**Dijlah Journal of Medical Sciences (DJMS)** Vol. 1, No. 1, August, 2024, pp. 25-32
ISSN: Pending, paper ID: 004

# The Study of lytA Genotyping and estimation of IL17A and TGF-β in Streptococcus pneumoniae patients

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**Article Info**

***Article history:***

Received June, 28, 2024
Revised July, 15, 2024
Accepted August, 02, 2024

***Keywords:***

Genotyping,
lytA,
IL17A,
TGF-β,
Streptococcus Pneumonia

**ABSTRACT**

Streptococcus pneumoniae is regarded as a main cause of CAP among other pathogens. In the current study, (60) patients infected with Streptococcus pneumoniae were included, from whom blood samples were collected. The patients attended to Baghdad Teaching Hospital during the period from 1st October 2023 to 20th April 2024. Blood sample also obtained from (60) apparently healthy individuals as controls. The results showed that the prevalence of streptococcus pneumonia infection was higher among the age groups (25-34) and (45-54) as compared to other age groups. Also, the distribution of streptococcus pneumonia infection was higher among males 31(62.0%) than females 19(38.0%) with no significant variation. The prevalence of streptococcus pneumonia infection according to residency was equal between rural and urban patients. A highly significant variation (P<0.01) was found between the mean Strept. IgM (2.1164±1.27) in the patient group and the healthy controls (0.05±0.04), and between the (Mean±Sd) of Strept. IgG (1.72±0.75) in the patients and the healthy controls (0.04±0.02). Also, the (Mean±Sd) IL17A was significantly higher (p<0.01) in the patient group (96.41±26.11) than the control group (19.68±6.52), and the (Mean±Sd) TGF was significantly higher (p<0.01) higher in the patient group (101.74±32.88) than the control group (23.59±10.86). There was a highly significant variation (p<0.01) between mean IL-17A and Anti Strept. IgM antibodies (25.423). There was a highly significant variation (p<0.01) between mean value IL-17A and Anti Streptococcus pneumoniae IgG antibodies (25.684), and a highly significant variation (p<0.01) between mean value TGF and Anti Strept. IgM antibodies (2.104). Also there was a highly significant variation (p<0.01) between mean value TGF and Anti Strept IgG antibodies (21.298), and a highly significant difference (p<0.01) between mean value of TGF and IL-17A (21.592). Results of amplification of specific region of lytA gene of Streptococcus pneumonia. The 1.5% agarose gel electrophoresis was used for fractionation of species of human samples and stained by ethidium bromide M: 100bp ladder markers. Lane 1-15 and 1-15 resembled 1023bp PCR product.

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## 1- INTRODUCTION

Streptococcus pneumoniae is regarded as a main reason for CAP among other pathogens. The multidrug-resistant (MDR) Streptococcus pneumoniae were found in different countries since 1977 when the antibiotic-resistant S. pneumoniae was first detected [1]. Furthermore, previous studies reported that Chinese multidrug resistant S. pneumoniae was the highest among 11 Asian countries, suggesting an urgent need to control antimicrobial resistances [1]. It is well recognized that the molecular characteristics analysis exerts important roles in clinical treatments. More than 90 S. pneumoniae serotypes were recognized until the present time. The diversity depends on the difference in structure of repeated unit of capsular polysaccharides [2]. Human's upper respiratory system is usually colonized by Streptococcus pneumoniae, and former studies showed prevalence of S. pneumoniae in 16.6% of healthy children in Shanghai, and about 70% in healthy children in Xinjiang, in China [3]. The nasopharyngeal colonization is an important initial step for pneumococcal pathogenesis, indicating that investigation of characteristics of S. pneumoniae in asymptomatic individuals is necessary. Little information is available on S. pneumoniae genotypic characteristics from asymptomatic individuals and CAP patients [4], despite many epidemiological studies carried out on this bacteria among patients with CAP and IPD in China [1]. IL-17A-producing Th17 cells mediate protection against pneumococcal colonization. The activations and recruitments of macrophages and neutrophils to the nasopharynx are signaled by IL-17A, resulting in pneumococcal clearance [5]. Several studies mentioned the CD4+ Th17 cells roles in decreasing pneumococcal colonization in animal models. Naive mice which lack the CD4+ Th17 cells are not protected against pneumococcal carriages, nevertheless, immunity is restored with the consequent transfers of the IL-17-secreting CD4+ T cell [6]. In a study by Lu et al., they also showed the important roles of IL-17A, since mice that lack IL-17A receptors had no protection against pneumococcal diseases compared to mice which lacked IL-4 or IFNγ, that had protection [7]. Activation of TGF-β results in functional immune-modulatory impacts in accordance with environmental circumstances. TGF-β function in arthritis development in murine models was widely studied with controversial results. Recently, the results indicated no relevant roles of TGF-β in mice models for collagen-induced arthritis. On the other hand, studies on TGF-β on T-cell response showed controversial results as a promoter or inhibitor of the inflammatory responses. This paper reviewed the TGF-β role in mice models with arthritis [8]. Our study aimed to detect the genotypes of the lytA gene with measurement of IL17A and TGF-β in Streptococcus pneumoniae patients.

