Molecular Detection of 16SrRNA of *Chlamydia pneumoniae* and specific IgE in Asthmatic Patients

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**ABSTRACT**

**Background:** *Chlamydia pneumoniae* is an obligate intracellular organism and it is one of the common causes of upper respiratory tract infection. Microbes were blamed to trigger asthma in different mechanisms, one of these microbes is *Chlamydia pneumoniae*.

**Aims of the study:** This study was done to show the rate of *Chlamydia pneumoniae* infection in asthmatic patients through detection of 16SrRNA of *Chlamydia pneumoniae* in blood and throat wash of patients using PCR test.

**Methods:** One hundred (120) asthmatic patients from both sexes and different age groups (2-84 years) were included in this study. These patients were attending the Clinic of Allergy and Asthma in Ramadi General Teaching Hospital, during the period extended from January to March 2011. Thirty (30) healthy individuals from both sexes were selected randomly to be considered as negative control group. Blood specimens (5ml) and throat wash were taken from each patient, were employed for the detection of 16SrRNA of *Chlamydia pneumoniae* using PCR test. Pooled sera from 70 patients were employed for detection of IgE specific for *Chlamydia pneumoniae* using ELIZA test.

**Results:**

**PCR test results of Blood specimens:**
Among of 70 tested sera from asthmatic patients from both attack and remittance, thirty five (35), (50%) of the tested specimens were showing positive PCR test. Among patients, adult females were showing more positive PCR results for 16s r RNA of *Chlamydia pneumoniae* in their blood specimens 22 females (10, at attack and 12 at remittance), while all tested specimens from control group individuals were showing negative PCR results. Out of (50) tested throat wash specimens, five (5), (10%) of them were showing positive PCR for 16s r RNA of *Chlamydia pneumoniae*. Four (4) (80%) of them were from adult patients at attack status and all control group individuals were showing negative PCR results of *Chlamydia pneumoniae* antigen, out of (54) tested sera from asthmatic patients, 37 of them were showing positive ELISA test for IgE specific for *Chlamydia pneumoniae* antigen, Adult females (24, (64. 8) were showing higher IgE positive results than males (P < 0.05). All tested sera (15) from control group individuals were showing negative IgE *Chlamydia pneumoniae* antigen. Positive correlation was found between the results of IgE specific for *Chlamydia pneumoniae* and PCR results for blood and throat wash specimens in both attack and remittance.

**Conclusion:** We can conclude from this study that *Chlamydia pneumoniae* is involved with asthma post infection to the lower respiratory tract and induction of allergy mediators like IgE in both sexes.

**Keywords:** Asthma, PCR, *Chlamydia pneumoniae*. 
INTRODUCTION

Chlamydiapneumoniae is an obligate intracellular organism and it is one of the common causes of upper respiratory tract infection (Cunningham 1998, Han 2005, Kocabas 2008). Microbial infections showed an increased importance in asthma pathogenesis and exacerbation (Kraft 2000, Fernandez, 2001, Lafi, 2004, Sutherland et al., 2004, Kocabas, 2008). Many bacterial types are able to release histamine from human mast cells and basophiles in vitro, this was suggested to be as pathological mechanism in intrinsic asthma (Brada et al., 1996, Holt and Bjorkeston 1997, Fernandez 2001).


Few reports were done in this category for asthmatic patients in Iraq, particularly West of Iraq, Al-Anbar Governorate so this study is devoted.

Patients and Methods:

One hundred (120) asthmatic patients from both sexes and different age groups (2-84 years) were included in this study. These patients were attending the Clinic of Allergy and Asthma in Ramadi General Teaching Hospital during the period extended from January to March 2011. Patients were examined by senior physician to follow up their affection, some patients were suffering from asthmatic attack while other at remission.

Thirty (30) healthy individuals from both sexes were selected randomly to be considered as negative control group, these individuals were examined in the same way of asthmatic individuals.

Specimen collection:

Blood specimens (5ml) were taken from each patient, (3 ml) of them were employed for serum pooling and (2ml) were used for DNA extraction soon. Pooled sera were kept frozen at -20 C to be employed for IgE Specific for Chlamydiapneumoniae estimation.

