

Research Article

Level of IgE and Leukotriene – B4 in Chronic Tonsillitis Bacterial Infection

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Received: 22.02.20, Revised: 19.03.20, Accepted: 26.04.20

ABSTRACT

This study aims to determine the levels of IgE and Leukotriene- B4 in chronic tonsillitis patients infected with *S. pyogenes* and *S. aureus* that age ranges between 3-54 years in Thi-Qar province and their relationship with gender, age.

This study included 75 patients with chronic tonsillitis infected with *S. pyogenes* and *S. aureus* diagnosed with the use selective and differential media and biochemical tests, and diagnostic methods have also been used as API 20 and VITEK2 compact system, from both sex and with different age at Al-Haboubi Teaching Hospital in Al-Nasiriya city, IgE levels were evaluated by Cobas® and Leukotriene- B4 by three generation ELISA system.

The results shows the high prevalence bacteria was *Streptococcus pyogenes* with 50.7%, and 26.7% of patients have a double infection of both, while *Staphylococcus aureus* were 22.6%. The results also showed that the highest incidence in the first age group and the lowest in the fourth age group, and in male higher than female. The level of IgE decreased in the patients infected with *S. pyogenes*, the results also show there are non-significant difference between them at p. value (≤ 0.05), while the Leukotriene- B4 level was not indicated when compared to the control group, only in the first age group there are significant differences at ($p \leq 0.05$).

Keywords: Leukotriene-B4, IgE, Tonsillitis, Pathogenic Bacteria

INTRODUCTION

Tonsils are important components of the immune system and their infections are very frequent, they are immunologically more active in the first years of life. During aging, whereas lymphoid tissue regresses, sub epithelial tissue changes into fibrotic tissue and crypts alter into cavities filled with keratin. In case of infection, bacteria that inhabit the crypts spread into the tonsil and leave their toxins and other products in it, eventually leading to polymorphonuclear leukocyte infiltration, swelling, necrosis and surface ulceration in tonsils. Consequently, after acute infection, bacteria may inoculate into the core [1]. Tonsillitis is inflammation of the pharyngeal tonsils, the inflammation usually extends to the adenoid and the lingual tonsils, therefore, the term pharyngitis may also be used. Most cases of bacterial tonsillitis are caused by group A beta-hemolytic *Streptococcus pyogenes*. In the first century AD. Bacterial population is observed in most cases of chronic tonsillitis, with alpha- and beta-hemolytic streptococcal species, *H. influenza*, *S. aureus*, and Bacteroides species having been recognized. [2]. bacterial colonization of the palatine tonsil, a specialized lymphoid tissue that contains a number of cell types with innate immune defense roles, including epithelial cells,

neutrophils, macrophages and dendritic cells. In response to group A *Strep. pyogenes* infection, these cell types secrete a number of inflammatory mediators including cytokines, chemokines, antimicrobial peptides (AMPs) and prostaglandins, as part of a highly coordinated host process. These mediators orchestrate the ensuing inflammatory response to group A. *Strep. pyogenes*, and also instruct the resulting adaptive immune response. [3,4].

MATERIALS & METHODS

Tonsil swabs samples were collected from 75 patients of both sex of different ages who suffered from chronic tonsillitis and who were diagnosed by a physician examination. Sterile swab stick is used, swab the posterior pharynx and tonsillar arches. All data forms and laboratory request forms were clearly labeled with each patient's name and identification number. Samples were properly labeled at the collection point and immediately transported to the investigated laboratory for microscopy, with the use selective and differential media and biochemical tests, diagnostic methods have also been used as API 20 and VITEK2 compact system. Five ml of venous blood samples were collected by from volunteer patients. Four ml of Blood has been

allowed to clot at room temperature for 30 minutes, then it was centrifuged for 5 minutes at 4000 RPM, serum was collected and distributed into man eppendorf tubes and give the same number in the form ,samples were frozen at -20 C° until used.. The serum was frozen for estimation the immunological parameters included (Immunoglobulin Epsilon (IgE), and Leukotriene B4 (LUE -B4)). IgE levels were evaluated by Cobas, Roche system / English, and LUE -B4 by three generation ELISA Creative Diagnostics / USA. Patients took care and medication at AL- Habouobi Teaching Hospital in Thi-Qar province, South Iraq from February to July 2019.

STATISTICAL ANALYSIS

Statistical analysis of the data was performed using the Statistical Package for Social Sciences (SPSS) for Windows 10 (SPSS Inc., Chicago, IL) (t-test, and ANOVA tests). Values of $p \leq 0.05$ were considered to be significant.

