

DOI: [http://dx.doi.org/10.28936/jmracpc12.1.2020.\(14\)](http://dx.doi.org/10.28936/jmracpc12.1.2020.(14))STUDY OF SOME IMMUNOLOGICAL PARAMETERS IN PATIENT THAT INFUCTED WITH *Streptococcus pyogenes*

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## ABSTRACT

A streptococci has recognized as *Streptococcus spp.*, associate with acute pharyngitis. *S. pyogenes* infection Has detected in the Hospital and Health Center in Tikrit city. Throat swabs sample has obtained and cultured on a sheep blood agar plate. Identification of *S. pyogenes* was performed by using the VITEK 2 automatic system. It detected of 50 samples from children included 25 were positive for *S. pyogenes* infection. were aged 10-35 years old and included 30 male and 20 female. . While 25 sample Negative of *S. pyogenes* infection (The control group included 25 clinically healthy children without *S. pyogenes* infection matched for age and sex with cases pateints). No significant differences was reported between tonsillitis patients and healthy control in terms of Complement 4 proteins ( $33.21 \pm 14.18726$  ,  $35.57 \pm 15.56254$  ) , C3 ( $268.5 \pm 14.34$  ,  $146.06 \pm 17.83$ ) although increased complement level in patient group. The present study revealed no significant decrease levels of C4 of tonsillitis patients with negative ASOT than those with positive. the disease duration and ASOT have significant effect on CRP and complements components serum levels in patients with tonsillitis.

Keywords: Complement 3, complement 4, c-reactive protein, anti streptolysin O, *S. Pyogenes*.[http://dx.doi.org/10.28936/jmracpc12.1.2020.\(14\)](http://dx.doi.org/10.28936/jmracpc12.1.2020.(14))دراسة بعض المتغيرات المناعية لدى المرضى المصابين ببكتريا المكورات العنقودية *Streptococcus pyogenes*

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## الخلاصة

تعد المكورات العنقودية مميزة كإناوع *Streptococcus spp.* مرتبطة مع التهاب البلعوم الحاد لفترة طويلة، وان الإصابة ببكتريا *Streptococcus pyogenes* اكتشفت في المستشفى والمركز الصحي بمدينة تكريت، إذ زرعت عينات مسحات الحنجرة على طبق أكار دم أغنام وتم التشخيص باستخدام نظام VITEK 2 الآلي، حيث جمعت 50 عينة من الأطفال من بينهم 25 كانت إيجابية لعدوى *S. pyogenes*، وقد تراوحت أعمارهم بين 10-35 سنة، وشملوا 30 ذكراً و20 أنثى، في حين أن 25 عينة كانت سلبية من عدوى تلك المكورات (شملت مجموعة السيطرة 25 طفلاً يتمتعون بصحة جيدة سريريا دون الإصابة بالتهاب تلك المكورات، وقد تناسبت مع العمر والجنس مع مجموعة المرضى ولم يلاحظ وجود فروقات معنوية مسجلة بين مرضى التهاب اللوزتين ومجموعة السيطرة من حيث بروتينات نظام المتمم الرابع ( $33.21 \pm 14.18726$ ،  $35.57 \pm 15.56254$ )، فيما سجلت زيادة معنوية في مستوى المتمم البروتيني الثالث لمجموعة المرضى ( $268.5 \pm 14.34$ ،  $146.06 \pm 17.83$ )، وسجلت الدراسة الحالية إنخفاض غير معنوي لمستويات المتمم الرابع لدى المرضى المصابين بالتهاب اللوزتين مع عدم إيجابية التفاعل الجسم المضاد للسربتولايسين O أكثر من تلك



المجاميع الايجابية للتفاعل، كما اثرت مدة المرض على الجسم المضاد للستربتولايسين O وسجلت تأثيرات معنوية على البروتين الفعال C ومستويات المصل لمكونات المتمم في المرضى المصابين بالتهاب اللوزتين. الكلمات المفتاحية: المتمم الثالث، المتمم الرابع، البروتين الفعال C، مضاد الستربتولايسين O، المكورات العنقودية.

