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Salivary IL-10 and TNF-α levels in Dental Caries Detection in Pediatric β-Thalassemia Major Patients

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Abstract:

β-Thalassemia Major, a severe genetic blood disorder, necessitates lifelong blood transfusions and can lead to various complications, including oxidative stress and dental caries. This study aims to evaluate the salivary levels of cytokines Interleukin-10 (IL-10) and Tumor Necrosis Factor-alpha (TNF-α) in pediatric β-thalassemia Major patients and their association with dental caries. With 80 participants aged 8-12, split equally between patients and healthy controls, it found significantly higher cytokine levels in patients, indicating an altered immune response. Despite higher dental caries severity in patients, no significant correlation with cytokine levels was noted. The findings underscore the necessity for specialized dental care for β-thalassemia Major patients due to their increased risk of dental caries, highlighting the potential impact of their condition on oral health.

Keywords: β-Thalassemia Major, Dental Caries, Salivary Cytokines, Interleukin-10, Tumor Necrosis Factor-alpha, Pediatric Dentistry.

Introduction:

Thalassemia is a genetic disorder that involves abnormal hemoglobin formation. Based upon the affected globin chain, thalassemia may be classified into α- or β-thalassemia that are then divided into further subcategories. Alpha thalassemia occurs when one or more of the four alpha globin genes are either abnormal or not present in an individual. Beta thalassemia occurs when one or both Beta globin genes are either abnormal or are absent (Helmi et al.,2017).

In most of the cases, mutations in the alpha or beta globin genes result in the development of thalassemia in the patient. Some of these gene mutations (heterozygous gene) result in mild versions of thalassemia that may even go unnoticed and only cause mild anemia and iron deficiency problems (Farashi et al.,2018), while others (homozygous gene) result in extremely severe forms of thalassemia. β-thalassemia major is one of the most common inherited blood diseases caused by a defect in the gene, which is responsible for making the mature hemoglobin (Sirivadhanakul et al., 2019).

Beta-thalassemia (β-thalassemia) major patients are severely anemic and require life-long blood transfusions for survival. The iron overload can cause oxidative damage and pro-inflammation and therefore, hasten mortality. Thus, regular monitoring of the oxidative stress and pro-inflammation status may be useful in these patients (Wlaschek et al., 2019). Frequent blood transfusion to compensate for anemia increases their excess load and consequently leads to oxidative stress through free radical production (Araujo et al., 2020). Saliva can be the first defensive line against oxidative stress created by free radicals (AlAnazi et al., 2018; Kazhaal et al., 2020).

Enhanced susceptibility to infections in β-thalassemia patients is associated with the interplay of several complex biological processes. β-thalassemia-related abnormalities of the innate immune system include decreased levels of complement, properdin, and lysozyme, reduced absorption and phagocytic ability of polymorphonuclear neutrophils, disturbed chemotaxis, and altered intracellular metabolism processes. Immunological abnormalities observed in patients with thalassemia can be caused by both the disease itself as well as therapies (Kazem et al., 2023).

Several pro- and anti-inflammatory cytokines are produced in response to bacterial components which modulate immune homeostasis, resulting in potentially protective inflammatory responses (Kareem et al., 2023).

Tumor necrosis factor alpha (TNF-α) plays a pivotal function in various immune and inflammatory processes, including cellular activation, proliferation and survival, as well as cell death by necrosis and apoptosis. TNF-α cellular source depends on the nature of the stimulus (Croft et al., 2017).

Interleukin 10 (IL-10) has been studied in human melioidosis as a potent anti-inflammatory cytokine to counter-balance enhancement of immune functions (Kessler et al., 2017).

Regarding oral health, the occurrence of dental caries in thalassemic patients may be attributed to several factors like improper diet, presence of malocclusion, lack of adequate knowledge regarding oral and dental health, reduction in the salivary concentration of urea, as well as levels of salivary immunoglobulin A (IgA) (Nabi et al., 2022).

Marital and methods:

2.1 Study Design and Participants:

This case-control study was conducted at a thalassemia center in Missan, Iraq, between December 2022 and March 2023. The study involved 80 children aged between 8 and 12 years, divided into two groups: 40 children with β-thalassemia Major (Patient group) and 40 healthy controls. Ethical approval was obtained from the scientific committee at the Basic Science Department/College of Dentistry/University of Baghdad (Project No. 674822).

