

# Longitudinal Analysis of Oral Health, Dental Plaque Microfilm and Salivary (IL-1 $\beta$ and TNF-A) after Tonsillectomy Among Iraqi Adolescents

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## Abstract:

**Background:** In general, the maturation of oral immune status of the upper aero-digestive tract are prominently depending on regional lymphoid tissues for both laryngeal and pharyngeal tonsils. Tonsillectomy would result in changes of the local immune defenses and causes both dental caries and periodontal diseases like gingivitis.

**Objective:** To investigate the impact of the tonsillectomy on oral health, microbial structure dental plaque and salivary levels of both IL-1 $\beta$  and TNF - $\alpha$ . In addition, to detect relationship between cariogenic bacteria and dental caries grades among adolescents aged 14-16 years.

**Patients and Methods:** The sample of thirty-five adolescents with Chronic Tonsillitis presenting for tonsillectomy were recruited. Caries experience was recorded according to Mühlemann (D1-4 MFD) Index. The determination of Chronic Tonsillitis performed through a clinical and histopathological examination after tonsillectomy. Dental microbial flora was cultured for semi-quantitative estimation of growth of aerobic and facultative organisms (preoperatively and at one month postoperatively).

**Results:** Finding of this study revealed decreasing of quantity of *Strep. mutans*, *Lactobacillus spp*, *Strept. salivarius*, *P. gingivalis* and *Actinomyces* a after tonsillectomy. Moreover, direct relations between (D4 and DMFS) and *Strep. mutans* and *Strep. mitis* was significant pre-operatively. Similarly, direct relations between *P. gingivalis* and both Salivary IL-1 $\beta$  TNF- $\alpha$  and gingivitis postoperatively.

**Conclusion:** The dental plaque of microbial structure act as reservoir for Chronic Tonsillitis among adolescents aged 14-16 years and it seems to be an association between declining in the account of cariogenic bacteria and tonsillectomy among adolescents aged 14-16 years old. And there is feedback between Chronic Tonsillitis and dental caries.

**Keywords:** Dental Caries, Adolescents, Chronic Tonsillitis, Oral Microflora, Dental Plaque

## Introduction:

Dental caries is a post-eruptive multifactorial disease results from interaction of three main group factors that must exist simultaneously including host (tooth structure), microorganisms (*Strep. mutans*, *Lactobacillus*, *Strep. mitis*, *Strept. salivarius* and others), and environment factors (e.g., diet, saliva composition and flow rate), for a period of time (1,2). Moreover, salivary antibacterial properties play a role in preventing bacteria colonization on tooth surfaces and gingiva which may affect the oral health condition such as dental caries and gingivitis (3-6).

Many researchers observed a correlation between salivary interleukin-1 $\beta$ , IL-6, and tumor necrosis factor- $\alpha$  as immunologic mediators and gingival health condition (7-9). Tonsils support oral cavity with immunoglobulins to a great degree, that play an important role in immune defense mechanism of the upper aero-digestive tract. Moreover, it can act as an essential role in suppression of cariogenic micro-flora, to act as caries prophylaxis or vice versa. Tonsillectomy is recommended for person to reduce the frequent episodes (7 or more time) of acute Tonsillitis documented clinically episodes in each of the 2 preceding years (10-14). It is still unknown about the direct connection by bacterial action, if dental caries come from Tonsillitis, or the reverse.

The present study represents the pioneering aspect. especially when no previous research in Iraq and Middle east carried out to assess the effect of tonsillectomy on the oral health, microflora, and

level of salivary IL-1 $\beta$  and TNF- $\alpha$  among adolescents.

This study aimed to investigate the impact of the tonsillectomy on oral health, microbial structure dental plaque and salivary levels of both IL-1 $\beta$  and TNF - $\alpha$ . In addition, to detect relationship between cariogenic bacteria and dental caries grades among Iraqi adolescents aged 14-16 years.

## Patients and methods:

This study was carried out among thirty-five (23 males and 12 females) Adolescents with chronic tonsillitis aged 14-16 years, they were randomly selected from 83 voluntary adolescents in Baghdad medical city Hospital ENT department / Iraq (15). The Chronic Tonsillitis diagnosis carried out through clinical examination done by ENT specialists were represented to whom with frequent episodes of Tonsillitis for 7 or more time of acute Tonsillitis that documented at least two preceding years. The sample of thirty-five Adolescents with Chronic Tonsillitis were indicated for Tonsillectomy. Finally, surgical removed of tonsils were sent for histopathological diagnosis through which tonsils were divided up in half and then fixed in 10% buffered neutral formalin, later after dehydration embedded in a paraffin block followed by sectioning and trimming. Lastly, they were stained by using Eosin stain and Hematoxylin (11).