Throat wash sample was taken following (Cadman, H. 2010), each patient was advised to brush his teeth with clean brush and water then advised to do throat wash and gargle with sterile normal saline. Gargles throat wash water was divided into four aliquots then centrifuged at 3000 rpm for 10 minutes. The supernatant was discarded and the precipitate was pooled and kept frozen at -20C to be used for DNA extraction and were employed for the isolation of 16SrRNA of Chlamydiapneumoniae using PCR test.

Detection of Chlamydiapneumoniae 16SrRNA Using PCR

Nucleic acid extraction:

DNA Sorb -B, DNA extraction kit (Sacace Biotechnology, ITALY) was employed for DNA extraction from each blood and throat wash specimens of patients and control group. Purity test was done for each extracted DNA samples. DNA samples were kept frozen at -20 to be used for PCR running later. Extracted DNA of patients and control individuals were amplified at Molecular Biology Unit of Microbiology Department, College of Medicine using PCR test:

a- Conventional PCR System.

PCR Premix –Accupower (Master mix, Bioneer, Korea).

Forward primer (CPn A)-------------for 16SrRNA
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Reverse primer (CPn B)-----------------for 16SrRNA (Alpha - DNA Canada). Running PCR Program of (cycles for minutes) was employed. PCR result was analyzed using gel electrophoresis for amplified specimens of nucleic acid in contrast to standard Molecular weight marker, DNA ladder (Promega, USA), and Bench Top PCR marker (Promega USA).

The PCR cycles consisted of an initial denaturation for 5 min. at 94\textdegree C followed by 50 cycles of denaturation at 94\textdegree C for 45 seconds, annealing at 55\textdegree C and extension at 72\textdegree C for 1 min. The final extension was for 10 min. at 72\textdegree C. The PCR products were visualized by agarose gel electrophoresis with redsafe dye. Results were calculated and analyzed using statistical methods.

**Specific IgE detection:**

Serum specimens from 70 patients were employed for detection of IgE specific for \textit{Chlamydia pneumoniae} using ELIZA test using (Biotek ELISA system, Spain). Antigen of \textit{Chlamydia pneumoniae} obtained from (virion/ serion GMBHI, Germany). Antigen Disk impregnation method described by (Lafi 2004\textsubscript{11}) was used to prepare antigen disks for ELiza test. Other required items for ELISA test (IgE conjugate, washing solution, stop solution etc.) were obtained from (Diagnostic Automation; INC, USA). ELISA test was done as Described by (Fernandez-Botran and Vetvicka, 2000).

RESULTS

1- Age and sex distribution of patients:

One hundred twenty (120) asthmatic patients from both sexes were included in this study, sixty seven (67) (55.87\%) of them were females and fifty three (53) (44.2\%) were males male to female ratio was 1.26. Forty nine (49) (40.8\%) of patients were in attack while seventy one (71) (59.2\%) were at remittance stage.

Seventeen 17(14.1\%) children were included in this study within age group (1-17 years), eleven (11) (64.7\%) were males and six (35.3\%) were females. Thirty (30) intact individuals from both sexes were included in this study resembling control group seven (23.4\%) children and twenty three (23) (76.6\%) were adults, eleven (11) (36.4\%) of them were males and nineteen (19) (63.4\%) were females (Table-1 and Fig. 1).
Table 1: Number and sex of the study patients and control group.