RESULTS

The results shows the high prevalence bacteria was *Streptococcus pyogen* with 50.7%, and 26.7% of patients have a double infection, while *Staphylococcus aureus* were 22.6%. The results also showed that the highest incidence in the first age group and the lowest is in the fourth age group, and in male higher than female. The results noticed that the level of IgE increased significantly when compared to the control group, while Leukotriene B4 level was not indicated when compared to the control group at P . value ≤ 0.05

Distribution of *Staphylococcus aureus* and *Streptococcus pyogen* in Chronic Tonsillitis by using culture Media

The results shows distribution of infection according to the type of pathogen. The high percentage of patients infected with *Streptococcus pyogen* was 38(50.7%), and 20 (26.7%) of patients have a double infection with both *Streptococcus pyogen* and *Staphylococcus aureus*, and low infection with *Staphylococcus aureus* was 17 (22.6%) as listed in the Figure(1).

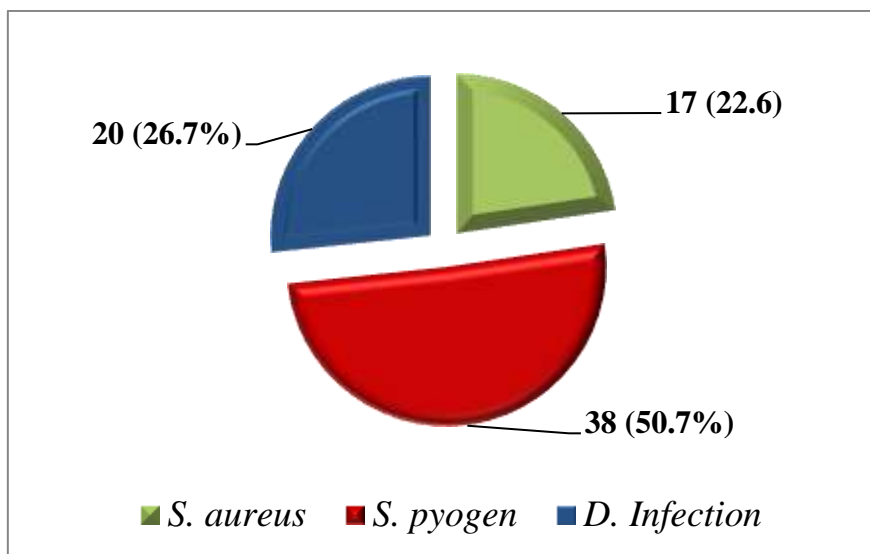


Fig.1: Distribution of *Staphylococcus aureus* and *Streptococcus pyogen* in Chronic Tonsillitis by using culture Media

Distribution of the infected patients with *Staphylococcus aureus* and *Streptococcus pyogen* According to Age Group

The results showed the higher infection was in first age group infected with *Streptococcus pyogen* was 15(20%), *Staphylococcus aureus* was 7(9.3%), and double infection was 8(10.7%). The results also showed that the highest incidence in

the first age group and the lowest is in the fourth age group, the lowest infection in the fourth age group infected with *Streptococcus pyogen* was 4(5.3%), *Staphylococcus aureus* was 0(0%),and double infection of both bacteria was 2(52.3%). The results showed there are non-significant statistical difference according to age groups at p . value ($P \leq 0.05$). as listed in the Table(1).

Table 1: Distribution of *Staphylococcus aureus* and *Streptococcus pyogen* in Chronic Tonsillitis According to Age Group.

Pathogen Groups	Staph aureus		Strep pyogen		Double Infection		Total	
	No.	%	No.	%	No.	%	No.	%
Less than 16 years	7	9.30	15	20.0	8	10.7	30	40.0
16 – 30 years	5	6.70	12	16.0	6	8.00	23	30.7
31 – 45 years	5	6.70	7	9.30	4	5.30	16	21.3
More than 45 years	0	0.00	4	5.30	2	2.7	6	8.00
Total	17	22.7	38	50.6	20	26.7	75	100%
Calx ² = 2.48		Tabx ² = 12.59		Df = 6		P. Value = (0.871)		

Note: G1= Less than 16 years, G2= 16 – 30 years, G3= 31 – 45 years, and G4= More than 45 years

Distribution of the Infected Patients with Staphylococcus aureus and Streptococcus pyogen According to Gender

The results showed the patients that infected with Staphylococcus aureus according to gender highest in the males 9 (12%) compare with infection in the females 8 (10.7%). The infection

with Streptococcus pyogen, in males 26 (34.7%), while the infection in females 12 (16%), and the last 11 (14.6%) males, and 9 (12%) females, have double infection.

The results also show there are non-significant statistical difference according to gender at p. value (P ≤ 0.05) as listed in the table (2).