Before tonsillectomy, the whole permanent teeth were implemented according to the basic method proposed through WHO (1997) (16). Caries experience was measured through the use of (D1-

4MFS) Index described by Mühlemann (1976) (17) by using the dental mirror and dental explorer. Gingival index was used for measuring gingival health condition (18).

Total of 35 adolescents (preoperatively and at 4 weeks postoperatively) underwent microbiological investigations and sample of dental plaque were taken from the first permanent molars' vestibular and frontal surfaces of lower jaw. Then it placed directly in transport medium "Amies transport medium to investigate. Dental plaque was collected 7 days prior to Tonsillectomy to make sure that the patient was not taking antibiotics that may interfere with results of microbiological investigations. For identification of aerobic microorganisms for dental plaque specimens were cultured on Blood agar, Chocolate agar, MacConkey agar, and Mannitol salt agar, Trypticase soy agar (supplemented with 10% serum and 75  $\mu$ g of bacitracin per ml), then plates were incubated under aerobic conditions at 37 C for 24-48 hours (19). Furthermore, Mitis salivaris bacitracin selective media agar (MSB agar) was used for isolation of *Strept. mutans* bacteria confirmed the diagnosis of bacterial species by biochemical test (20). A standard bacteria counting method according to Miller, 1972 (21).

The whole unstimulated salivary samples collections from the volunteer were performed under standardized conditions and if the samples containing blood it should be discarded. Before collection of saliva sample. The volunteer should: sit in a relaxed position on an ordinary chair, not eat and not drink except water one hour, not smoke and undergo heavy physical stress (3,22). Three milliliter of saliva sample were collected between 9-12 a.m. into a sterile plastic tube at two different time preoperatively (one week before tonsillectomy) and postoperatively (one month after tonsillectomy), then these sample were centrifuged at 3000 rpm for 10 minutes, the supernatants layer were collected and stored in a deep freeze (at -20 $^{\circ}$ c) until processed (23). Following the manufactures' instructions, ELISA kits were used to estimate the levels of both salivary IL-1 $\beta$  and TNF-  $\alpha$  at two different times preoperatively and postoperatively.

Statistical analyses were performed through using SPSS package version 16. Student's t-test were applied for comparisons between the pre-operative and post-operative time. (P < 0.05) was considered statistically significant.

## Results

Table 1 illustrates the mean dental caries experience (DMFS), components (DS, MS and FS)

and grades at Pre-operative time among adolescents aged 14-16 years. The table shows that the decayed fraction D3 contribute the higher component than other MS and FS. However, concerning the severity of dental caries, this table demonstrated that the sever grade of dental caries represented by D4 was found to be higher than lower grade.

Table 2 illustrates the mean value of dental plaque biofilm of bacteria count, at Pre-operative and Post-operative times among adolescents aged 14-16 years. Concerning bacteriological analysis of dental plaque, data reported that the mean values of *Strep. pyogenes* and *Lactobacillus spp* at postoperative time were highly significantly higher than at preoperative time with statistically highly significant difference (P< 0.01) and significant difference (P< 0.05) for *Strep. mutans* and *Strept. salivarius*. While it was not significant among *Strep. mitis* (P> 0.05).

Table 3 illustrates the mean value of IL-1 $\beta$ , TNF- $\alpha$  and gingivitis Pre-operative and Post-operative times among adolescents aged 14-16 years. Data reported that the mean values of IL-1 $\beta$  and TNF- $\alpha$  postoperatively were highly significantly higher than preoperatively with statistically highly significant difference (P< 0.01). Concerning the gingivitis, data reported that the mean values of gingival health condition postoperatively was highly significantly lower than preoperatively with statistically highly significant difference (P< 0.01).

The correlation coefficient between dental caries experience and grades of decay fraction and cariogenic bacteria Pre-operatively are illustrated in Table 4. Results showed that the relations between (both D4 and DMFS) and bacteria (*Strep. mutans* and *Strep. mitis*) were significant in positive direction. Similar finding was reported between Missing and *Strep. mutans*, *Lactobacillus spp* and *Strep. mitis*, while it was not among other grades of decay fraction and cariogenic bacteria.

The correlation coefficient between microflora bacteria & gingival health condition, Salivary IL-1 $\beta$  and TNF- $\alpha$  at Pre-operative and Post-operative among adolescents aged 14-16 years are illustrated in Table 5. Pre-operatively, results showed no relation between microflora and Salivary IL-1 $\beta$ , TNF- $\alpha$  and gingivitis. Post-operatively, results showed a positive relation between *P. gingivalis* and both Salivary IL-1 $\beta$ , TNF- $\alpha$  and gingivitis were highly significant and significant respectively. Also results showed a positive relation between both *Actinomyces A* & *Strep. Sangius* and Salivary TNF- $\alpha$  were highly significant. while it was not for salivary IL-1 $\beta$  and gingivitis.