## 2- MATERIALS AND METHODS

In the current study, (60) patients infected with Streptococcus pneumoniae were included, from whom blood samples were collected. The patients attended Baghdad Teaching Hospital during the period from 1st October 2023 to 20th April 2024. Streptococcus Pneumoniae IgM, IgG as well as levels of IL-17A and TGF-β were measured using the enzyme-linked immunosorbent assay (ELISA) method. The (10 µl) mixture of PCR was composed of 0.2 mM concentration of each of deoxyribonucleoside triphosphates, 10 mM of Tris-HCl buffer (pH 8.3), 50 mM of KCl, 2 mM of MgCl2, 1 U of Ex Taq DNA polymerases, 0.5 µM concentration of each primer, and 1 µl of template DNA. PCR was done using a thermal cycler for 30 cycles. Each cycle was done in 1 minute at 94°C, 1 minute at 55°C and in 1 minute at 72°C for the ply genes and in 15 seconds at 94°C, and in 15 seconds at 53°C and in 15 seconds at 72°C for the lytA genes. On 3% agarose gel, a sample of (2 µl) of the PCR amplification product was subjected to electrophoresis. The sequences of the primers used for PCR are as follows: lytA:

lytA F 5′-CAGCGGTTGAACTGATTGA-′3 251 to 269

lytA R 5′-TGGTTGGTTATTCGTGCAA-′3 423 to 405

Analysis: For data analysis, the SPSS program version-20 (Faculty version) was used, involving Mean ± SD and t-tests. A p-value less than (0.05) is regarded as significant. PCR amplification of lytA gene.

## 3- RESULTS

The results showed that the prevalence of streptococcus pneumonia infection was higher among the age groups (25-34) and (45-54) as compared to other age groups. Also, the distribution of streptococcus pneumonia infection was higher among males 31(62.0%) than females 19(38.0%) with no significant variation. The prevalence of streptococcus pneumonia infection according to residency was equal between rural and urban patients as shown in table 1.

**Table (1): Distribution of study groups according to age, sex and residence**

| | | | Groups study | |
|---|---|---|---|---|
| | | | Control(n=50) | Cases(n=50) |
| Age groups | (15-24) | No. | 7 | 14 |
| | | % | 14.0% | 28.0% |
| | (25-34) | No. | 19 | 15 |
| | | % | 38.0% | 30.0% |
| | (35-44) | No. | 6 | 9 |
| | | % | 12.0% | 18.0% |
| | (45-54) | No. | 9 | 10 |
| | | % | 18.0% | 20.0% |
| | (55-65) | No. | 9 | 2 |
| | | % | 18.0% | 4.0% |
| Sex | Male | No. | 31 | 31 |
| | | % | 62.0% | 62.0% |
| | Female | No. | 19 | 19 |
| | | % | 38.0% | 38.0% |
| Residence | Urban | No. | 25 | 24 |
| | | % | 50.0% | 48.0% |
| | Rural | No. | 25 | 26 |
| | | % | 50.0% | 52.0% |

