

Original Research Article

Changes of Salivary Concentration of IL-6 and TNF-A in Early Childhood Caries

Sarah Jamal Kareem^{1*}

¹Oral Microbiology, Iraqi Ministry of Health, Iraq

***Corresponding Author:** Sarah Jamal Kareem
Oral Microbiology, Iraqi Ministry of Health, Iraq

Article History

Received: 11.01.2024

Accepted: 19.02.2024

Published: 26.02.2024

Abstract: **Background:** Early childhood caries is a biofilm-dependent, critical, widespread health problem in both developing and industrialized countries. The interleukin-6 (IL-6) has several roles in the body's response to inflammation and tissue damage. TNF- α is a pleiotropic cytokine that acts as a pro-inflammatory cytokine. **Aim of Study:** The purpose of this research was to assess the salivary concentrations of TNF- α and IL-6 in children with dental caries and study the correlation between cytokines and DMFs index. **Materials and Methods:** Eighty children aged four and five took part in the study. Forty children with ECC served as the research group, whereas 40 caries-free children of the same age and gender served as the control group. Clinical parameters were assessed after an oral examination and measurement. All participants had saliva samples obtained. IL-6 and TNF- α concentrations in saliva were estimated using an ELISA test. **Results:** The current study found a significant elevation ($P < 0.05$) in mean levels of TNF- α and IL-6 among children with ECC in comparison to caries free children. Moreover, there is significant positive correlation between IL-6 and TNF- α levels in caries children. **Conclusion:** Based on these results, it seems likely that elevated levels of TNF- α and IL-6 are involved in ECC.

Keywords: Early childhood caries, cytokines, TNF- α , IL-6.

INTRODUCTION

The condition and the ensuing lesion are both known by the same name: dental caries. Lesion in the dental hard tissues results from the caries process, which takes place in the biofilm and is always active with every pH variation. When a person consumes a lot of sugar, the biofilm bacteria that typically dwell in the oral cavity disrupt homeostasis and transform into an acidogenic, aciduric, and cariogenic population, causing dental caries. Depending on how much mineral is lost inside the tooth's hard structures, the ensuing carious lesion may be undetectable by a clinician or clearly evident, as with diseases, genetics has an important effect on the etiology of dental caries [1-4]. Early childhood caries (ECC) is a biofilm-dependent, critical, widespread health problem in both developing and industrialized countries, usually targets children from poor socio-economic families and racial/ ethnic minority backgrounds, this type of dental caries doesn't vary from the usual carious process, but; mainly associated with incorrect feeding practice so called bottle feeding or nursing caries [5-7]. Child's attitude, social experience and general health might be affected if this health problem is left untreated, but; early diagnosis prevents the disease progression [8]. Caries is only one of numerous illnesses of the oral cavity that may be diagnosed and monitored with the use of cytokines due to their role in influencing the immune and inflammatory response [9, 10].

Interleukin-4 (IL-4), interleukin-6 (IL-6), interleukin-8 (IL-8) and tumor necrosis factor-alpha (TNF-alpha) are only a few of the cytokines being studied for their possible role in the diagnosis of caries. In addition, pro-inflammatory cytokines were shown to be sensitive systemic indicators of tissue damage and predictive of future adverse cardiac events in individuals who otherwise seemed to be in good condition. Periapical lesions and the livers of produced periapical

Copyright © 2024 The Author(s): This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International License (CC BY-NC 4.0) which permits unrestricted use, distribution, and reproduction in any medium for non-commercial use provided the original author and source are credited.

Citation: Sarah Jamal Kareem (2024). Changes of Salivary Concentration of IL-6 and TNF-A in Early Childhood Caries. 14
South Asian Res J Oral Dent Sci, 6(1), 14-18.

abscess rats have been demonstrated to have elevated levels of IL-6 and TNF- α [11]. Tumor necrosis factor alpha and interleukin-6 are important inflammatory mediators.

The cytokine interleukin-6 (IL-6) has several roles in the body's response to inflammation and tissue damage. Higher concentrations are associated with an increased risk of death from any cause, unstable angina, diabetes, and its complications, hypertension, and obesity [10-12]. TNF- α , or tumor necrosis factor alpha, is a cytokine with pleiotropic biological powers that was first identified as a protein with necrotizing effects in some transplantable animal tumors. The development, differentiation, and/or function of practically every cell type studied is affected by TNF- α , and this includes its cytostatic and cytotoxic effects on some tumor cells. More than that, TNF- α is assumed to be a cog in a network of interconnected signals that directs both inflammation and immunity [13-15]. The purpose of this research was to examine the relationship between cytokines and the DMFs index, as well as to measure the amounts of IL-6 and TNF- α in the dental caries-affected children's saliva.

