PCR مقارنة مع 4 حالات تم الكشف عنها في الجسم المضاد المناعيIgM في عينة الدم الأولى . تم تشخيص أكثر الالتهابات الرئوية بواسطة المايكوبلازما خلال فصل الشتاء (10.4٪). ولم يعثر على أي اختلاف في معدل انتشار العدوى الرئوية فيما يتعلق بنوع الجنس.

وكانت أعلى نسبه عدوى (11.3٪) من المايكوبلازما الرئوية في الجهاز التنفسي في الفئة العمرية 25–64 سنة، تليها الفئة العمرية بين سن 0–9 (9.1٪) واخيرا من 10–24 (7٪).

لم يتم الكشف عن اختلافات كبيرة في تكرار علامات وأعراض التهابات الجهاز التنفسي في المرضى الذين يعانون من عدوى المايكوبلازما الرئوية مقارنة مع الذين يعانون من الاصابة بالعوامل المعدية الأخرى.

وأظهرت نتائج الفحوصات المخبرية ان اعلى تكرار كان في زيادة الخلايا الليمفاوية في المرضى الذين يعانون من عدوى المايكوبلازما الرئوية بالمقارنة مع المرضى الذين يعانون من ميكروبات مرضية اخرى, وكان الاختلاف ذات دلالة احصائية ($P = 0.001$).

المايكوبلازما الرئوية تبدو عامل مهم مسببا لالتهابات الجهاز التنفسي في المنطقة، وبالتالي مطلوب المزيد من الاهتمام في طرق تشخيص الاصابة بهذا النوع من البكتريا وسياسة الأدوية المستخدمة.