Table (2) showed that a highly significant variation (P<0.01) was found between the mean Strept. IgM (2.1164±1.27) in the patient group and the healthy controls (0.05±0.04), and between the (Mean±Sd) of Strept. IgG (1.72±0.75) in the patients and the healthy controls (0.04±0.02). Also, the (Mean±Sd) IL17A was significantly higher (p<0.01) in the patient group (96.41±26.11) than the control group (19.68±6.52), and the (Mean±Sd) TGF was significantly higher (p<0.01) higher in the patient group (101.74±32.88) than the control group (23.59±10.86).

**Table (2): Comparison between the studied groups according to Strep IgM, IgG, IL-17A and TGF**

| Study groups | | Mean ±Sd. | F-test | P-Value | C.S |
|---|---|---|---|---|---|
| Control | Strip IgM | 0.05±0.04 | 65.015 | P=.000 | P< 0.01 (HS) |
| Cases | | 2.1164±1.27 | | | |
| Control | Strip IgG | 0.04±0.02 | 47.755 | P=.000 | P< 0.01 (HS) |
| Cases | | 1.72±0.75 | | | |
| Control | IL17A | 19.68±6.52 | 32.341 | P=.000 | P< 0.01 (HS) |
| Cases | | 96.41±26.11 | | | |
| Control | TGF | 23.59±10.86 | 22.921 | P=.000 | P< 0.01 (HS) |
| Cases | | 101.74±32.88 | | | |

There was a highly significant variation (p<0.01) between mean IL-17A and Anti Strept. IgM antibodies (25.423). There was a highly significant variation (p<0.01) between mean value IL-17A and Anti Streptococcus pneumoniae IgG antibodies (25.684) as shown in table (3).

**Table (3): Comparison between mean value of IL17A in cases group with Strep IgM and Strep IgG**

| | t-test | P-Value | C.S |
|---|---|---|---|
| Strip IgM | 25.423 | P=.000 | P< 0.01 (HS) |
| Strip IgG | 25.684 | P=.000 | P< 0.01 (HS) |

A highly significant variation (p<0.01) was found between mean value TGF and Anti Strept. IgM antibodies (2.104). Also there was a highly significant variation (p<0.01) between mean value TGF and Anti Strept IgG antibodies (21.298), and a highly significant difference (p<0.01) between mean value of TGF and IL-17A (21.592), as shown in table (4).

**Table (4): Comparison between mean value of TGF in cases group with Strep IgM, IgG and IL-17A**

| | t-test | P-Value | C.S |
|---|---|---|---|
| Strip IgM | 2.104 | P=.041 | P< 0.05 (S) |
| Strip IgG | 21.298 | P=.000 | P< 0.01 (HS) |
| IL17A | 21.592 | P=.000 | P< 0.01 (HS) |

Results of amplification of specific region of lytA gene of Streptococcus pneumonia. The 1.5% agarose gel electrophoresis was used for fractionation of species of human samples and stained by ethidium bromide M: 100bp ladder markers. Lane 1-15 and 1-15 resembled 1023bp PCR product.