METHODS

Subjects: Eighty children aged four and five took part in the study. Last birthday used to determine age. Forty children diagnosed with ECC according to the criteria established by Wyne served as the study group, whereas forty children found to be caries-free according to the criteria established by WHO served as the control group.

Children with the following conditions were excluded from the study; with unapproved consent, who were taking medication (antibiotic or any medication), who ate during the hour before sample collection, who had flu or any systemic disease and uncooperative child, crying, fearful and anxious.

Sample Size:

Using Franz-Faul's (Kiel University, Germany) G power 3.1.9.7 software. Under these circumstances, and assuming an effect size of 0.6 (medium) between the two groups, the sample size was 80 (40 in each group). The statistical test used was a two-sample T-test with 85% power and a 5% likelihood of error.

Ethical Approval:

All participants were given extensive background on the research and its protocols. They signed an informed consent form that had been reviewed and approved by the University of Baghdad's College of Dentistry's ethics board.

Saliva Collection:

Drooling was used to collect 3 cc of unstimulated saliva, by giving the children simple instructions in a clear way to drool the saliva by tilting the head and putting the plane tube close to the lips to permit the saliva drooling^(16,17). After numbering the plane tube with a special code for each child which is the same that recorded on the child's case sheet, then the samples were transported to an ice box. The whole collected samples were carried to the laboratory immediately for centrifuging to inhibit the hydrolysis of proteins. Each sample was centrifuged for 3000 rpm for 10 minutes. The supernatant was collected in Eppendorf's tubes and stored at - 20°C for further analysis.

Measurement of Salivary TNF- α and IL-6: Salivary levels of two biomarkers (MyBioSource, USA) were determined by ELISA.

Statistical analysis: Statistical significance of differences between groups was determined using the T-test and chi-square test. Correlations between biomarkers and clinical factors were analyzed using the Pearson correlation coefficient test. The cutoff for statistical significance is set at $P < 0.05$.

RESULTS

The current study showed highly significant elevation ($P < 0.01$) in mean salivary levels of IL-6 among children with ECC in comparison to caries free children, as shown in table (1) and figure (1). Moreover, the level of TNF- α in ECC and caries free groups was shown in table (2) and figure (2) which illustrates that the mean level of salivary TNF- α among children with ECC was higher than in the caries free children with statistically significant differences ($P < 0.05$). In addition, there is a positive correlation between IL-6 and TNF- α , as shown in Table (3)

Table 1: The mean values of IL-6 levels in study and control groups

IL-6	Study group, n=40	Control group, n=40	T-test (P-value)
Minimum	10.03	7.47	0.040*
Maximum	44.31	11.68	
Mean	18.38	6.76	
SD	7.50	0.98	

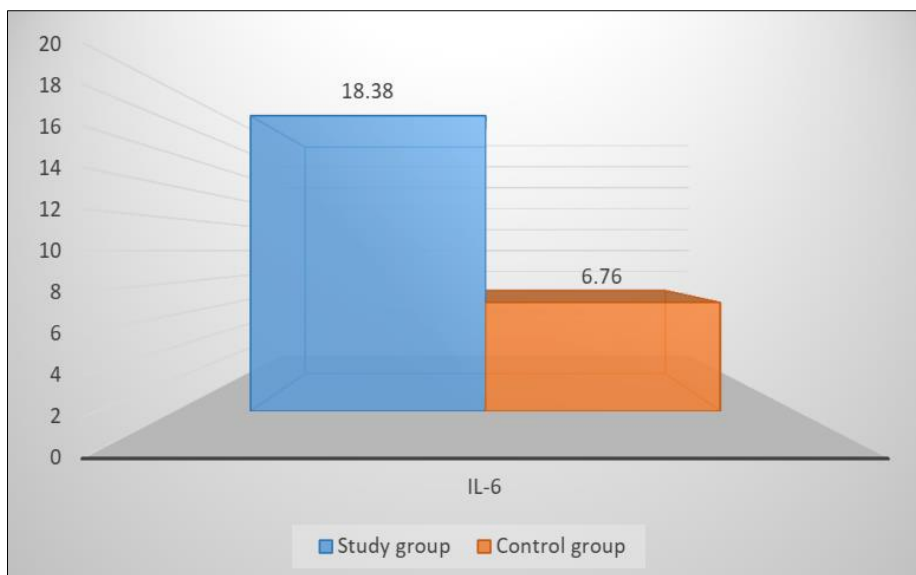


Figure 1: Salivary mean values of IL-6 in study and control groups.