## INTRODUCTION

*S. pyogenes* was gram-positive bacterium group A streptococcus, is an serious mankind sickened that colonized skin and tonsils, causative an sicknesses ambit from abstemious sore throat and satin to life-threatening and invasive contagious (Bencardino *et al.*, 2016; Terao, 2012). It was an mankind sickened recorded in the summit 10 infections reasons of mankind Fatality (Laabei & Ermert, 2018). Like most pathogen, the generate many virulence agents, presently, eleven virulence agents have been set as super antigens (Bencardino *et al.*, 2016). The most important virulence factor in GAS pathogenesis, including the M protein, are location in the fibronectin-collagen-T antigen (FCT) region (Kratovvac *et al.*, 2007). The complement system is a major component of innate immunity where it's act as a preemptive buckler in the untimous stages of injury. After injury with sickened, each agent of accomplisher system will lived in a serial pattern under particular domination of accomplisher orderly agents consisted : C1-INH (Honda-Ogawa *et al.*, 2013; Kuo *et al.*, 2008). The complement system of human composed of more than 30 proteins found in host cells and plasma. depended on various distinctive particles to sense exogenous material, there are many distinct complement pathways are: classical router, Lectin router and alternative router (Kuo *et al.*, 2008). The reactivate of first router start with recognition and binding of microbial outer layer wherein the antibodies, activation of Lectin pathway accomplished through recognition of carbohydrate ligands exhibit on the microbial outer layer wherein MBLs (mannose binding lectins), in contrast to the pathways of classical and lectin router, the active protein act as a intrinsic active system spontaneous hydrolysis of a thioester bond with in C3 create a conformationally altered Complement 3 molecule (Laabei & Ermert, 2018). All three pathway lead to opsonization of target pathogens with the C3 complement derivative C3b, the releasing of the inflammatory mediators C3a and C5a and the formation of the membrane attack complex on the target cell membrane (Merle *et al.*, 2015). So, the study was aimed of some immunological proteins that affected with *streptococcus pyogenes* infections.

## MATERIALS AND METHODS

### Specimen collection and preparation

A throat swab sample was obtained from each participant and cultured on a sheep blood agar plate. Identification of *S. pyogenes* was performed by using the VITEK 2 automatic system (bioMérieux, Marcy l'Etoile, France). Of these 50 children 25 were positive for *S. pyogenes* infection. While 25 sample Negative of *S. pyogenes* infection were aged 10-35 years old and included 30 male and 20 female. The control group included 25 clinically healthy children without *S. pyogenes* infection matched for age and sex with cases

### Serum preparation

Peripheral blood samples from cases and controls were collected by venipuncture using vacuum tubes (Vacutainer). Whole blood and plasma aliquots were obtained and stored at 4°C and -20°C, respectively until analyzed, and we use deep frozen or fresh serum samples. The specimens was obtained by lode, The specimen after centrifugation was overstocked for up to 48 hours at 2-8°C before assay and for along storage period. The samples thawing and repeated freezing must be impossible. The collection of peripheral blood samples from by venipuncture using EDTA vacuum tubes. Whole blood and plasma aliquots were obtained and stored at 4°C and -20°C.

### Evaluation of ASOT and CRP



The assay latex agglutination test for semi quantitative determination of ASO and CRP kits (Plasmatic Laboratory Products LTD/U.K).

#### Detection of C3 and C4 concentration

The radio immune diffusion micro plates were embossed for 24 to 72h at room temperature. These technique essentially of (Fahey & McKelvey, 1965). Calibrating viewer was used to gauged the distance of the ring, this technique is called Mancini method. In this reaction Ag-Ab spread on in semisolid phase, where antigen aspur will form into these phase. The reactants spread toward each other on the semi solid phase even they concurs, The distance of the ring is a calculated of antigen amount, the ring diameter micro plates are read after of incubation 24-72 hr (Parija, 2012).

#### Statistical analysis

The data analyzed by SPSS program (statistical package for social science) version (Xie *et al.*; 2004). Quantitative variables were represented as mean±SD. P-value less than 0.05 (< 0.05) consider statistically significant. The relationship between studied variables was assessed by using Spearman correlation.

