

قسم الميكروبيولوجيا  
كلية الطب - جامعة أسيوط  
رئيس القسم : أ.د/ ماهر مختار زكي •

النقص السيروولوجي عن وجود الميكوبلازما نيمونيا في الصعيد

اسماعيل صديق ، نبيلة رشوان ، مختار الطرابيلي ، عبدالخالق الطماوي ،  
أماني ثابت

لقد استخدم اختبار تثبيت المكمل في مجموعتين للبحث عن الأجسام المضادة  
للميكوبلازما نيمونيا • مجموعة من الأصحاء مظهريا والمجموعة الأخرى من المرضى  
بالجهاز التنفسي •  
وكانت الحالة المرضية لهؤلاء النزلاء في الأقسام المختلفة هي الالتهاب الشعبي  
والرئوي والالتهاب الشعبي الرئوي •

وقد صنف الأجسام المضادة لهذا الميكروب موضوعا في الاعتبار عامل السن  
وفصول السنة والحالة المظهرية • وقد لوحظ وجود الأجسام المضادة بنسبة ٢٥% في  
الأشخاص المصابين بالالتهاب الرئوي و ٨٧,٥% من المرضى بالالتهاب الشعبي  
و ٤٣,٨% فقط من المرضى بالالتهاب الشعبي الرئوي وتم اكتشاف معظم هذه الحالات  
في فصل الربيع ( ١٤٣٨% ) وفصل الخريف ( ١٢٥٠% ) وكان معظم الحالات في سن  
قبل البلوغ ( ١٨٧٥% ) وسن المرحلة التعليمية الأولى ( ١٦٨٨% ) •

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**A SEROLOGICAL INVESTIGATION ON THE OCCURRENCE  
OF MYCOPLASMA PNEUMONIAE IN UPPER EGYPT**  
(With 5 Tables)

By  
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(Received at 20/8/1987)

**SUMMARY**

The complement fixation test was carried out for Mycoplasma Pneumoniae in 2 groups, one group of apparently healthy and the other group of patients with respiratory illness. The clinical picture of the patients admitted to different departments varied from bronchitis, pneumonia, bronchopneumonia. Patients showed antibodies against M.pneumoniae were analysed with respect to age, seasonal incidence and clinical picture. The antibody was noted in 25% of patients with pneumonic illness, in 8.75% of patients with bronchitis and in 4.38% of patients with bronchopneumonia. Most cases were seen in spring (14.38%) and autumn (12.50%). The disease was prevalent in the young adults (18.75%) and School age group (16.88%).

**INTRODUCTION**

Mycoplasma Pneumoniae is one of the most common causes of community acquired pneumonia. IMAM, et al. (1968) and similarly HASSAN, et al. (1972) noticed that pneumonia due to Mycoplasma Pneumoniae occurred mainly in Egypt in February and March. AWATEF AWWAD (1976) stated that the organism was usually endemic in a given population through out the year. MUFSON, et al. (1979) reported Mycoplasma Pneumoniae infection in 2% of infants with lower respiratory tract infections. FOY, et al. (1973) isolated mycoplasma from children infections. They isolated the organism in 7.2% of pneumonia cases, 2.6% of cases of bronchitis and 0.5% of cases with bronchopneumonia.

This work was planned to determine the role of M.pneumoniae in chest infections in Upper Egypt through the detection of complement fixing antibodies in the patients sera.

**MATERIAL and METHODS**

A total number of one hundred and sixty cases admitted in chest department, Assiut University Hospitals and chest hospital were studied. Their age onset and course of the disease as well as the season of the clinical presentation were quoted for interpretation (Table 1).

Serum samples were collected from these cases as well as from thirty apparently normal healthy persons. Detection of complement fixing antibodies against M.pneumoniae antigen was done by microtitration technique after inactivation of the collected Sera at 56°C for 30 minutes.

