Estimation of Complement Components C3, C4 and Immunoglobulins IgA, IgM and IgG Among Patients with Tonsillitis

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Abstract

Background: Tonsillitis is inflammation of the tonsils. It's usually caused by a viral infection or, less commonly, a bacterial infection. Bacterial causes are mainly through group A beta-hemolytic *Streptococcus pyogenes* that leads to strep throat. Tonsils are involved in the development of immune defense mechanisms by both local immunity and immune surveillance.

Objective: To evaluate the serum levels of complement component C3, C4, immunoglobulin A, IgG, IgM, anti streptolysin O and C-reactive protein in sera of tonsillitis patients and healthy controls. The association between circulating levels of these biomarkers were investigated.

Patients and Methods: This study involved thirty-eight patients with tonsillitis (18 males and 20 females), their age range from 1-35 years. Blood samples collected from Rizgary teaching hospital/Erbil during the period from 4th of November 2015 to 13th of May 2016. They were examined and compared with 18 healthy control individuals of similar ages and gender. Sera were separated for the following tests ASO, CRP, IgA, IgG, IgM,C3 and C4.

Results: Among total patient group, 6(15.8%) and 23(60.5%) presented with positive ASO and CRP tests compared with 32(84.2%) and 15(39.55%)negative tests with significant results. Patients and healthy controls were compared and there were no significant differences between the groups in serum level of 3 and C4 ($P>0.05$), although an increase in complements levels. While, differences were significant between the group in term of IgG($P<0.05$) and highly significant in term of IgA ($P<0.01$).There was no significant differences in mean concentration of the above parameters regarding to age ($P>0.05$), however there was highly significant difference regarding to IgG($P<0.01$)in all the above mentioned terms. Indeed C4 also recorded highly significant result in the CRP parameter. Lastly the study was reported significant and highly significant positive and negative correlation among some above parameters like C3:C4 and C4:IgA.

Conclusion: Study immunological parameters increased in tonsillitis patients, whose age below 10 years, disease duration less than 2 years and their ASO negative.

Key words: Tonsillitis, GABHS, complement, immunoglobulins, CRP, ASO.

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Received: 22th May 2016

Accepted: 12th June 2016

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Introduction

Tonsillitis means viral and /or bacterial infection that lead to inflammation of the tonsils, is the main source, when caused by a bacterium belonging to the group A beta hemolytic *Streptococcus pyogenes*

(GABHS), it is usually called strept throat in approximately15-30% of patients [1]. The disease is characterized by fever and sore throat and the incidence high among children, the treatment includes either anti inflammatory or antibiotics or combinations. The most patients improve completely, with or without treatment. During three days symptoms will resolve in 40%,, and within one week in 85% of people, despite if streptococcal infection is present or not [1].

Children with chronic tonsillitis or recurrent, complications may rarely include dehydration and kidney failure due to inflammation, and pharyngitis due to the spread of infection. Complications like rheumatic fever or glomerulonephritis can happened in very rare cases of strep throat. These complications are remaining a significant problem in poorer nations [2,3].

The majority of mucus associated lymphoid tissue (MALT) lymphocytes secrete immunoglobulin A (IgA) which acts as a 'mucosal antiseptic paint' and combines with foreign molecules, either blocking their attachment or rendering them harmless, so that they are phagocytosed in specific manner. Tonsillar imunoglobulin secretion differs from the usual MALT pattern. IgG-producing cells and nasopharyngeal IgA dimmers secreted by plasma cells bind to membrane receptors on the internal surface of epithelial cells. Both IgA and IgG pass out directly into the pharyngeal secretions by leaking between epithelial cells; this is enhanced when there is inflammation [4].

C-reactive protein (CRP) is a biomarkers that, in combination with other signs of sepsis, can be useful as indicators of infection[5]. Different studies have reported high serum levels of immunoglobulin A and IgG in chronic tonsillitis patients[6]. conversely, some studies reported that decreased in level of immunoglobulins IgM, IgG and IgA [7]. Others have found that no significant difference between the levels of immunoglobulins and complements mong the children after tonsillectomy[8,9].

This study aims to assess serum levels of complement component C3, C4, immunoglobulin IgA, IgG, IgM, anti streptolysin O test (ASOT) and CRP in sera of patients with tonsillitis and healthy controls.

Patients and Methods

The groups in present study consisted of thirty-eight tonsillitis patients were entered Rizgare Teaching Hospital in Erbil during the period from 4thof November 2015 to 13thof May 2016. Also, 18 apparently healthy controls were ages and genders matched were enrolled in the study. The general immunolaboratory assessments included ASOT, CRP, IgA, IgG and IgM, C3 and C4 tests. A blood sample (five ml venous blood) was aspirated from all patients and healthy controls. Each sample was transferred to 10 ml sterile tubes, centrifuged for 15 min at 3000 round per minute. Thereafter the obtained serum dispensed into several Eppendorf tubes and immediately frozen at –20 ºC till tested. At the start of the study, permission of local health authorities to access patients obtained from Erbil Health directory, in addition informed consent of patients obtained verbally too. Patients interviewed and filled out a designed questionnaire for the purpose of this study. Data collected from each patient using special designed questionnaire. Demographic data include information about the name, age, gender, disease duration residence, ASOT, CRP, IgA, IgG, IgM, C3 and C4 tests results were also recorded.