2.2 Inclusion and Exclusion Criteria:

The patient group consisted of children previously diagnosed with β-thalassemia Major. Participants in both groups were cooperative and had no systemic diseases influencing dental health. Children with a history of systemic diseases like diabetes, uncooperative children, and those whose parents refused participation were excluded.

2.3 Saliva Collection:

Saliva samples were collected in the morning between 9-11 AM using passive drooling into collection tubes, as per the University of South California School of Dentistry Guidelines. The procedure emphasized participant relaxation and required oral rinsing with sterilized water before collection (Navazesh et al., 2008). Samples contaminated with blood were discarded. Collected samples were centrifuged at 3000 rpm for 10 minutes, and the supernatant was stored at -20˚C for analysis.

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2.4 Cytokine Analysis:

Salivary levels of IL-10 and TNF-α were determined using commercially available ELISA kits. The analysis was conducted following the manufacturer's instructions, ensuring accuracy and reliability of the results.

2.5 Dental Caries Assessment:

Dental caries severity was evaluated in both groups. The dmfs index (decayed, missing, and filled surfaces in primary teeth) was used to assess the presence and extent of dental caries.

2-6 Ethical Considerations:

Informed consent was obtained from all participants' parents after providing them with detailed information sheets. All procedures were conducted in accordance with ethical standards and guidelines to ensure participant safety and data confidentiality.

2-7 Statistical Analysis:

In this investigation, SPSS version 26, and Microsoft Excel 2010 were employed. The present study's data was carefully examined to evaluate the difference between groups using normality tests to determine if it was parametric or non-parametric. As a result, appropriate statistical tests were employed Chi-Square test, T. test and Person correlation.

3. Results and Discussion:

3.1 Demographic characteristic:

The current study included 80 children aged between 8 and 12 years, divided into two groups. The patient group comprised 40 children previously diagnosed with β-thalassemia major syndrome; the mean age was 10.614±1.687 years, all of whom were dependent on blood transfusions. The control group consisted of the remaining 40 children, and the mean age was 10.659±1.670 years. The result showed a statistically non-significant difference between males and females. Furthermore, there is no significant difference in age for both groups. As recorded in Table 1

Table 1: Descriptive and statistical test of gender and age among groups.

			Groups		p-value
			C	P	
Gender (Chi square=0.000)	Male	N	21	21	1.000 NS
		%	47.73	47.73	
	Female	N	23	23	
		%	52.27	52.27	
Age (T test=0.127)	mean		10.659	10.614	0.899 NS
	±SD		1.670	1.687	

Table 2 and Figure 1 illustrated the grades of disease severity in primary teeth for both the patient and control groups. For the (d1, d2) division, the mean value was lower in the patient group than in the control group, with a statistically significant difference between the two groups ($P < 0.05$). However, in the (d3, d4) division, the mean value was higher for the patient group compared to their controls, and this difference was also significant ($P < 0.05$).

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Table 2: Descriptive and statistical test of caries severity of primary dentition (d_{1-4}).

Groups		d1	d2	d3	d4
C	Min.	0.000	0.000	.000	0.000
	Max.	2.000	4.000	3.000	0.000
	Mean	0.171	1.257	0.943	0.000
	±SD	0.453	1.039	1.027	0.000
	±SE	0.077	0.176	0.174	0.000
P	Min.	0.000	0.000	0.000	0.000
	Max.	0.000	2.000	10.000	30.000
	Mean	0.000	0.333	3.103	7.462
	±SD	0.000	0.662	2.614	5.844
	±SE	0.000	0.106	0.419	0.936
T test		2.240	4.504	4.766	7.974
P value		0.032	0.000	0.000	0.000

