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Study of the Relationship Between Blood Group and Group A Beta - Hemolytic *Streptococci* Isolated from Patients with Tonsillitis

Rana Talib Al-Ani*

Department of Biochemistry, Medicine College, AL Anbar University, Ramadi, Iraq

Abstract

The aim of this study is the relationship between blood group and group A Beta-Hemolytic *Streptococci*, Ninety patients with tonsillitis were included in this Study from both genders and different age group for blood group study. They were attended at Ramadi Teaching Hospital during the period extended from January 2011 to June 2011. Their ages range from 3-35years. The control group included 20 apparently healthy persons, while patient divided into, chronic and recurrent acute tonsillitis. Diagnostic tools were Bacteriological methods (Bacitracin method to identify and count *Streptococcus pyogenes* group A beta hemolytic streptococci (GABHS)). Results of blood grouping showed a reduced frequency of blood group AB- with tonsillitis and increased frequency of blood group O + with tonsillitis.

Keywords: GABHS, tonsillitis, blood group.

دراسة العلاقة بين فصائل الدم ومجاميع بكتريا الميسجيات العنقودية المحللة للدم نوع بيتا لمرضى التهاب اللوزتين

رنا طالب العاني*

قسم الكيمياء الحياتية، كلية الطب، جامعة الانبار، الرمادي، العراق

الخلاصة

إن الهدف من هذه الدراسة هو العلاقة بين مجاميع الدم ويكتريا الميسجيات العنقودية، حيث تم دراسة 90 مريض يعانون من التهاب اللوزتين حيث تضمنت الدراسة لكلا الجنسين ولمختلف الأعمار وعلاقتها بفصيلة الدم. المرضى كانوا را قدين في مستشفى الرمادي التعليمي ضمن فترة كانون الثاني الى حزيران 2011. و لأعمار اختلفت من 3-35 سنة. عينات السيطرة تضمنت 20 شخصا أصحاء ظاهريا، تم تقسيم المرضى إلى مجموعتين التهاب اللوزتين المزمن والحاد المتكرر، تضمن التشخيص الطريقة البكتيرية لتشخيص (GABHS)، النتائج لمجاميع الدم أظهرت انخفاض مجموعة دم AB- مع التهاب اللوزتين وزيادة مجموعة الدم O+ مع التهاب اللوزتين.

Introduction

Tonsillitis is one of the most prevalent infections in children and adolescents [1]. The signs are similar in bacterial and viral pharyngitis. The etiologic agents might be viral or bacterial [2]. Acute tonsillitis lasts for about four to six days Recurrent tonsillitis is an infection of acute tonsillitis which is diagnosed when an individual has seven episodes in one year, five infections in two consecutive years, or three infections each year for three years consecutively [2,3].

The most important bacterial cause is group A *streptococcus* (GAS), which is responsible for about 15% of all cases, and 80-90% of cases are caused by viruses particularly adenovirus [3]. A lack of knowledge of these bacteria could result in ineffective treatment for patients. Only streptococcal

*Email: mmphysisc361@gmail.com

tonsillitis justifies the use of antibiotics. Antibiotic treatment of non – bacterial tonsillitis adds greatly to the potential for the Emergence of resistant strains in or pharyngeal human flora [4] Within the tonsils, white blood cells of the immune system destroy the viruses or bacteria by producing inflammatory cytokines like phospholipase A2,[5].

The changing epidemiology of group A streptococci and rheumatic fever is related to changes in the distribution of serotypes, where certain virulent M types have been associated with invasive disease [6].

The study was relationship between blood group and group A Beta-Hemolytic *Streptococci*.

Materials and Method

Samples of Study

Over a period of 5 months, hematological study of tonsillitis was conducted between January 2011 to June 2011 in patients with high clinical suspicion of Recurrent Acute and chronic tonsillitis who were referred to AL-Ramadi Teaching Hospital ENT Department.

The total number of patients was 90, Group One consists of 40 patients with recurrent acute tonsillitis, and Group two consists of 50 chronic tonsillitis.

Control Group:

This includes 20 (apparently) healthy individuals (10 males and 10 females) with no previous history of tonsillitis.

Hematological investigation study:

About 2 ml of blood sample were collected in EDTA tubes which were obtained by 10 ml sterile disposable syringe from each individual and left for assessment of ABO blood groups and Rh factor.