The assay latex agglutination test for semi quantitative determination of ASO and CRP kits (Plasmatic Laboratory Products LTD/U.K). Single radial immunediffusion assay for quantitative determination of compliments C3, C4 and immunoglobulins

IgA, IgG and IgM (Intermedical/ Italy) were used to detect study parameters in the serum.

Statistical analysis

The data analyzed by statistical package for social science (SPSS) version [18]. Quantitative variables summarized by finding mean ±SD. P-value of < 0.05 consider as statistically significant, non quantitative variables were analyzed by using Chi square test. The relationship between studied variables was assessed by using Spearman correlation.

Results

The present study was designed to estimate some immunological biomarkers (C3, C4, IgA, IgG, IgM, ASO and CRP) in studied groups; 38 tonsillitis patients and 18 healthy controls. The association between circulating levels of these biomarkers were investigated. Table (1) lists the demographic characteristics of the patients with tonsillitis, including the relationship of different variables with the disease. The age of the patients in this study, ranged from (1-35) years with a mean age of (4.05) years, in which 18(47%) were male and 20 (53%) patients were female, p>0.05 and male to female ratio was (1:1.1). Among total patient group, lower frequency of ASOT positive results 6 (15.8%) while higher frequency of CRP positive patients 23 (60.5%) with significant difference (p<0.05) Duration of tonsillitis revealed that the majority of patients were below 2 years 30 (78.9%) while only 8 (21.1%) were above 2 years with highly statistical significant difference (p<0.01).

Table (1): Demographic profile of 38 patients with tonsillitis

Patient characteristics	Value	Probability
Age (years): Mean ±SE	7.70±1.53	-
Sex: No (%)Male/female	18 (47) / 20 (53)	p>0.05
ASOT (mg/dl):		-
< 200: No (%)	32 (84.2)	P<0.01**
≥ 200: No (%)	6 (15.8)	
CRP (mg/dl):		-
< 6: No (%)	15(39.5)	P<0.05*
≥ 6: No (%)	23(60.5)	
Duration: No. (%) ≤ 2 years	30(78.9)	P<0.01**
>2 years	8 (21.1)	

ASOT: Anti-Streptolysine O test; **CRP**: C-reactive protein;** **Highly 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). Both groups were compared and there were no significant differences between the groups in terms of age, sex, C3 and C4 (P>0.05). While differences were significant between the group in term of IgG(P<0.05) and highly significant in term of IgA (P<0.01).

Table (2): Demographic comparison between HC patients with tonsillitis

Characters	Healthy control Mean ±SE No.18	Tonsillitis patients Mean ±SE No.38	P value
Age (years):	6.4±2	7.7±1	P≥0.05
Sex: No. (%)M /F	9(50)9/(50)	18 (47)/ 20 (53)	P≥0.05
C3/ Mean ±SE	260.3±41	314.5±14	P≥0.05
C4/ Mean ±SE	97.9±21	97.6±6	P≥0.05
IgA/ Mean ±SE	435±24	935±32	P<0.05
IgG/ Mean ±SE	3239±339	4120±327	P<0.01
IgM/ Mean ±SE	295±35	479±32	P≥0.05

HC: Healthy control; **C:** complement; **Ig:** Immunoglobulin; **p<0.01(t test);** Highly significant**; P<0.05**: Significant; **P≥ 0.05;** No-significant**.; M/F**: Male/female ;
Normal values:C3:95-163 mg/dl ;**C4**:26-66 mg/dl ;**IgA**:89-445 mg/dl ;**IgG**:870-1960 mg/dl ;**IgM**:52-245 mg/dl

Table (3) showed complements (C3 and C4) and immunoglobulns(IgA, IgG and IgM) levels according to following terms gender, age, duration, ASOT and CRP. There was no significant differences in mean concentration of the above parameters regarding to age (P>0.05), however there was highly significant difference regarding to IgG(P<0.01)in all the above mentioned terms. Indeed C4 also recorded highly significant result in the last parameter.

Table (4) depicted the Pearson correlation tests to explain the powerful relationship between different biomarkers. There was positive highly significant correlation observed between different studied biomarkers (C3, C4 and C4, IgA) (P<0.01). Moreover positive significant correlation observed between (C3 with C4), (C4 with IgA) and (IgG with IgM) (P<0.05).