<table>
<thead>
<tr>
<th>Age group</th>
<th>Patients group</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>F</td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>F</td>
<td></td>
</tr>
<tr>
<td>No. (%)</td>
<td>No. (%)</td>
<td>No. (%)</td>
<td>No. (%)</td>
<td>No. (%)</td>
<td>No. (%)</td>
<td></td>
</tr>
<tr>
<td>1 – 17 year</td>
<td>5 (26.3%)</td>
<td>2 (6.76%)</td>
<td>6 (17.6%)</td>
<td>4 (10.8%)</td>
<td>1 (9.1%)</td>
<td>6 (31.6%)</td>
</tr>
<tr>
<td>18 – 40 year</td>
<td>8 (42.1%)</td>
<td>13 (43.3%)</td>
<td>13 (58.3%)</td>
<td>19 (51.4%)</td>
<td>8 (72.7%)</td>
<td>9 (47.4%)</td>
</tr>
<tr>
<td>≥41 year</td>
<td>6 (31.6%)</td>
<td>15 (50%)</td>
<td>15 (44.1%)</td>
<td>14 (37.8%)</td>
<td>2 (18.2%)</td>
<td>4 (21%)</td>
</tr>
<tr>
<td>Total</td>
<td>19 (38.7%)</td>
<td>30 (61.3%)</td>
<td>34 (47.9%)</td>
<td>37 (52.1%)</td>
<td>11 (36.6%)</td>
<td>19 (63.4%)</td>
</tr>
</tbody>
</table>

2- PCR test results:

A- Blood specimens:

Among of 70 tested blood sample from asthmatic patients from both attack and remittance stage, thirty five (35) (50%) of the tested specimens showed positive PCR test. Among patients, adult females showed more positive PCR results for 16SsRNA of *Chlamydia pneumoinae* in their blood specimens, 22 females (10 at attack and 12 at remittance), significant difference was found between them and males (P =0.001). Non significant difference (P= 0.61) was found between patients in attack (16 patients) and remittance status (19 patients). While all tested specimens from control group individuals showed negative PCR results, (Table 2 and Fig. 2).
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Table 2: Age and sex distribution of PCR results in blood samples for patients and control group.

<table>
<thead>
<tr>
<th>Age group</th>
<th>+ve PCR</th>
<th>-ve PCR</th>
<th>+ve PCR</th>
<th>-ve PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M &amp; F</td>
<td>M &amp; F</td>
<td>M &amp; F</td>
<td>M &amp; F</td>
</tr>
<tr>
<td>1-17 year</td>
<td>0 &amp; 1%</td>
<td>0 &amp; 0%</td>
<td>1 &amp; 0%</td>
<td>0 &amp; 0%</td>
</tr>
<tr>
<td>18-40 year</td>
<td>1 &amp; 10%</td>
<td>4 &amp; 4%</td>
<td>3 &amp; 3%</td>
<td>2 &amp; 2%</td>
</tr>
<tr>
<td>≥41 year</td>
<td>4 &amp; 4%</td>
<td>3 &amp; 3%</td>
<td>2 &amp; 2%</td>
<td>1 &amp; 1%</td>
</tr>
<tr>
<td>Total</td>
<td>9 &amp; 45%</td>
<td>10 &amp; 54%</td>
<td>6 &amp; 33%</td>
<td>4 &amp; 20%</td>
</tr>
</tbody>
</table>

After amplification of 16SrRNA gene target by polymerase chain reaction and electrophoresis by 2% agarose gel, bands of amplified gene of *Chlamydia pneumoniae* were showed in Figure (3) for patients and (4) for control group.

Fig. 3: The result of agarose gel electrophoresis (2%) with redsafe staining. Bands of amplified 16SrRNA gene of *Chlamydia pneumoniae* obtained from blood samples of patients. Note that (L2, L3, L4, L5, L6, L7, L8, L9, L10, L11, L12, L14, L15) were positive while (L3) were negative. DNA ladder with (100-1500 bp) on the left (L1) was used as DNA molecular weight marker.

Fig. 4: The result of agarose gel electrophoresis (2%) with redsafe staining. Bands of amplified 16SrRNA gene of *Chlamydia pneumoniae* obtained from blood samples of control group. Note that all of samples were negative. DNA ladder with (100-1500 bp) on the left (L1), was used as DNA molecular weight marker.
Table 3: Age and sex distribution of PCR results of throat wash samples for patients and control group.