Table 2: The mean values of TNF- α levels in study and control groups

TNF- α	Study group, n=40	Control group, n=40	T-test (P-value)
Minimum	132.29	59.12	0.000*
Maximum	242.51	114.63	
Mean	188.38	81.08	
SD	27.54	15.94	

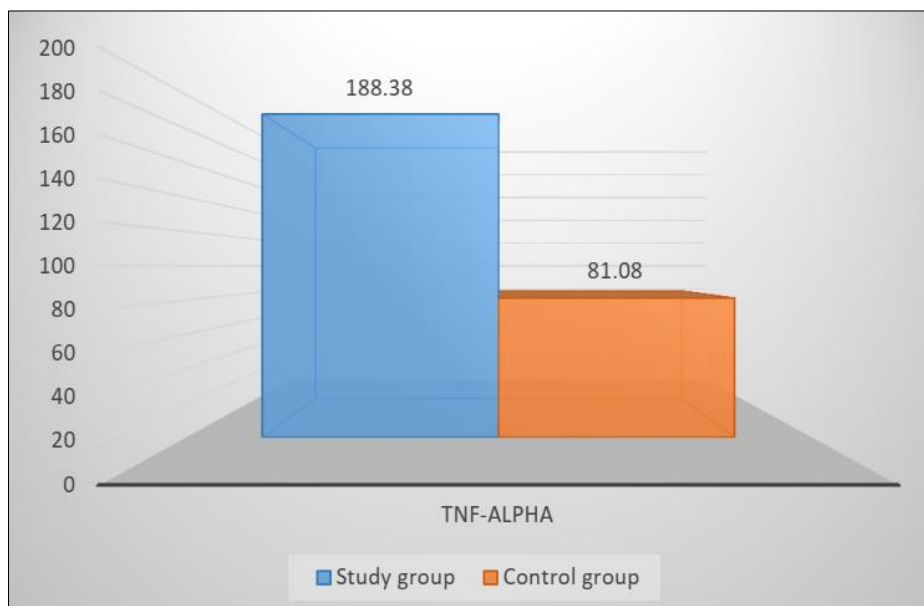


Figure 2: Mean values of TNF- α in study and control groups

Table 3: Correlation between IL-6 and TNF- α in study group

Biomarkers	Salivary IL-6	
TNF- α	R	P
	0.482	0.002

DISCUSSION

Salivary cytokine levels may reveal disease activity, severity, and prognosis, and may alert patients to the need for therapy either now or in the future [18]. It is well accepted that IL-6 and TNF- α are two of the most important cytokines in the generation of specialized immune responses [13-19].

The current findings revealed a significant increase in the salivary levels of TNF- α and IL-6 among ECC children when compared to those who are caries free, this agrees with prior research [20-25]. Who found significant elevation of IL-6 and TNF- α levels in the presence of high caries severity. Similarly, previous studies [26, 27]. In a study analyzing the levels of the cytokines TNF- α and IL-6 in the saliva of patients with dental caries, it was shown that all individuals with dental caries had considerably increased levels of these cytokines, indicating that decay may also trigger the inflammatory process in the immune system via a destructive process. resulting in the release of TNF- α .

Similarly, [26], noted that abnormalities in the oral cavity may lead to an elevated salivary cytokine level and hypothesized that a breakdown in the triangular relationship between the oral mucosa, immune cells, and saliva triggers the development of illnesses of the mucosa. However, as demonstrated by Kurtis and colleagues [28], individuals with dental caries have greater levels of TNF- and IL-6, which promotes demineralization of teeth and the dental caries process by decreasing the number of osteoblasts and fibroblasts. Together with other variables, IL-6 triggers bone resorption and increases chemokine production [29]. Locally generated TNF- α by osteoclasts has been shown to play a role in resorption processes and regulate osteoclast development [30]. In addition, [10], shown that IL-6 levels are inversely proportional to the size and severity of carious lesions, and that these levels considerably decreased after successful treatment of caries and restoration. Children's cytokine levels may rise due to poor plaque management, plaque buildup, and gingival inflammation, as shown by [10]. Based on these results, it seems likely that elevated levels of TNF- α and IL-6 are involved in ECC.