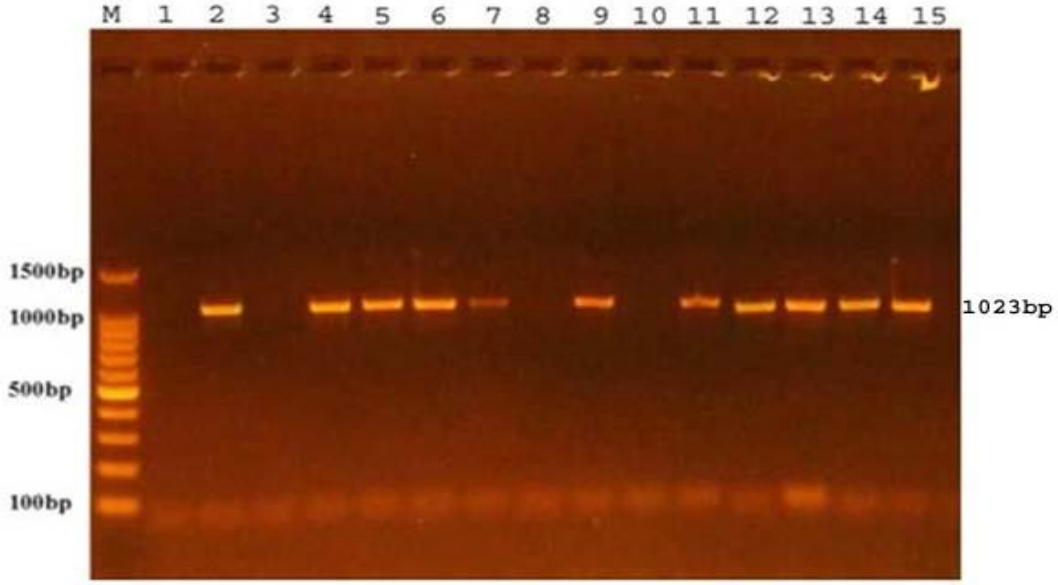

**Figure (1): Banding patterns of lytA gene in Streptococcus pneumonia, with molecular size marker (100bp); lytA gene in samples of Streptococcus pneumonia, numbered (2, 4, 5, 6, 7, 9, 11, 12, 13, 14 and 15) at the molecular weight (1023bp), after electrophoresis done on the agarose gel.**

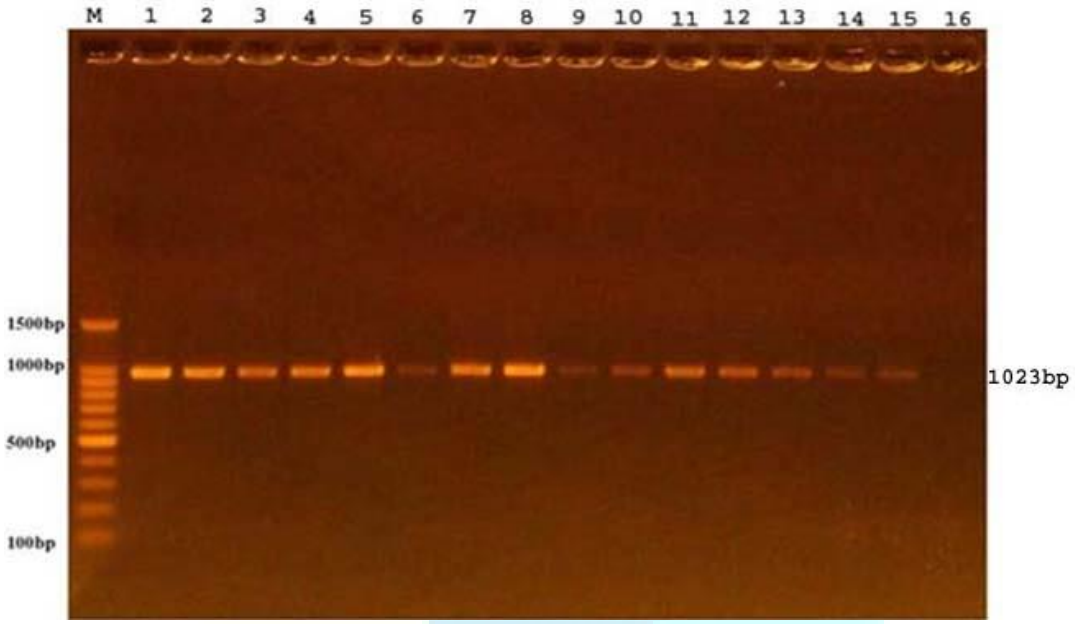


**Figure (2): Banding patterns of lytA gene in Streptococcus pneumonia, with molecular size marker (100bp); lytA gene in samples of Streptococcus pneumonia, numbered (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 and 15) at the molecular weight (1023bp), after electrophoresis done on the agarose gel.**