<table>
<thead>
<tr>
<th>Age group</th>
<th>+ve PCR</th>
<th>-ve PCR</th>
<th>+ve PCR</th>
<th>-ve PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>control</td>
<td></td>
</tr>
<tr>
<td>patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>remittance</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-17 year</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>18-40 year</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>≥ 41 year</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

| 1-17 year | 0     | 0     | 0     | 0     |
| 18-40 year | 2   | 0     | 0     | 0     |
| ≥ 41 year | 100  | 0     | 0     | 0     |
| Total     | 100  | 0     | 0     | 0     |

B- Throat washes specimens:

Out of fifteen tested throat wash specimens, five (5) (10%) of them showed positive PCR for 16SrRNA of *Chlamydia pneumoniae*. Four (80%) of them were from adult patients at attack status and one (20%) of them in remittance stage. Throat wash specimens from adults showed more positive result from child and most of them were in attack status.

Regarding this category, non significant difference, (P > 0.5) was found between males and females, and all control group individuals showed negative PCR results, Table (3) and Fig.(5).

After amplification of 16SrRNA gene target by polymerase chain reaction and electrophoresis by 2% agarose gel, bands of amplified gene of *Chlamydia pneumoniae* were showed in Figure (6) for patients and (7) for patient and control group.
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3- **IgE Specific for *Chlamydia pneumoniae***:

Out of (54) tested sera from asthmatic patients, 37 of them showed positive ELISA test for IgE specific for *Chlamydia pneumoniae*, (17 patients at attack and 20 of them were at remittance). Adult females (24) (64.8%) showed higher IgE positive results than males (P=0.003). All tested serum samples (15) from control group individuals showed negative IgE specific for *Chlamydia pneumoniae* (Table-4). No significant difference was found between attack and remittance asthmatic patients who showed positive ELISA for specific IgE for *Chlamydia pneumoniae*, (P=0.62). Positive correlation was found between the results of IgE specific for *Chlamydia pneumoniae* and PCR results for blood and throat wash specimens in both attack and remittance patients (P=0.00), Table (4) and Figure (8).
Table 4: Age and sex distribution of specific IgE for *Chlamydia pneumoniae* in sera of patients and control group.

<table>
<thead>
<tr>
<th>Age group</th>
<th>positive</th>
<th>Remittance</th>
<th>negative</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 – 17 year</td>
<td>0%</td>
<td>1%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>18 – 40 year</td>
<td>25%</td>
<td>53.9%</td>
<td>4%</td>
<td>33.3%</td>
</tr>
<tr>
<td>≥ 41 year</td>
<td>75%</td>
<td>38.4%</td>
<td>4%</td>
<td>8%</td>
</tr>
<tr>
<td>Total</td>
<td>23.5%</td>
<td>76.5%</td>
<td>12%</td>
<td>60%</td>
</tr>
</tbody>
</table>

M*: male  
F*: female

Figure (6): Age and sex distribution of specific IgE for *Chlamydia pneumoniae* in patients and control group.

**DISCUSSION**

Infective asthma (Asthma imposed by infective agent mediators and allergens) had been well recognized by many pioneers who found that asthma could be induced by one or more of different bacterial agents, viruses, fungi and even parasites (Oiling, BRADA 1996, Cunningham 1998, Kraft, 2000, Fernandez 2001, Lafi 2004, Lafi 2008, Mnadero and Lprize 2010).

We found increased rate of asthma in female adults (55.8%) in contrast to males ratio (44.2%), this was in accordance with that reported by (Lafi 2004, 21 Zhao et al. 2001, Shames et al. 1999). This difference among sexes might be due to, environmental, domestic and occupation effect on females (makes them more exposed to infective and non infective environmental allergens than males as well as the effect of sex effect).
hormones that modulates immune status in females (Zhao et al. 2001). Decreased number of asthmatic children in this study was attributed to the method of sampling, patients included in this study were attending Ramadi General Teaching Hospital and the Clinics at this hospital were dealing with adult patients. So in order to include more asthmatic children data, specimens should be collected from patients were attending child hospital.