Blood Groups and Rh Typing:

One drop of anti –A was placed on the left side of slide, one drop of anti–B was placed on the right side of slide, and one drop of anti –Rh was placed in the center of slide (ABO blood groups and Rh, Biotest ,UK) .Three Blood drops were obtained and placed one drop on each side of slide and a drop on center. Quickly each three positions mixed with a fresh wooden applicator stick. The slide was rocked gently back and forth. After two minutes, all three blood drops were observed for evidence of clumping .The agglutination that occurred in each sample was reported according to `Ndamba [7].

Bacterial Isolation and Identification:

A loopful from the blood culture bottles was taken and sub cultured on sheep blood agar and MacConkey's agar media. The plates were incubated in ordinary atmospheric conditions at 37°C and in candle jar at the same temperature [8].

Subcultures were done on chocolate agar and blood agar media which will be accentuated by stabbing the agar after streaking increases activity of streptolysin O which is oxygen labile after 18-24 hours of incubation due to the streptococci which tend to undergo autolysis and die very rapidly [9] and the second subculture is done after 72 hours of incubation .The sample was considered negative and discarded after one week. If any suspected streptococcal colonies appeared, it will be subjected for biochemical and confirmatory tests.

The recognition of β - or α – hemolytic pinpoint colonies was improved and their presumptive identification were examined firstly by Gram's stain for vary chain of Gram positive cocci . Also the growth was examined by adding H₂O₂ (3%) for catalase test, which is a negative result for streptococci.

Tetra methyl paraphenyline diamine hydrochloride reagent (oxidase reagent) was added to the colonies placed on filter paper and yielded violet color, which indicates a positive reaction , *Streptococcus* has negative result for oxidase test .In addition to that the β -hemolytic colonies can be improved by susceptibility test to lower concentration of bacitracin (0.02 – 0.05) IU , A β – hemolytic streptococci showing any zone of inhibition around the disc should be reported as S . pyogenes . The – hemolytic colonies were improved by adding the optochin disc on a blood agar streaking plate. Zone of inhibition of growth around the disc of Optochin indicate *Streptococcus pneumoniae* and no zone of inhibition observed around the disc, this may be due to *Streptococcus viridians* [9, 10].

Results

There were 50 patients who undergo from chronic tonsillitis divided into 24 (48.0%) for males and 25 (52.0)% for females , there was the high rate of chronic tonsillitis and the recurrent acute tonsillitis in the age 6-15 years, 29(58%) and 12(30%) . while the lowest rate was in the age group 26-35 years 1(2%) and(2.5%),respectively as shown in figure1 and 2 The statistical analyses showed no significant

difference between males and females for each age group according to $X^2 = 32.08$, $P > 0.05$. The distribution of age group in relation to GBHS infected individuals in chronic tonsillitis which was demonstrated in Table-1 and Figure-1.

Table 1- The distribution of chronic tonsillitis individuals according to age group and sex

Age group(year)	Chronic tonsillitis			X^2	P value
	Male (%)	Female (%)	Total (%)		
3-5	5(10)	4(8)	9(18)	0.11	0.74
6-15	16(32)	13(26)	29(58)	0.31	0.57
16-25	3(6)	7(6)	10(22)	1.0	0.31
26-35	0	2(4)	2(2)	-	-
Total	24(48)	26(52)	50(100)	0.08	0.77
X^2	12.25	10.61	32.08		
P value	0.002	0.014	0.00		