## RESULTS AND DISCUSSION

The present study was designed to estimate some immunological biomarkers (C3, C4, ASO and CRP) in studied groups; 25 tonsillitis patients and 25 healthy controls. The association between circulating levels of these biomarkers were investigated. The complement protein calculated by using method encompass radially diffusing of antigen that found in well through agarose gel including monoclonal Ab. Intricate of Ag-Ab are configured under the circumstances proportioned to these reactions will constitutes ring that configured made of reactions. The diameter of ring will proportion suited ejective between the diameter and concentration in accordance with data sheet that provided with the kit. The diameter of ring will proportion. (Table 1) display demographic characteristics of the patients with tonsillitis, as well as relationship between disease and different variables. The lower frequency of ASOT positive results 6 (15.8) while higher frequency of CRP positive patients 19 (60.5%) with significant difference (p<0.05).

**Table (1):** Demographic profile of 25 patients with tonsillitis .data include information about the name, age, gender, disease duration residence, ASOT and CRP.

Patient characteristics	Value	Probability
Age (years):	(10-29) years	-
Sex:	(Male/Female)	-
ASO (%)		
< 200: No (%)	19 (84.2)	P<0.05*
≥ 200: No (%)	6 (15.8)	
CRP (mg/dl):		
< 6: No (%)	9 (39.5)	P<0.05*
≥ 6: No (%)	16(60.5)	
ASOT: Anti-Streptolysine O test; CRP: C-reactive protein significant: p<0.01(Chi square); * Significant: p<0.05(Chi square)		

**Table (2):** C3, C4 Concentration in studied group.

Characters	Tonsillitis patients Mean ±SE No.25	Healthy control Mean ±SE No.15	P value
C3/ Mean ±SE	268.5±14.34	146.06±17.83	P≥0.05
C4/ Mean ±SE	33.21 ±14.18726	35.57±15.56254	P≥0.05



HC: Healthy control; C: complement;;  $p < 0.01$  (t test); Highly significant;  $P < 0.05$ : Significant;  $P \geq 0.05$ ; No-significant.

Characteristics of patients with tonsillitis and healthy control are detailed in (Table 2). showed significant decrease in C4 concentration compared with healthy control which non significant level ( $P > 0.05$ ), while C3 protein showed significant increase in concentration compared with healthy control in significant level ( $P > 0.05$ ). The present study was designed to correlation between some of immunological biomarkers (C3, C4, ASO and CRP) in studied groups. Tonsils are involved in the development of immune defense mechanisms by both local immunity and immune surveillance (**Faramarzi et al., 2006**). No significant differences was reported between tonsillitis patients and healthy control in terms of Complement 4 proteins, Complement 3 proteins although increased complement level in patient group. The male to female ratio in the present study was the same that Reported by (**Hessan & Abbas, 2012**) in Babylon. differences were significant and highly significant in terms of C3 and CRP involved in the clearance of microorganisms, and run up serum echelons of complement 3 are combination with acute phase of inflammatory interactions (**Povoa, 2002**). Complements and some serological markers levels were elevated in tonsillitis patients with healthy control, but some of these decline compared with healthy control however. The above results revealed that in patient, tonsils are essential as a local immunological defense mechanism. On senility, complement 3 in serum and plasma is quickly pasted enzymatically to lethargic C3c (**Xie et al., 2004**). The present study revealed no significant decrease levels of C4 of tonsillitis patients with negative ASOT than those with positive. release in above study parameters in positive ASOT test may be due to present of stress, it has either internal, external, chemical and physical effects that irritate nerve cells underneath thalamus to increase the secretion of corticotropic releasing hormone at a higher average (**Al-Sultan et al., 2013**). This result increase of adrenocorticals in which delay adherence of white blood cells to the vessel wall, it is therefore delayed their migration towards the inflammatory foci within vasculature and in the meantime affect the chemotaxis process. This in turn inhibits the process of phagocytoses plus affixing the lysosomes that will keep the intercellular phagocytised agents inside the cells, also this process will delay the interaction with antigens and the weakness of immune level and function will allow the pathogenic microorganisms to adhere, colonize and invade upper respiratory tract, pharynx and tonsils to amplify the disease (**Al-Sultan et al., 2013**). Finally, the disease duration and ASOT have significant effect on CRP and complements components serum levels in patients with tonsillitis.

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