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### Mycoplasma pneumoniae antigens:

For C.f.t. is extracted from M.pneumoniae by means of a special process and supplied by Behring institute.

Complement fixation test adopted in the present work was carried out in accordance with the procedure described by EDWIN (1969). The scheme for its performance is presented in Table II.

A preliminary screening C.f. test was conducted on each of 160 serum samples at a dilution of 1/4 against M.pneumoniae antigen. Sera showing positive reaction were retested using serial dilutions starting from 1/4 up to 1/128.

## RESULTS

The results of complement fixing antibodies of Mycoplasma pneumonia in 160 patients and 30 healthy individual were analysed in Table 3.

From the table it was noticed that 40 cases were positive at 1/4 titre out of 60 cases with pneumonic illness and 14 out of 50 cases with Bronchitis symptoms while in patients with bronchopneumonic symptoms only 7 out of 50 showed the same antibody titre. The sera of 30 healthy persons did not show any complement fixing antibodies for Mycoplasma pneumonia.

From the same table it was observed that the number of positive reactors decreases with the increase of antibody titre.

Mycoplasma infection was more prevalent in young adults and school age children but was less common under 5-years of age and in adults (table 4).

As regards the seasonal variation it was observed that positive cases for mycoplasma were more frequent in spring and early autumn as shown in table 5.

## DISCUSSION

Mycoplasma play an important role in chest infection specially M. pneumoniae which is considered to be one of the most common causes of community-acquired pneumonia. HILL (1979) showed that various mycoplasma spp. could infect various animal species including man.

Our findings are similar to those of IMAN, et al. (1969) and HASSAN, et al. (1972). They noticed that pneumonia occurring in Egypt mainly in February and March is due to Mycoplasma pneumoniae. HASSAN, et al. (1972) mentioned that not only the incidence of the disease was higher in winter but also the incidence of Mycoplasma pneumonia was higher in children and adults as revealed from our investigation. In our study it was noted that the disease is more prevalent in spring, autumn and winter and less frequent in summer.

As regards the patients with pneumonia, bronchitis and bronchopneumonic respiratory infection, our findings were higher than that observed by MUFSON, et al. (1970) and FOY, et al. (1973). Since MUFSON, et al. (1970) obtained serological evidence of M. pneumoniae infection in 2% of infants with lower respiratory tract infection with Mycoplasma pneumoniae, while in our study 10% positivity rate was recorded.

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FOY, *et al.* (1973) found that there were 4 folds or greater rising c.f. antibody titre against Myc. pneumoniae in 4% of cases of pneumonia, 3% of cases of bronchitis.

In our study, c.f. antibodies was detected in 25% of those patients suffering from pneumonic respiratory infection and in 8.35% of those with bronchitis symptoms. These findings were higher than that obtained by BINAZZI and SERRA (1968), since they noticed that M. pneumoniae C.f. antibodies were found in 6% of hospital patients and in 10% of Military recruits. Also BERNI, *et al.* (1968) examined normal children and those with pneumonic respiratory conditions to evaluate the incidence of complement fixing anti Mycoplasma pneumoniae antibodies in the 2 groups, and the authors reported that 7.2% were positive in normal children while 21.3% were positive in those with pneumonic respiratory infection.

FRANSEN, *et al.* (1969) recorded a significant rise in titre against M. pneumoniae antigen in 22% of patients with pneumonia and in 4% of patients with acute respiratory illness other than pneumonia. Also, GRIFFIN and GRAWFORD (1969) noted that with the use of seroconversion of complement fixing antibodies as a criterion of infection, M. pneumoniae was implicated in 20% of patients with pneumonia.

The results of BOSSHARD, *et al.* (1969) were higher than that obtained in our investigation by using purified lipidic antigen in diagnosis of acute respiratory illness and reported that 66% of the patient were positive to complement fixation test.

In this study it was noted that the use of complement fixation test is essential for the serodiagnosis of suspected cases of pneumonia and the respiratory tract infection.