Table 2: Distribution of the Infected Patients According to gender

Pathogen Groups	Staph aureus		Strep pyogen		Double Infection		Total	
	No.	%	No.	%	No.	%	No.	%
Males	9	12.0	26	34.7	11	14.6	46	61.3
Females	8	10.7	12	16.0	9	12.0	29	38.7
Total	17	22.7	38	50.7	20	26.6	75	100%
Calx ² = 1.64		Tabx ² = 5.99		Df = 2		P. Value = (0.43)		

Immunoglobulin Epsilon (IgE)

The result show the highest level of IgE is in the fourth age group with level 394.4 ± 67.2, also there is increase in the another age groups.

These results also shows that there are significant differences at (p ≤ 0.05) between IgE levels in the patient compared to their level in the corresponding age of healthy control in all age groups. as listed in the table (3).

Table 3: Level of IgE for both tonsillitis patients and control

Groups	Parameter	No.	IgE mg/dl Mean ± SD	P-Value
	G1	Control	9	
Patient		30	213.3 ± 176.73	
G2	Control	8	20.01 ± 8.69	0.002
	Patient	23	281.2 ± 56.8	
G3	Control	5	23.59 ± 8.61	0.001
	Patient	16	341.9 ± 96.6	
G4	Control	3	30.60 ± 6.3	0.018
	Patient	6	394.4 ± 67.2	

Level of IgE in Patients Infected with S. aureus according to age Groups

The current study show the level of IgE increased significantly in the second age group, while the

low level in the first age group, the result also show there are significant difference at p. value (≤ 0.05). as listed in the table (4).

Table 4: Level of IgE in Patients Infected with S. aureus

Level of IgE	No. of Cases	IgE (mg/dl)	LSD
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Patients infect with <i>S. aureus</i>		Mean + SD	
1 - 15 years	7	138 ± 19.0	P. value = 0.007
16 - 30years	5	202 ± 90.5	
31 - 45 years	5	159 ± 71.5	

Level of IgE in Patients Infected with *S. aureus* according to Age Groups

The current study show the level of IgE increased in the second group, while the low level in the first

age group, the result also show there are significant difference at p. value (≤ 0.05). as in table (5).

Table 5: Level of IgE in Patients Infected with *Stre. pyogen*

Level of IgE Patients infect with <i>S. pyogen</i>		No. of Cases	Mg/dl Mean + SD	LSD
1 - 15 years		15	159 ± 75.6	P. value = 0.026
16 - 30years		12	242 ± 70.0	
31 - 45 years		7	171 ± 64.6	
> 45 years		4	220 ± 90.8	

Levels of IgE according to type of bacterial Infection

The result of the current study show the level of IgE non- increased in patients infected with *S.*

aureus, while the low level in the patients infected with *S. pyogen*, the results also show there are non-significant difference at p. value (≤ 0.05) as in table (6)

Table 6: Levels of IgE according to type of bacterial Infection

Level of IgE Patients infect with <i>bacteria</i>		No. of Cases	Mg/dl Mean + SD	LSD
<i>S. aureus</i>		17	319 ± 101.9	P. value = 0.612
<i>S. pyogen</i>		38	260 ± 90.8	
D. Infection		20	275 ± 93.4	

Leukotriene B4 (LEU-B4)

These results showed there are significant differences at ($p \leq 0.05$) between LEU- B4 level in the first age group only, as in the table (7).

Table 7: Level of LEU-B4 for both tonsillitis patients and control.

Parameter Groups		No.	LEU B--4 IU/L Mean ± SD	P-Value
G1	Control	9	0.13 ± 0.04	0.001
	Patient	30	0.20 ± 0.04	
G2	Control	8	0.21 ± 0.01	0.743
	Patient	23	0.22 ± 0.05	
G3	Control	5	0.21 ± 0.38	0.232
	Patient	16	0.17 ± 0.07	
G4	Control	3	0.21 ± 0.02	0.203
	Patient	6	0.16 ± 0.05	

Legend Tables 1.

Level of LEU-B4 at Different Levels of IgE

The results showed that, there are significant differences at ($p \leq 0.05$) LEU -B4 levels in

patients and control at different levels of IgE. as listed in the table (8)

Table 8: Level of LEU-B4 at Different Levels of IgE.

Level of IgE	No. of Cases	LEU-4 (IU/L) (M±SE)
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Below 151	21	0.20 ± 0.07
151-300	32	0.19 ± 0.06
Above 300	22	0.20 ± 0.05
Control	25	0.18 ± 0.05
LSD	100	1.52
		p. value= 0.42

The results of the current study showed that the highest level of IgE in patients infected with *S. aureus* bacteria. While the lowest level in the patients infected with *S. pyogen*.

The results also showed that non-significant statistical difference between the levels of IgE according to type of bacterial infection at P. value ≤ 0.05.