CONCLUSION

The significant elevation of cytokines levels (IL-6 and TNF- α) in ECC may indicate that caries plays an important role in increasing proinflammatory cytokines.

Conflict of Interest: The author has no conflicts of interest to declare.

Informed Consent: Informed consent was obtained from all individuals or their guardians included in this study.

REFERENCES

1. Schwendicke, F., Frencken, J. E., Bjørndal, L., Maltz, M., Manton, D. J., Ricketts, D., ... & Innes, N. P. T. (2016). Managing carious lesions: consensus recommendations on carious tissue removal. *Advances in dental research*, 28(2), 58-67.
2. Duangthip, D., Gao, S. S., Chen, K. J., Lo, E. C. M., & Chu, C. H. (2020). Oral health-related quality of life and caries experience of Hong Kong preschool children. *International dental journal*, 70(2), 100-107.
3. Cogulu, D., & Saglam, C. (2022). Genetic aspects of dental caries. *Frontiers in Dental Medicine*, 3, 1060177. doi: 10.3389/fdmed.2022.1060177
4. Al-Taie, L. H., Al-Ghurabi, B. H., Al-Hassan, A. A., & Dager, A. J. (2012). Frequency of HLA-A and B antigens in Iraqi patients with end-stage renal disease preparing for transplantation. *Iraqi Postgraduate Med J*, 11, 642-648.
5. Çolak, H., Dülgergil, Ç. T., Dalli, M., & Hamidi, M. M. (2013). Early childhood caries update: A review of causes, diagnoses, and treatments. *Journal of natural science, biology, and medicine*, 4(1), 29.
6. Khaudyer, A. T., Mohammed, A. T., & Al-Ghurabi, B. H. (2019). Oral health condition among Kindergarten children in Relation to Salivary soluble Cluster of Differentiation 14 and Tool Like Receptor 2 (comparative study). *Indian Journal of Public Health*, 10(9), 1407.
7. Al-Ali, G. M., Jafar, Z. J., & AL-Ghurabi, B. H. (2021). The relation of salivary cathelicidin and beta-defensin with dental caries of schoolchildren. *J Res Med Dent Sci*, 9(4), 30-5.
8. Al-Ghurabi, B. H. (2021). The Role of Soluble TLR-2 in the Immunopathogenesis of Gingivitis. *International Medical Journal*, 28(1), 37-39.
9. Kareem, S. J., & Al-Ghurabi, B. H. (2023). Regulatory Role of Human Neutrophil Peptides (HNP1-3) on Interleukin-6 Production in Early Childhood Caries. *Journal of Emergency Medicine, Trauma & Acute Care*, 2023(3), 11. <https://doi.org/10.5339/jemtac.2023.midc.11>
10. Fontana, M., Jackson, R., Eckert, G., Swigonski, N., Chin, J., Zandona, A. F., ... & Zero, D. T. (2011). Identification of caries risk factors in toddlers. *Journal of dental research*, 90(2), 209-214.
11. Al-Ghurabei, B. H., Shaker, Z. F., Fadhel, R., Al-Khayli, N. G., & Mustafa, L. K. (2012). Serum levels of interleukine-1beta and interleukine-2 in chronic periodontitis. *Al-Mustansiriya J. Sci*, 23(3), 55-62.
12. Govula, K., Anumula, L., Swapna, S., & Kirubakaran, R. (2021). Interleukin-6: A Potential Salivary Biomarker for Dental Caries Progression—A Cross-sectional Study. *International Journal of Experimental Dental Science*, 10(1), 8-13.
13. Al-Ghurabei, B. H. (2012). Evaluation of serum anti-cardiolipin antibody, hs-CRP and IL-6 levels in chronic periodontitis as possible risk factors for cardiovascular diseases. *Journal of baghdad college of dentistry*, 24(2), 161-165.
14. Lo Giudice, R., Militi, A., Nicita, F., Bruno, G., Tamà, C., Lo Giudice, F., ... & Mannucci, C. (2020). Correlation between oral hygiene and IL-6 in children. *Dentistry Journal*, 8(3), 91.