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Table (1)  
 Distribution of 160 patients suffering from respiratory  
 infection according to nature of disease, age and season

| Factor                               | No. of patients | Percentage % |
|--------------------------------------|-----------------|--------------|
| <u>Nature of disease:</u>            |                 |              |
| pneumonia<br>(Chest department)      | 60              | 37.50        |
| bronchitis<br>(Medicine Dept.)       | 50              | 31.25        |
| bronchopneumonia<br>(Chest hospital) | 50              | 31.25        |
| -----                                |                 |              |
| <u>Age group:</u>                    |                 |              |
| - Preschool age (1-6 years)          | 10              | 6.25         |
| - School age (6-12 years)            | 70              | 43.75        |
| - Young adult (12-20 years)          | 65              | 40.62        |
| - Adult (above 20 years)             | 15              | 9.37         |
| -----                                |                 |              |
| <u>Season:</u>                       |                 |              |
| Winter                               | 45              | 20.125       |
| Spring                               | 50              | 31.250       |
| Summer                               | 20              | 12.500       |
| Autumn                               | 45              | 28.125       |

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Table (2)  
Scheme for performance of the microtitre complement fixation test (Edwin, 1968)

| Tube of   | Serum<br>ml                | Saline<br>ml            | Antigen<br>ml | Non specific<br>antigen<br>ml | Complement                                  | Sensitized cells<br>ml  |
|---|----------------------------|-------------------------|---------------|-------------------------------|---|---|
|   |                            |                         |               |                               |   | 15-30 minutes at 37°C   |
| Serum under test  | 0.025                      | 0                       | 0.025         | 0                             | 0.025                                       | 0.050   |
| Serum control<br>(tested for A.C.)                              | 0.025                      | 0.025                   | 0             | 0                             | 0.025                                       | 0.050   |
| Serum nonspecific<br>antigen control                            | 0.025                      | 0                       | 0             | 0.025                         | 0.025                                       | 0.050   |
| Reagent controls  |                            |                         |               |                               |   |   |
| Complement controls<br>for specific and<br>non specific antigen | units<br>2.0<br>1.5<br>1.0 | 0.025<br>0.025<br>0.025 | 0.025         | 0<br>0.025<br>0               | 0.025<br>0.025<br>(1:1.5)<br>0.025<br>(1:2) | 0.050<br>0.050<br>0.050   |
|   | 0.5                        | 0.025                   | 0.025         | 0                             | 0.025<br>(1:4)                              | 0.050   |
| Haemolytic control  | 0                          | 0.050                   | 0             | 0                             | 0.025                                       | 0.050   |
| Sheep cell control  | 0                          | 0.075                   | 0             | 0                             | 0   | 0.050   |
|   |                            |                         |               |                               |   | Overnight incubation at 4 C followed by 15<br>minutes at room temperature |
|   |                            |                         |               |                               |   | Shaken and the following was added  |

Wells containing 2 and 1.5 units of complement showed complete haemolysis, while the wells containing 1.0 unit showed complete to nearly complete haemolysis and the well containing 0.5 unit showed no haemolysis.

Table (3)  
Comparison between different titre of Mycoplasma antibodies of positive cases  
in relation to diseased and healthy person

