



Microbial Science Archives (ISSN: 2583-1666)

Journal homepage: www.microbial.sciencearchives.org



Research Article

<http://dx.doi.org/10.47587/MSA.2022.2101>



Study of some immunological parameters in patient that infected with *Streptococcus pyogenes*

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Received: Jan 31, 2022 / Revised: Feb 18, 2022/ Accepted: Feb 28, 2022

Abstract

For a long period, *Streptococci* has been recognized as *Streptococcus sp.* associated with acute pharyngitis. *S. pyogenes* infection has been 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 males and 20 females. While 25 samples were Negative of *S. pyogenes* infection (The control group included 25 clinically healthy children without *S. pyogenes* infection matched for age and sex with cases patients). No significant differences were reported between tonsillitis patients and healthy control in terms of Complement 4 proteins (33.21 ± 14.18726 , 35.57 ± 15.56254), C3 (268.5 ± 14.34 , 146.06 ± 17.83) although increased complement level in the patient group. The present study revealed no significant decrease in levels of C4 of tonsillitis patients with negative ASOT than those with positive. the disease duration and ASOT have a significant effect on CRP and complement components serum levels in patients with tonsillitis.

Keywords Complement 3, Complement 4, C-reactive protein, Antistreptolysin O, *S. pyogenes*

Introduction

Streptococcus pyogenes gram-positive bacterium group A streptococcus, is serious mankind sickened that colonized skin and Tonsils, Causative and sicknesses ambit from abstemious sore throat and satin to life-threatening and invasive contagious (Bencardino et al., 2016; Terao, 2012). *streptococcus pyogenes* and mankind sickened recorded in the summit 10 infections reasons of mankind Fatality (Laabei & Ermert, 2018). Like most pathogens, *streptococcus pyogenes* generates many virulence agents, presently, eleven virulence agents have been set as superantigens (Bencardino et al., 2016). The most important virulence factor in GAS pathogenesis, including the M protein, is located in the fibronectin-collagen-T antigen (FCT) region (Kratovvac et al., 2007).

The complement system is a major component of innate immunity where it acts as a preemptive buckler in the untimous stages of injury. After injury with sickened, each agent of accomplisher system will livened in a serial pattern under particular domination of accomplisher orderly agents consisting of C1-INH (Honda-Ogawa et al., 2013 ; Kuo et al., 2008). The complement system of humans is composed of more than 30 proteins found in host cells and plasma. Depending 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 the 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 an intrinsic active system spontaneous hydrolysis of athioester bond within C3 create a conformationally altered Complement 3 molecule (Laabei & Ermert, 2018). All three pathways 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). The study was aimed at 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 samples Negative of *S. pyogenes* infection were aged 10-35 years old and included 30 males and 20 females. 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 were 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 sample's thawing and repeated freezing must be impossible. the collection of Peripheral blood samples from 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 C3and C4 concentration

The radio immunodiffusion microplates were embosomed for 24 to 72 h at room temperature. This technique is essential (Fahey and McKelvey, 1965). The calibrating viewer was used to gauge the distance of the ring, this technique is called Mancini method. In this reaction, Ag-Ab spread in the semisolid phase, where antigen aspur will form into these phases. The reactants spread toward each other on the semi solid-phase even they concur, The distance of the ring is a calculated antigen amount, the ring diameter microplates are read after incubation 24-72 hrs (Parija, 2012).

Statistical analysis

The data were 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

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 was investigated.

The complement protein calculated by using method encompasses 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 constitute ring that configured made of reactions. The Diameter of the ring will proportion suited ejective between the diameter and concentration following the datasheet provided with the kit. The Diameter of the ring will be a proportion. (Table 1) display demographic characteristics of the patients with tonsillitis, as well as the relationship between disease and different variables. The lower frequency of ASOT positive results 6 (15.8) while the 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 includes information about the name, age, gender, disease duration residence, ASOT, 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)		

Characteristics of patients with tonsillitis and healthy control are detailed in (Table 2). Showed Significant decrease in C4 concentration compared with healthy control wick nonsignificant level (P>0.05), While C3 protein showed Significant increase in concentration compared with healthy control in insignificant level(P>0.05).

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≥ 0.05; No-significant			

Discussion

The present study was designed to the correlation between some of the immunological biomarkers (C3, C4, ASO.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 were reported between tonsillitis patients and healthy control in terms of Complement 4 proteins, Complement 3 proteins although increased complement level in the patient group. The male to female ratio in the present study was the same as that Reported by (Hessan and Abbas, 2012) in Babylon. differences were significant and highly significant in terms of C3 ., CRP involved in the clearance of microorganisms, and run-up serum echelons of complement 3 are a combination with the acute phase of inflammatory interactions (Povoa, 2002). Complements and Some serological markers levels were elevated in tonsillitis patients with Healthy controle, But some of these decline Compared with healthy control, however, The above results revealed that in Patients, 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 Y *et al.*, 2004).

The present study revealed no significant decrease in levels of C4 of tonsillitis patients with negative ASOT than those with positive. release in the above study parameters in positive ASOT test may be due to the presence of stress, it has either internal, external, chemical, or physical effects that irritate nerve cells underneath thalamus to increase the secretion of the corticotropic releasing hormone at a higher average (Al-Sultan *et al.*, 2013). This result increase adrenocorticals in which delay adherence of white blood cells to the vessel wall, it is therefore delayed their migration towards the inflammatory foci within the 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 a significant effect on CRPand complements components serum levels in patients with tonsillit

Conflict of Interest

The author hereby declares no conflict of interest.

Consent for publication

The author declares that the work has consent for publication.

Funding support

The author declares that they have no funding support for this study.

Ethical Considerations

The study was approved by the institutional ethical committee

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How to cite this article

Alobadi, E.G.Y. (2022). Study of some immunological parameters in a patient that infected with *Streptococcus pyogenes*. *Microbial Science Archives*, Vol. 2 (1), 1-4. <http://dx.doi.org/10.47587/MSA.2022.2101>

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