**Table 1:** The dental caries experience (DMFS), components (DS, MS and FS) and grades at Pre-operative time among patient.

	Mean	SE
D1	0.54	$\pm$ 0.16
D2	2.11	$\pm$ 0.18

D3	2.54	± 0.24
D4	7.6	± 0.29
Decayed	12.8	± 0.64
Missing	0.2	± 0.08
Filling	0.34	± 0.1
DMFS	13.34	± 0.67

**Table 2:** The dental plaque biofilm bacteria count at pre-operative and post-operative times among patients.

	Pre-operative time			Post-operative time			Group time difference (t-test)		
	No.	Mean	±SE	No.	Mean	±SE	t-value	df	P value
<i>Strep. mutans</i>	35	291.34 X 10 <sup>2</sup>	± 1.49 X 10 <sup>2</sup>	35	285.8 X 10 <sup>2</sup>	± 1.87 X 10 <sup>2</sup>	2.322	68	0.02*
<i>Strep. pyogenes</i>	35	303.57 X 10 <sup>3</sup>	± 1.52 X 10 <sup>3</sup>	35	215.31 X 10 <sup>3</sup>	± 1.28 X 10 <sup>3</sup>	44.3	68	0.000**
<i>Lactobacillus spp.</i>	35	163.51 X 10 <sup>1</sup>	± 1.46 X 10 <sup>1</sup>	35	153.14 X 10 <sup>1</sup>	± 2.05 X 10 <sup>1</sup>	4.13	68	0.000**
<i>Strep. mitis</i>	35	158.63 X 10 <sup>4</sup>	± 2.32 X 10 <sup>4</sup>	35	151.34 X 10 <sup>4</sup>	± 2.08 X 10 <sup>4</sup>	2.34	68	0.22
<i>Strep. salivarius</i>	35	256.23 X 10 <sup>4</sup>	± 0.76 X 10 <sup>4</sup>	35	250.26 X 10 <sup>4</sup>	± 2.86 X 10 <sup>4</sup>	2.02	68	0.04*
<i>P. Gingivalis</i>	35	126.57 X 10 <sup>2</sup>	± 1.30 X 10 <sup>2</sup>	35	57.66 X 10 <sup>2</sup>	± 2.06 X 10 <sup>2</sup>	28.32	68	0.000**
<i>Actinomyces A</i>	35	42.91 X 10 <sup>3</sup>	± 1.93 X 10 <sup>3</sup>	35	35.77 X 10 <sup>3</sup>	± 1.45 X 10 <sup>3</sup>	2.96	68	0.004**
<i>Strep. Sangius</i>	35	140.49 X 10 <sup>4</sup>	± 1.42 X 10 <sup>4</sup>	35	137.26 X 10 <sup>4</sup>	± 2.12 X 10 <sup>4</sup>	1.27	68	0.21

\* (Significant)= P<0.05 (between group times) \*\* (Highly significant) = P<0.01 (between group times)

**Table 3:** The salivary IL-1β, TNF-α and gingivitis at pre-operative and post-operative times among patients.

	Pre-operative time			Post-operative time			Group times difference (t-test)		
	No.	Mean	±SE	No.	Mean	±SE	t-value	df	P value
IL-1β (pg/ml)	35	157.35	± 0.97	35	168.34	± 0.98	-7.99	68	0.000**
TNF-α (pg/ml)	35	3.19	± 0.01	35	3.91	± 0.01	-11.9	68	0.000**
Gingivitis	35	0.31	± 0.07	35	0.04	± 0.02	3.81	68	0.00**

\*\* (Highly significant) = P value<0.01 (between group times)

**Table 4:** The correlation coefficient (r) between cariogenic bacteria & dental caries experience (DMFS), components (DS, MS and FS) and grades Pre-operative among patients

	<i>Strep. mutans</i>		<i>Lactobacillus spp.</i>		<i>Strep. mitis</i>		<i>Strep. salivarius</i>	
	r	P value	r	P value	R	P value	r	P value
D1	-0.02	0.91	0.07	0.69	0.03	0.85	-0.32	0.07
D2	-0.06	0.74	-0.22	0.20	0.11	0.52	-0.35	0.04
D3	0.05	0.77	-0.07	0.70	0.12	0.5	-0.29	0.10
D4	0.81	0.000**	0.27	0.12	0.71	0.00**	0.19	0.28
Decayed	0.37	0.031*	0.05	0.78	0.41	0.016	-0.19	0.26
Missing	0.51	0.002**	0.38	0.025*	0.45	0.006*	-0.08	0.66
Filling	0.14	0.44	0.17	0.32	0.18	0.30	-0.04	0.81
DMFS	0.43	0.01*	0.12	0.49	0.47	0.005*	-0.20	0.24

\* (Significant)= P value<0.05 \*\* (Highly significant)= P value<0.01

**Table 5:** The correlation coefficient (r) between microflora bacteria & gingival health condition among, Salivary IL-1β and TNF-α at Pre-operative and Post-operative times among patients.