| Number of person      | Number of positive cases at different titre |       |      |       |      |       |     |       |     |      |     |      |     |     |
|-----------------------|---|-------|------|-------|------|-------|-----|-------|-----|------|-----|------|-----|-----|
|                       | 1/4   | 1/8   | 1/16 | 1/32  | 1/64 | 1/128 |     |       |     |      |     |      |     |     |
|                       | No.   | %     | No.  | %     | No.  | %     | No. | %     | No. | %    | No. | %    | No. | %   |
| Pneumonic (60)        | 40  | 25.00 | 32   | 20.00 | 25   | 15.63 | 10  | 6.25  | 4   | 2.50 | 4   | 2.50 |     |     |
| Bronchitis (50)       | 14  | 8.75  | 12   | 7.50  | 7    | 5.63  | 6   | 3.75  | 2   | 1.25 | -   | -    |     |     |
| Bronchopneumonia (50) | 7   | 4.38  | 5    | 3.13  | 4    | 2.50  | 1   | 0.63  | 1   | 0.63 | 1   | 0.63 |     |     |
| Total diseased (160)  | 61  | 38.13 | 49   | 30.63 | 38   | 23.75 | 17  | 10.63 | 7   | 4.38 | 5   | 3.13 |     |     |
| Healthy (30)          | 0   | 0.0   | 0    | 0.0   | 0    | 0.0   | 0   | 0.0   | 0   | 0.0  | 0   | 0.0  | 0   | 0.0 |

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Table (4)  
Comparison between the titre of Mycoplasma antibodies of positive cases in relation to age group

| Number of patient at | No. of positive cases | Number of positive cases at different titre |       |     |       |      |       |      |       |      |      |       |      |   |   |
|----------------------|-----------------------|---|-------|-----|-------|------|-------|------|-------|------|------|-------|------|---|---|
|                      |                       | 1/4   |       | 1/8 |       | 1/16 |       | 1/32 |       | 1/64 |      | 1/128 |      |   |   |
|                      |                       | No.   | %     | No. | %     | No.  | %     | No.  | %     | No.  | %    | No.   | %    |   |   |
| Preschool age (10)   | 1                     | 9   | 10.0  | 1   | 10.00 | -    | -     | -    | -     | -    | -    | -     | -    | - | - |
| School age (70)      | 27                    | 43  | 38.57 | 25  | 35.71 | 20   | 28.57 | 10   | 14.29 | 5    | 7.14 | 3     | 4.29 |   |   |
| Young adult (65)     | 30                    | 35  | 46.15 | 21  | 32.31 | 17   | 26.15 | 17   | 26.15 | 2    | 3.08 | 2     | 3.08 |   |   |
| Adult (15)           | 3                     | 12  | 20.00 | 2   | 13.33 | 1    | 6.67  | -    | -     | -    | -    | -     | -    |   |   |
| Total (160)          | 61                    | 99  | 38.13 | 49  | 30.63 | 38   | 23.75 | 17   | 10.63 | 7    | 4.38 | 5     | 3.13 |   |   |

Table (5)  
Comparison between the titre of Mycoplasma antibodies of positive cases in relation to seasonal variations

| Season | No. of positive cases | No. of negative cases | Titre |    |       |    |       |    |       |   |      |   |       |   |  |
|--------|-----------------------|-----------------------|-------|----|-------|----|-------|----|-------|---|------|---|-------|---|--|
|        |                       |                       | 1/4   |    | 1/8   |    | 1/16  |    | 1/32  |   | 1/64 |   | 1/128 |   |  |
|        |                       |                       | No.   | %  | No.   | %  | No.   | %  | No.   | % | No.  | % | No.   | % |  |
| Spring | 23                    | 37                    | 46.00 | 20 | 40.00 | 16 | 32.00 | 10 | 20.0  | 4 | 8.00 | 2 | 4.00  |   |  |
| Summer | 2                     | 18                    | 10.00 | 2  | 10.00 | 1  | 5.00  | 1  | 5.0   | - | -    | - | -     |   |  |
| Autumn | 20                    | 25                    | 44.44 | 15 | 33.33 | 12 | 26.66 | 3  | 6.67  | 1 | 2.22 | 1 | 2.22  |   |  |
| Winter | 16                    | 29                    | 35.56 | 12 | 26.67 | 9  | 20.00 | 3  | 6.67  | 2 | 4.44 | 2 | 4.44  |   |  |
| Total  | 61                    | 99                    | 38.13 | 49 | 30.63 | 38 | 23.75 | 17 | 10.63 | 7 | 4.38 | 5 | 3.13  |   |  |