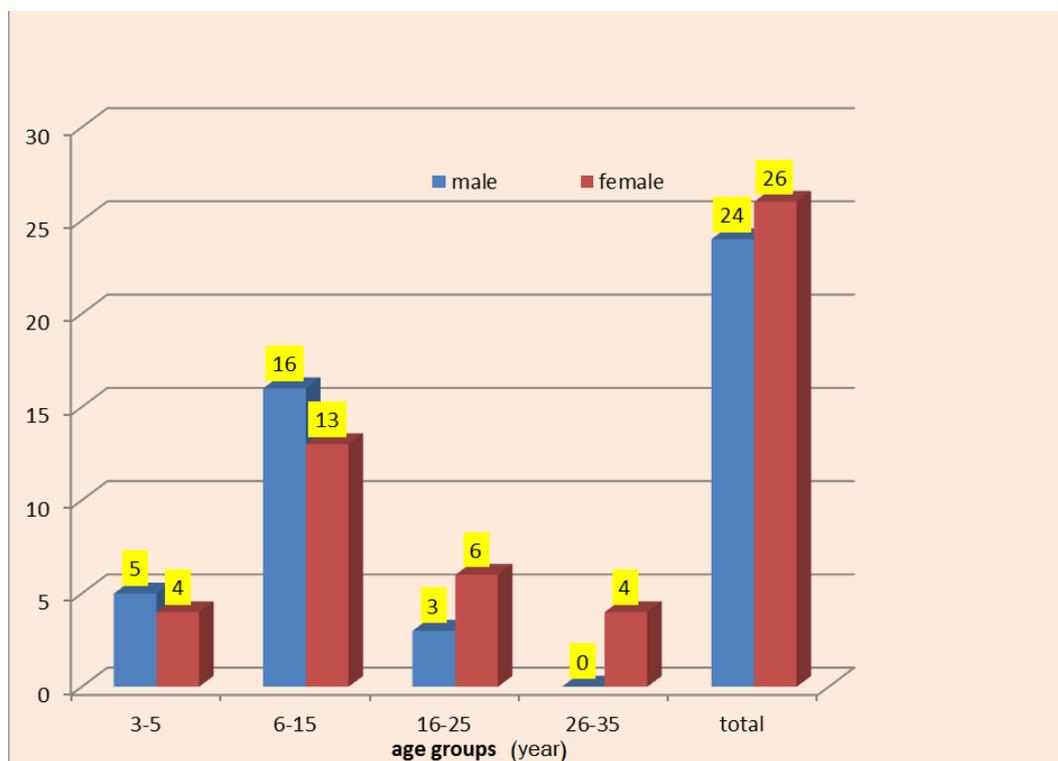


Figure 1- The distribution of chronic tonsillitis individuals according to age group and sex.

There was no significant statistical difference between GBHS infected the tonsils and age group $X^2 = 18.9$, $P > 0.05$. The distribution of age group in relation to GBHS infected individuals in recurrent acute tonsillitis which was demonstrated in Table-2 and Figure-2.

Table 2- The distribution of recurrent acute tonsillitis individuals according to age group and sex

Age group(year)	Recurrent Acute Tonsillitis			X^2	P value
	Male (%)	Female (%)	Total (%)		
3-5	4(10)	4(10)	8(20)	-	-
6-15	12(30)	11(27.5)	23(57.5)	0.043	0.83
16-25	3(7.5)	5(12.5)	8(20)	0.50	0.48
26-35	1(2.5)	0	1(2.5)	-	-
Total	20(50)	20(50)	40(100)	-	-
X^2	14.00	4.3	18.9		
P value	0.003	0.11	0.00		

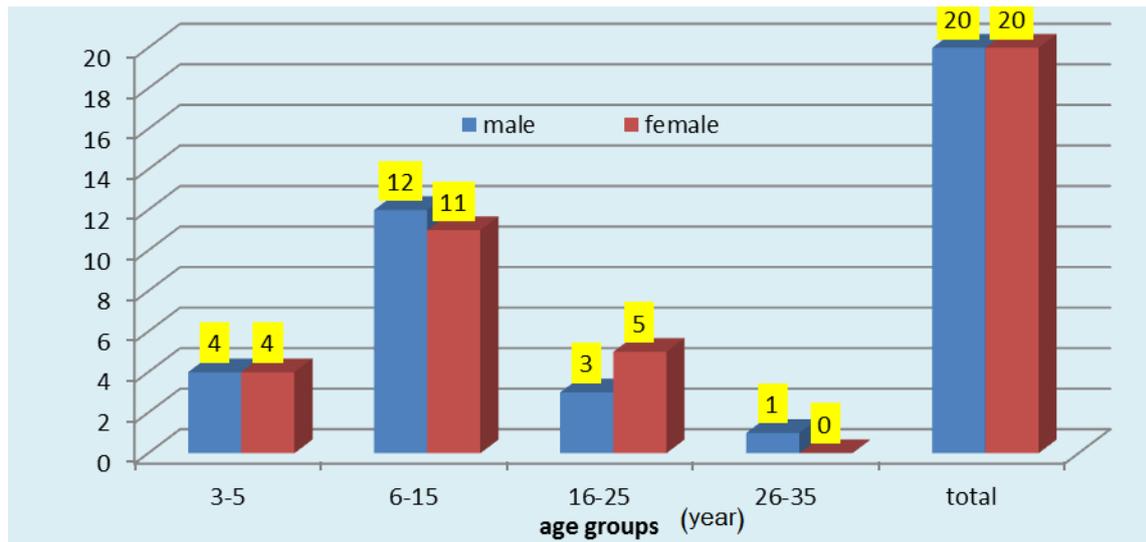


Figure 2- The distribution of recurrent acute tonsillitis individuals according to age group and sex.