15. Al-Ghurabei, B. H. (2011). Role of salivary tumor necrosis factor-alpha and immunoglobulin-a in recurrent aphthous stomatitis. *Journal of the Faculty of Medicine Baghdad*, 53(2), 207-210.
16. Navazesh, M. (1993). Methods for collecting saliva. *Annals of the New York Academy of Sciences*, 694(1), 72-77.
17. Al-Hindawi, S. H., Luaibi, N. M., & Al-Ghurabei, B. H. (2017). Possible use of saliva as a diagnostic tool in hypothyroidism. *J Biosci Biotech*, 6(3), 539-542.
18. Hall, B. E., Zhang, L., Sun, Z. J., Utreras, E., Prochazkova, M., Cho, A., ... & Kulkarni, A. B. (2016). Conditional TNF- α overexpression in the tooth and alveolar bone results in painful pulpitis and osteitis. *Journal of dental research*, 95(2), 188-195.
19. Morand, D. N., Davideau, J. L., Clauss, F., Jessel, N., Tenenbaum, H., & Huck, O. (2017). Cytokines during periodontal wound healing: potential application for new therapeutic approach. *Oral diseases*, 23(3), 300-311.
20. Mahmood, H. K., & Al-Ghurabi, B. H. (2020). Association between anti-CMV IgG and salivary levels of IL-6 and TNF- α in chronic periodontitis. *Journal of Baghdad College of Dentistry*, 32(2), 5-11.
21. Zehnder, M., Delaleu, N., Du, Y., & Bickel, M. (2003). Cytokine gene expression—part of host defence in pulpitis. *Cytokine*, 22(3-4), 84-88.
22. Pezelj-Ribaric, S., Prso, I. B., Abram, M., Glazar, I., Brumini, G., & Simunovic-Soskic, M. (2004). Salivary levels of tumor necrosis factor- α in oral lichen planus. *Mediators of Inflammation*, 13, 131-133.
23. Zhao, A., Blackburn, C., Chin, J., & Srinivasan, M. (2014). Soluble toll like receptor 2 (TLR-2) is increased in saliva of children with dental caries. *BMC Oral Health*, 14(1), 1-5.
24. Seyedmajidi, M., Khodadadi, E., Maliji, G., Zaghian, M., & Bijani, A. (2015). Neutrophil count and level of interleukin-1 β and interleukin-8 in the saliva of three to five year olds with and without dental caries. *Journal of Dentistry (Tehran, Iran)*, 12(9), 662.
25. Ramirez-De los Santos, S., López-Pulido, E. I., Medrano-Gonzalez, I. D. C., Becerra-Ruiz, J. S., Alonso-Sanchez, C. C., Vázquez-Jiménez, S. I., ... & Guzmán-Flores, J. M. (2021). Alteration of cytokines in saliva of children with caries and obesity. *Odontology*, 109, 11-17.
26. Gornowicz, A., Bielawska, A., Bielawski, K., Grabowska, S. Z., Wójcicka, A., Zalewska, M., & Maciorkowska, E. (2012). Pro-inflammatory cytokines in saliva of adolescents with dental caries disease. *Annals of agricultural and environmental medicine*, 19(4).
27. Sharma, V., Gupta, N., Srivastava, N., Rana, V., Chandna, P., Yadav, S., & Sharma, A. (2017). Diagnostic potential of inflammatory biomarkers in early childhood caries-A case control study. *Clinica Chimica Acta*, 471, 158-163.
28. Kurtiş, B., Tüter, G., Serdar, M., Akdemir, P., Uygur, C., Firatli, E., & Bal, B. (2005). Gingival crevicular fluid levels of monocyte chemoattractant protein-1 and tumor necrosis factor-alpha in patients with chronic and aggressive periodontitis. *Journal of periodontology*, 76(11), 1849-1855.
29. Gabay, C. (2006). Interleukin-6 and chronic inflammation. *Arthritis Res Ther*, 8(S2), S3-S6.
30. Tani-Ishii, N., Tsunoda, A., Teranaka, T., & Umemoto, T. (1999). Autocrine regulation of osteoclast formation and bone resorption by IL-1 α and TNF α . *Journal of dental research*, 78(10), 1617-1623.