	Bacteria Type	Salivary IL-1β		Salivary TNF-α		Gingivitis	
		r	P value	r	P value	r	P value
Pre-operative time	<i>Actinomyces A.</i>	0.04	0.83	0.21	0.23	0.21	0.23
	<i>P. Gingivalis</i>	0.20	0.25	-0.26	0.13	-0.09	0.63
	<i>Strep. Sangius</i>	0.11	0.51	-0.05	0.76	0.01	0.97
Post-operative Time	<i>Actinomyces A.</i>	0.2	0.25	0.48	0.00**	0.09	0.63
	<i>P. Gingivalis</i>	0.51	0.002**	0.84	0.00**	0.44	0.01*
	<i>Strep. Sangius</i>	0.11	0.55	0.45	0.00**	0.06	0.73

\*(Significant)= P<0.05 \*\* (Highly significant) = P<0.01

**Discussion:**

Frequently attack (more than five per year) of Tonsillitis for two continuous years was the commonest indication for Tonsillectomy in this study) (10). It is well documented that Tonsillitis and oral health can be represented as most important part of the general body health. Microbiologically, studies observed that *Strep. pyogenes* is a  $\beta$ -haemolytic bacterial pathogens play a role in pathogenesis of pharyngotonsillitis. And others represented that *Strep. mutans*, *Lactobacillus (LB)*, *Strep. mitis* and *Strep. salivarius* acts as cariogenic bacteria (2,24-26). Anatomically, tonsil and lower molar are in close relation to microbiological picture of Tonsillitis, dental caries and periodontal disease. In general, caries represent the most common oral diseases and it may act as reservoir for Chronic Tonsillitis. On the other hand, tonsils enlargement may result in mouth breathing and oral dryness that may have a harmful effect on dental caries, and explain a positive relationship between dental caries and Chronic Tonsillitis (27-29). This can explain the finding of present study which reported a higher mean value of *Strep. mutans*, *Lactobacillus*, *Strep. mitis* and *Strep. salivarius* at preoperative time in compare to postoperative time. However, it is difficult to compare present finding with other studies this may be due to differences in the criteria of sample selection and size in addition to the difference in definition Chronic Tonsillitis adopted in studies.

In general, both the IL-1 $\beta$  and TNF- $\alpha$  induce vascular dilatation which increased the permeability and enhancing inflammatory response(8). Tonsillectomy resulted in a short-term decrease in total levels of salivary IL-1 $\beta$  and TNF- $\alpha$ . While it observed controversial finding in a long-term. Results of present study observed a gradual decrease in the level of salivary IL-1 $\beta$  and TNF- $\alpha$  after tonsillectomy, this finding is in agreement with other studies(7,30-31). While disagree with other(32-34) that represented no significant change, this may be due to difference in the design of study and selection criteria of patient that involve: sample age group, size and type of salivary sample.

The primary role of plaque *Strep. mutans*, *Lactobacilli* *Strep. mitis* and *Strep. salivarius* account, determined is to predict the future incidence of dental caries. These bacteria has been detected in high number in both superficial and deep caries and play an important role in the development and progression of dental caries (35-36). Furthermore, data of present study observed a highly positive correlation between cariogenic bacteria of both *Strep. mutans* and *Strep. mitis* and D4, DMFS. The findings are in agreement with Fukuizumi and his coworkers study (37), while disagree with other study (38).

Tonsils with cooperation of salivary glands providing oral cavity with immunoglobulins, tonsils consequently play an essential role in suppression of dental plaque microflora, in that way providing oral

diseases prophylaxis or vice versa (12). Many researchers observed that the gingival index increases with age (9,39).With advancing age, *P. Gingivalis* reach to about 60 % of dental plaque structure and it is closely associated with the onset and severity of gingivitis among group of patients aged from 2 to 18 years (40). This may explain results of present study that observed a positive relation between *P. Gingivalis* and gingivitis.

Clinical results of this study agreed with concept that the dental plaque of microbial structure act as reservoir for Chronic Tonsillitis among adolescents aged 14-16 years and it seems to be an association between declining in the account of cariogenic bacteria and tonsillectomy among them. In addition there is feedback between Chronic Tonsillitis and dental caries. No such relevant study has been carried out on local oral flora for person so there is a need to evaluated both short and long term impact of tonsillectomy on oral health, microflora, and salivary immune system among adolescents.

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