Tonsillitis with Respect to Blood Groups and Rh factor

The distribution of ABO- Rh blood group in patients with recurrent, chronic and acute tonsillitis is listed to Table-3 and Figure-3. The predominance of ABO blood group types (Antigens) was studied in 40 patients with recurrent tonsillitis (group one of study), 50 patients with chronic tonsillitis (group two of study) and compared with 20 healthy apparently persons of control group. The present study showed that the frequency of blood group O⁺ was 38(42.2%) which was higher than in the three groups, and the lowest frequency rate was in AB⁻ 1(1.1%). The statistical analysis showed that there was no significant difference between tonsillitis and type group (A⁺, A⁻, B⁺, O⁻, AB⁺) by using $X^2 = 12.27$ ($P < 0.05$). While significant difference between tonsillitis and blood group (O⁺), $P < 0.05$ $X^2 = 7.081$

All groups were compared with healthy control group.

Table 3- Tonsillitis with Respect to Blood Groups and Rh factor.

Blood group and Rh	Number examined (%)	Recurrent acute tonsillitis (%)	Chronic tonsillitis (%)	Control number (%)	X^2	P value
A ⁺	19(21.1)	8(8.9)	11(12.2)	4(20)	3.17	0.0
A ⁻	3(3.4)	1(1.1)	2(2.2)	0	0.33	0.56
B ⁺	15(16.7)	6(6.7)	9(10.0)	4(20)	2.00	0.36
B ⁻	4(4.5)	3(3.3)	1(1.1)	0	1.0	0.31
O ⁺	38(42.2)	20(22.2)	18(20.0)	6(30)	7.081	0.02
O ⁻	3(3.3)	1(1.2)	2(2.2)	2(10)	1.80	0.18
AB ⁺	7(7.7)	1(1.1)	6(6.7)	4(20)	3.45	0.17
AB ⁻	1(1.1)	0	1(1.1)	0	-	-
Total patients	90(100)	40(44.5)	50(55.5)	20(100)	12.27	0.002
X^2		32.57	20.63			
P value		0.00	0.00			