The results of the current study showed that there were the highest level of the LU-B4 in the patients infected with *S. aureus* compare with levels of patients infected with *S. pyogen* or patients infected with both bacteria.

The results also showed that there were non-significant statistical difference between the levels of LU-B4 according to type of bacterial infection at P. value ≤ 0.05. as listed in the table (9)

Table 9: Levels of IgE and LU-B4 according to type of bacterial infection.

Parameters	No. of Cases	IgE mg/dl Mean + SD	LU-B4 IU/L Mean +SD
Patients infect with <i>S. aureus</i>			
<i>S. aureus</i>	17	319 ± 101.9	0.02 ± 0.07
<i>S. pyogen</i>	38	260 ± 90.8	0.19 ± 0.03
D. Infection	20	275 ± 93.4	0.19 ± 0.04
P. value		0.36	0.41
LSD		Non-Significant	Non-Significant

DISCUSSION

The present study gave an approximate results with previous study obtained by AL- Khafaji [5], in Thi-Qar province, he examined 73 patient with chronic tonsillitis and found 48.72% patient infect with *Streptococcus pyogen*, and 12.83% infect with *Staphylococcus aureus*, the current study also gave an approximate results with study obtained by Brook [6], in Washington, he found the most patient with chronic tonsillitis infected with *Streptococcus pyogen* and *Staph. aureus*, and the lowest percentage of pathogenic bacteria was isolated were *Moraxella catarrhalis* and *Klebsiella pneumonia*, Cavalcanti, et al [7], in Brazil, they examined 123 patients with chronic tonsillitis and found the proportion of infection caused by *Staphylococcus aureus* 40.7%, while they did not isolate *Streptococcus pyogen* from patients, and Alasmar, et al [2], they found the most bacteria isolate from patients with chronic tonsillitis is group A streptococci and no infected were reported by *Staphylococcus aureus*. The current study also show 26.7% of patients infected with both bacteria, this also find by Miramontes et al [8], in Benin City, they found 25.6% of patients have a double and mix infection. The reason between the current and previous studies may be due to the different location of the study as well as because of the patient's immune status, and the

emergence of this bacterium [9]. Group A beta-hemolytic *Streptococcus* (GABHS) is the most important cause of sore throat in children for obvious reasons, it is a causative organism of pharyngitis. [10].

According to gender the current study record the prevalence of chronic tonsillitis in the males is higher than females, Our study record 61.3% from chronic tonsillitis patients were males among them (34.7% infected with *S pyogen*, 12.0% *S aureus*, and 14.6% double infection), while in the females were 38.7% among them (16.0% infected with *S pyogen*, 10.7% *S aureus*, and 12.0% double infection), the current study give similar observations were reported by [11], in Cairo Egypt, they identify the prevalent bacterial pathogens reported among 100 patients were 58% males and 42% females, Our current study yielded slightly higher results than resulted obtained by [7] they study isolation of *Staphylococcus aureus* from recurrent tonsillitis and reported among 123 patient with chronic tonsillitis 53.7 males and 46.3 females, Probably because some patients admitted were more than female patients, as for as socio-economic condition concerned, up- per cases reported for the low-income group is perhaps due to their poverty, poor nourishment, unhygienic condition, illiteracy and improper medical care [12].

The study immune response and allergy with chronic tonsillitis, and record the patients with recurrent tonsillitis develop into allergies and this leads to the production of IgE [13], study the hypersensitivity in chronic tonsillitis patients, and record the patients with recurrent tonsillitis becomes less resistant to various allergen and improved antibody responses to antigens reflect deviations in the mucosa such as altered microorganisms and / or increased gut permeability, which are later seen as allergen [14]. Yadav et al [15] was showed mean serum IgE concentration significantly higher in children with chronic tonsillitis. Miramontes and colleagues in 2014 demonstrated a correlation between IgA and IgE values differed between genders also, which suggests a greater exposure to allergens in boys than girls.

human tonsil cells secrete TGF- β 1 following exposure to GAS, which, in concert with more than one interleukin secreted by GAS-infected epithelial cells and macrophages, drives CD4+ T helper 17 (Th17) cell differentiation [16,17].

The result of current study show non-significant difference in the levels of LEU- B4 at different levels of IgE.

Epithelial cells also secrete neutrophil chemoattractants such as IL-8 in response to Bacterial infection, though IL- 8 play role in the cytokine network its major pathophysiological role lies in affecting in mechanisms expression and effects on leukocytes [18]. Leukotriene B4 is a leukotriene involved in inflammation. It is produced from leukocytes in response to inflammatory mediators and is able to induce the adhesion and activation of leukocytes on the endothelium, allowing them to bind to and cross it into the tissue [19].

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