Regarding PCR test results, adult females showed more positive PCR results for 16S rRNA of *Chlamydia pneumoniae* in their blood specimens, and negative results in blood from control individuals, this indicates presence of *Chlamydia pneumoniae* in asthmatic patients. This was found also by (Harju et al., 2006; Mitchell et al., 2009, Zahang 2000) positive titers of IgE specific for *Chlamydia pneumoniae* antigen in adult asthmatic patients particularly females indicated the role of *Chlamydia pneumoniae* allergens in of Atopy and astma induction. This was in acceptance with that mentioned by (Brada 1996 Kraft 2000, Numazakei 1990, Krull et al. 2006, Kokabas 2008) who showed that microorganisms were imposed in infective asthma through their allergens.

Decreased rate of positive results of PCR test in throat wash was ought to the fact that Chlamydia pneumoniae is an obligate intracellular pathogen (Brook, and Caroll, 2007; Krull et al. 2006) and it need to bind host cells and free cells wash out with saliva, so it is difficult to see free chlamydial cells in saliva and throat wash specimens (Harvy, 2008). Our findings in PCR test for throat wash was nearly in accordance with finding of Harjull et al. 2006 and disagree with that of Zhang et al. 2000, Kokabas et al. 2008).

Positive correlation between PCR test results and IgE specific for *Chlamydia pneumoniae* confirm our findings through the importance of entrance of the pathoge n to the host cell and immune activation (Male et al. 2006, Krull 2006, Mora et al. 2009).

We can conclude that *Chlamydia pneumoniae* involved in upper respiratory tract infection through the presence in host tissue and immune induction through Th2 activation and release of IgE specific for its own antigens and later on induction of type one hypersensitivity (Anaphylaxis) as indicated by the positive titers of IgE specific for *Chlamydia pneumoniae*. We recommend screening tests for microbes (*Chlamydia pneumoniae*) in asthmatic patients and suiTable anti *Chlamydia pneumoniae* treatment should be used for asthmatic patients in addition to that we recommend further studies on other microorganisms role in asthma in both adults and children to relief infective asthma properly.

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Molecular Detection of 16SrRNA of *C. pneumoniae* and specific IgE in Asthmatic Patients


ARABIC SUMMARY

تشخيص الحمض النووي الرئيسي عيار 16S للكلاميديا الرونية والпад

حامد عبد الرزاق السه巴西 و شهاب أحمد لأفي ومشتاق طالب صالح المعييلي

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2- قسم الأحياء المخبرية كلية الطب جامعة الإباد غرب العراق
3- كلية الصيدلة جامعة الإباد غرب العراق

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توطنة:
كلاميديا الرونية هي واحدة من الجراثيم الجلدية داخل الخلايا والتي تسبب الالتهاب المجرم التنفسي العلاج.

أهداف الدراسة:
هذة الدراسة لبيان نسبة إصابة مرضى الروية بجرثومة الكلاميديا الرونية من خلال تحديد الحمض النووي عيار 16S الخاص بها باستخدام تفاعل سلسلة البلمرة الجينية PCR وكذلك بين ذكر هذه الجراثوما بحثية وفاة الروية من خلال الكشف عن الضد IgE.

طريقة العمل:

النتائج:
تبين من اختبار عينة من مرضى (60%) (25/35) عنوانها أبنته نتيجة موجبة لتفاعل سلسلة البلمرة 16S. بينما جمعت وتدريب العينات المتلفة ومن مجموعة الدهم وplies من المرضى في حالة نوبة الروية جينية، كما وكأن جميعهم من الإباث البلغاف (20%) عنوانها مريض، (210) من إباث الذين في حالة نوبة الروية جينية. بينما جمعت وتدريب العينات المتلفة ومن المجموعة الدهم وplies من المرضى في حالة نوبة الروية.

الاستنتاج:
تبين من الدراسة أن الكلاميديا الرونية لها دور في الروية التشيلي من خلال تفاعليات الكلاسيكية التنفسي المفيدة، وثورية الأحماض، والشمس، والجلود، والرد، IgE، بالإضافة إهلاء النوى من خلال تفاعليات الكلاسيكية التنفسي المفيدة، وثورية الأحماض، والشمس، والجلود، والرد، IgE.