## 4- DISCUSSION

The results showed that the prevalence of Streptococcus pneumonia infection was higher among the age groups (25-34) and (45-54) as compared to other age groups. Also, the distribution of Streptococcus pneumonia infection was higher among males 31 (62.0%) than females 19 (38.0%) with no significant variation. Avarvand et al. [9] reported that the ages ranging from 40 to 75 years are the most infected with Streptococcus pneumoniae among infected people in Iran. Also, the prevalence of Streptococcus pneumonia infection according to residency showed that it was equal. These results agreed with Cleary et al. [10] who also found no significant differences between Streptococcus pneumoniae infections in urban people and people who live in cities. The Anti-IgG anti-Streptococcus antibodies level was higher than the control group with highly important change. These findings matched with Genua et al. [11] who reported a significant increase in IgG levels of Streptococcus pneumoniae bacteria among infected patients compared to a control group. The IL-17A Levels and TGF Levels were high in Streptococcus pneumonia infections compared to healthy individuals. Mills et al. [12] demonstrated that interleukin 17A levels are very high in advanced infections of Streptococcus pneumoniae patients. On the other hand, Jing et al. [13] reported increased levels of IL-2, IL-17, and TGF-β in patients with CAP. Moreover, there was a positive correlation between IL-17 and WBCs, neutrophils, platelet/lymphocyte ratio, and neutrophil/lymphocyte ratio in CAP patients, and a negative correlation with lymphocytes. The mean of Strip IgM and Mean ± Sd Strip IgG was significantly higher than the control group, in Streptococcus pneumonia diseases. These results coincided with Gaultier et al. [14] who attributed this variation to the high exposures of indigenous persons to Streptococcus pneumoniae and/or cross-reactive antigen of other environmental organisms or plant. The comparison between mean value of IL-17A and Anti-Streptococcus pneumoniae IgG antibodies was highly significant. Li et al. [15] reported IL-17A blockade by anti-IL-17A antibodies which abrogated the protective effects of Streptococcal pre-infections. It is important to say that memory Th17 response induced by Streptococcal pre-infections could overcome viral-driven Th17 inhibitions and caused cross-protections against various Streptococcus serotypes after co-infection with IAV [16]. The comparison between mean value of IL-17A and Anti-Streptococcus pneumoniae IgM antibodies was highly significant. Also, the comparison between mean value of IL-17A and Anti-Strep IgG antibodies was highly significant. Gingerich et al. [17] reported that antibodies are important components of immunity against pneumococcal infection, that target the capsular

polysaccharides and protein antigen on the bacterial surfaces. Those antibodies were shown to play different roles such as elevation of opsono-phagocytic activities, toxin and enzymatic neutralizations, decreasing Streptococcus adherence, and changing Streptococcus gene expressions. Furthermore, Martinez et al. [18] revealed that Streptococcus immune-mediated consequences, such as acute rheumatic fevers are because of responses of humoral and cellular immune systems which target cardiac and cerebral antigens in addition to the cross-reactive antigens of group A Streptococcus. Also, Aguinagalde et al. [19] reported that mAbs are enabled by the hexamer-enhancing mutation for induction of great phagocytosis and intracellular killings by neutrophils in the human body. Lastly, passive immunizations with CPS6-IgG1-E345K caused protection of mice from severe pneumonia development. The amplification of specific region of lytA gene of Streptococcus pneumoniae. The 1.5% agarose gel electrophoresis was used for fractionation of species of human samples and stained by ethidium bromide M: 100bp ladder markers. Lane 1-15 and 1-15 resembled 1023bp PCR product. These findings agreed with Al-Saadi [20] who showed that results of PCR were: 6 (24%) isolates showing positive results for lytA gene existence, and 5 (20%) isolates demonstrated positive results for ply gene existence.