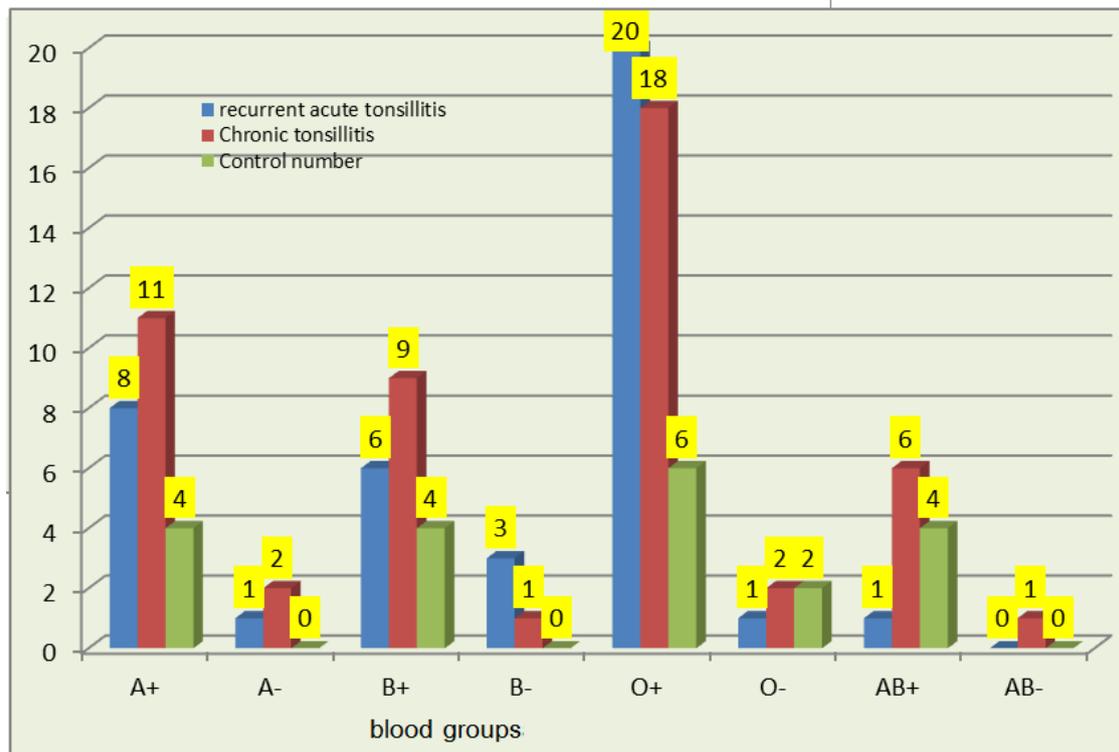


Figure 3- Tonsillitis with Respect to Blood Groups and Rh factor

Discussion

Tonsillitis and Gender Differences:

These results agreed with that reported by earlier studies on tonsillitis in Iraq that indicated that there were no differences between sex and tonsillitis infection [11] and also with studies laid down by (Abdul – Hameed; AL–Ani [12,13] and agreed with studies laid down by (Boukadida; Mzoughi [14,15]), who studied the tonsillitis infection in Tunisia and agreement with (Kurien [16]) who studied the tonsillitis in Singapore and agreement with (Chreithah [17]) While our study results were disagreed with those reported by AL–Gebori in Iraq that indicated male have more prevalence of infection than females [18,19] .

Age Groups of Individuals with Respect to Tonsillitis:

In our study, GBHS were distributed mostly in the (5-15) years old in case of Chronic and recurrent acute tonsillitis (58%, 57.5 %) respectively more than other age groups. This was confirmed by (Caffney; Fujimori [20, 21]) and (Behrman [22]) who found that the distribution of BHS were mostly in school – aged children.

Al–Gebori [18] in Iraq referred to that bacterial tonsillitis affected the children in the (5-15) years old (50.5%) and (59.2%) respectively , and our results disagreement with Al–Saaid [19] in Iraq who revealed that bacterial tonsillitis affected the first age group and Al–Shabieb [23] referred to the increasing of frequency of infection in first years old that may be due to the incomppliance to correct medical requirement of sucking in socioeconomic areas , in addition to the possibility of the presence of the bacteria in their mother (as a carrier), [24] who explained that the children are mostly subjected to infection due to the immaturity of immune system in addition to these explanation , children in this age are exposed to infection frequently as Influenza , Measles and other infectious disease , that would weaken the immune defense mechanisms of body against bacterial infection. GAS are prevalence in elementary school age children [25-27].

Tonsillitis with Respect to Blood Group and Rh Typing:

With regard to blood samples of individuals diagnosed for tonsillitis infection , the highest positive rate of infection in the present study was found in individuals with blood group O+ (42.2%) , A+ (21.1%) , B+ (16.7%) , AB + (7.7%) , B⁻ (4.5%) , ,A⁻ (3.4%) and O⁻ (3.3%) whereas the lowest positive rate was observed in those with blood group and AB⁻ (1.1%) .

These results showed no agreement with the results reported by Fadhil, (1989) who found the highest rate which was found in blood group B+ while the lowest rate of infection was observed in blood group A+ .

The reasons of studying the ABO blood groups in relation to infection that on erythrocyte there is a particular lipid – bound carbohydrate which is of interest because of its ability to act as an antigen. The gene specifies one of three forms of an enzyme, resulting in blood types known as A, B, AB, or O. Our immune system is able to recognize our own cells regardless of A- B- O type and avoid an immune response, The evasion mechanisms of a successful state of parasitism include rapid turnover of membrane component tegument structural development , the coating or (masquerade) of the bacterial surface with host antigenic acited by Al-Khafaji [29].

Conclusions

Tonsillitis caused by Beta–hemolytic streptococcal infects individuals at age (6-15) years more than other ages. The Positive rate of tonsillitis was found in blood group O + (42.2%) and the lowest frequency rate was in AB⁻ 1(1.1%).

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