Table (3): Complements and immunoglobulins levels according to gender, age, duration, ASOT and CRP tests in tonsillitis patients

Parameter	The Mean± SE of Complements(C3, C4) and Immunoglobulins (IgA, IgG, IgM) in 38 tonsillitis patients					
	No.	C3	C4	IgA	IgG	IgM
Gender	F(20)	308.30±24	91.53±9	955.83±44	4333.41±372	473.35±52
	M(18)	321.19±16	104.15±8	923.13±45	3816.13±458	488.49±21
	P value	0.89	0.88	0.86	0.001**	0.93
Age	>10(28)	283.45±15	76.41±8	975.62±29	3934.43±313	438.21±29
	≤10(10)	323.38±12	103.66±6	924.34±25	4262.43±371	503.52±15
	P value	0.74	0.82	0.78	0.08	0.73
Duration	>2(8)	332.24±17	96.55±11	926.16±47	2899.80±258	450.91±29
	≤2(30)	309.44±18	97.91±8	943.18±50	4316.52±257	483.04±37
	P value	0.81	0.98	0.86	0.001**	0.58
ASOT	+ve(12)	358.94±13	94.40±18	884.58±41	2912.85±13	356.70±21
	- ve(26)	306.78±16	98.16±7	955.83±40	4603.41±14[b]	526.07 ±20
	P value	0.34	0.9	0.38	0.001**	0.4
CRP	+ve(7)	343.7±12	103.6±8	923.8±89	4095±136	397.8±17
	-ve(31)	271.9±27	88.8±10.8	1003.3±44	4603±189	526±15
	P value	0.36	0.001**	0.60	0.001**	0.85

ASOT: Anti-Streptolysine O test; **CRP:** C-reactive protein;**C3** :Complements component 3; **IgA**: Immunoglobulin A; ** **Highly significant**: p<0.01(t test)

Table (4): Correlation between different biomarkers of tonsillitis patients

Parameters	C3	C4	IgA	IgG	IgM
C3 **Pearson correlation** **Sig. (2-tailed)**	-	0.46 **P<0.05**	0.29 P≥0.05	0.09 P≥0.05	0.43 P≥0.05
C4 **Pearson correlation** **Sig. (2-tailed)**	0.46 **P<0.01**	-	-0.81 **P<0.01**	-0.13 P≥0.05	-0.18 P≥0.05
IgA **Pearson correlation** **Sig. (2-tailed)**	0.29 P≥0.05	-0.81 **P<0.05**	-	0.39 P≥0.05	0.48 P≥0.05
IgG **Pearson correlation** **Sig. (2-tailed)**	0.09 P≥0.05	-0.13 P≥0.05	0.39 P≥0.05	-	0.79 **P<0.05**
IgM **Pearson correlation** **Sig. (2-tailed)**	0.43 P≥0.05	-0.18 P≥0.05	0.48 P≥0.05	0.79 **P<0.05**	-

Discussion

Tonsils are involved in the development of immune defense mechanisms by both local immunity and immune surveillance [6]. The male to female ratio in the present studywasthe same that Reported by Hessan and Abbas, 2012 in Babylon [10]. No significant differences was reported between tonsillitis patients and healthy control in terms of age, sex, C3 and C4 , although increased complement level in patient group. While differences were significant and highly significant in terms of IgG and IgA. Jointly with components of complement, CRP involved in the clearance of microorganisms, and raised serum levels of C3 are associated with acute inflammatory reactions [11].The increased Ig levels among tonsillitis patients in the present study is multi-factorial, IgM is a prominent antibody in initial response to most antigens and increased levels during frequent, chronic and acute infections while IgA is the second most abundant immunoglobulin was found in lymphoid tissues and increased during inflammations [12].Indeed, this result comes in agreement with an study done by Ikinciogullari *et al*., in 2002, who assisted an increased in serum immunoglobulin levels during the episodes of tonsillitis (recurrent) in children due to lymphocyte stimulation by repeating antigenic stimulation[13].

Complements and immunoglobulins levels were elevated in tonsillitis patients with age less than 10 years when compared with those more than 10 years. Similar result was reported by other researcher [14]. features of tonsils above 8 years age are less active than those below the age of 8 years, This was reported by immunohistochemical study. At the age of 5 to 7 years, the total number of IgA is the highest but it decline by age however, at the age of 11 to 13 years, the serum and salivary secretory IgA concentrations reach to adult's level. The above results revealed that in preschool children, tonsils are essential as a local immunological defense mechanism. On ageing, C3 in serum and plasma is rapidly cleaved enzymically to inactive C3c[15].

The present study revealed no significant increase levels of C3, C4, IgA and IgM of tonsillitis patients with negative ASOT than those with positive. However IgG increased significantly.

Down 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 [16]. 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 the upper respiratory tract, pharynx and tonsils to amplify the disease[16].Children who have IgA deficiency cannot manifest an appropriate immunologic response and, therefore, are susceptible to recurrent infections and allergies [17].

Finally, the age, disease duration and ASOT have significant effect on immunoglobulins and complements components serum levels in patients with tonsillitis.

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