## 5- CONCLUSION

The study revealed that the comparison between mean value of IL17A and Anti Streptococcus pneumoniae IgM antibodies was high with highly significant variation. Also the comparison between mean value of IL17A and Anti Strep IgG antibodies was high with highly significant variation**.** The amplification of specific region of lytA gene of Streptococcus pneumonia. The 1.5% agarose gel electrophoresis was used for fractionation of species of human samples and stained by ethidium bromide M: 100bp ladder markers. Lane 1-15 and 1-15 resembled 1023bp PCR product.

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**Dijlah Journal of Medical Sciences (DJMS)** Vol. 1, No. 1, August, 2024, pp. 25-32
ISSN: Pending, paper ID: 004

# دراسة التنميط الجيني لـلايت أي جين وتقدير انترلوكين 17 اي وعامل التنخر الورمي –بيتا في مرضى العقدية الرئوية

## الخلاصة

تعد العقدية الرئوية سببًا رئيسيًا لالتهاب الرئة المجتمعي من بين مسببات الأمراض الأخرى. في الدراسة الحالية، تم تضمنت (60) مريضًا مصابًا بـالعقدية الرئوية والذين تم أخذ عينات الدم منهم. المرضى الذين يرتادون إلى مستشفى بغداد التعليمي خلال الفترة من 1 أكتوبر 2023 إلى 20 أبريل 2024. كما تم الحصول على عينة دم من (60) فردًا سليمًا ظاهريًا كمجموعة سيطرة . أظهرت النتائج أن معدل انتشار عدوى العقدية الرئوية كان أعلى بين الفئات العمرية (25-34) و (45-54) مقارنة بالفئات العمرية الأخرى. كما كان توزيع عدوى العقدية الرئوية أعلى بين الذكور 31 (62.0٪) من الإناث 19 (38.0٪) مع عدم وجود اختلاف كبير. كان معدل انتشار عدوى العقدية الرئوية وفقًا للإقامة متساويًا بين المرضى الريفيين والحضريين. تم العثور على تباين شديد الأهمية (P <0.01) بين متوسط (2.1164 ± 1.27) Strept. IgM في مجموعة المرضى ومجموعة الأصحاء (0.05 ± 0.04) وبين (Mean ± Sd) لـ (1.72 ± 0.75) Strept. IgG في المرضى ومجموعة الأصحاء (0.04 ± 0.02). كما كان (متوسط ± الانحراف المعياري IL17A (أعلى بشكل ملحوظ (p<0.01) في مجموعة المرضى (96.41±26.11) من مجموعة التحكم (19.68±6.52)، وكان (متوسط ± الانحراف المعياري TGF (أعلى بشكل ملحوظ (p<0.01) في مجموعة المرضى (101.74±32.88) من مجموعة التحكم (23.59±10.86). كان هناك تباين كبير للغاية (p<0.01) بين متوسط IL-17A والأجسام المضادة لـ Streptococcus. IgM (25.423). كان هناك تباين كبير للغاية (p<0.01) بين متوسط قيمة IL-17A والأجسام المضادة لـ Streptococcus pneumoniae IgG (25.684)، وتباين كبير للغاية (p<0.01) بين متوسط قيمة TGF والأجسام المضادة لـ Streptococcus. IgM (2.104). كما كان هناك تباين كبير (p<0.01) بين متوسط قيمة TGFوالأجسام المضادة لـ Streptococcus IgG (21.298) ، واختلاف كبير (p<0.01) بين متوسط قيمة TGFو IL-17A (21.592.) ونتائج تضخيم منطقة معينة من جين lytA لـ Streptococcus pneumonia. تم استخدام 1.5% من هلام الأجاروز الكهربي لتجزئة أنواع العينات البشرية وتم صبغها بعلامات سلم بروميد الإيثيديوم .M: 100bp المسار 1-15 و1-15 يشبهان ناتج تفاعل البوليميراز المتسلسل 1023pb.