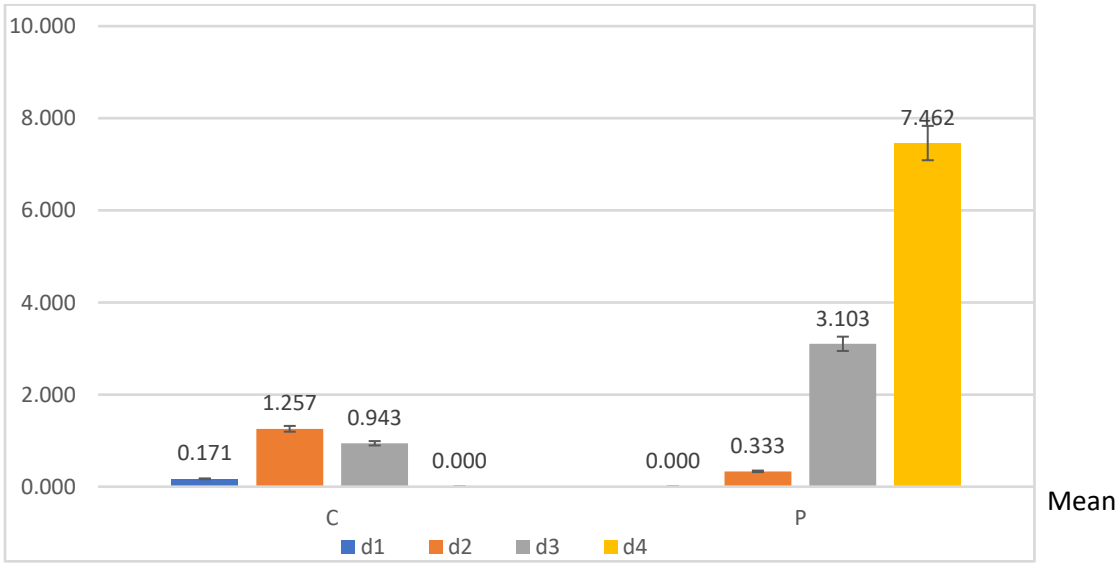

Figure 1: mean value of caries severity of primary dentition (d_{1-4}).

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Table 3 and Figure 2 illustrations the statistical analysis of salivary Interleukin-10 (IL-10) levels between the patient and control groups. The results revealed that the mean value of salivary IL-10 was higher in the patient group compared to the control subjects, with a statistically significant difference between the two groups ($P < 0.05$).

Table 3: Descriptive and statistical test of IL-10 pg/ml among groups.

Statistics	Groups	
	C	P
Minimum	10.643	27.071
Maximum	384.929	1031.357
Mean	106.089	282.652
±SD	41.176	100.104
±SE	12.835	37.110
T test	4.170	
P value	0.000 Sig.	

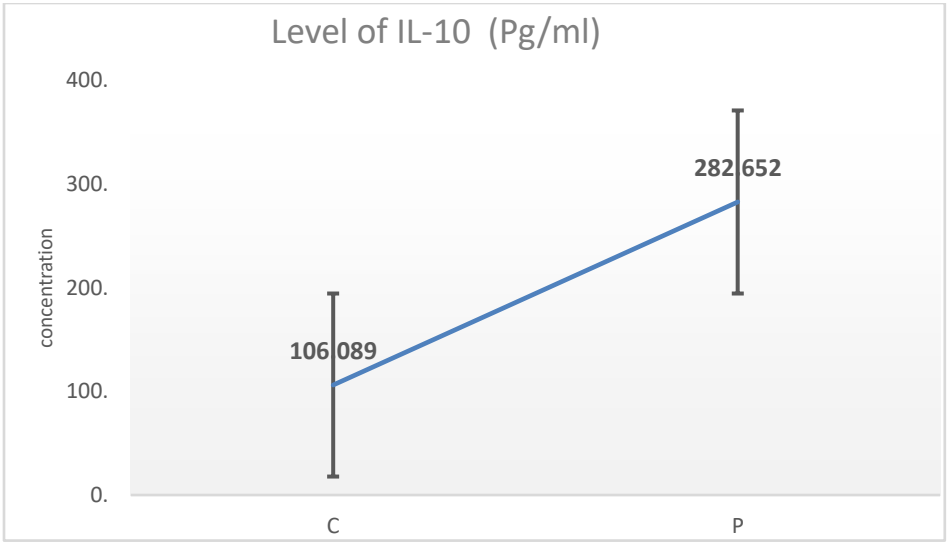

Figure 2: mean value of salivary Interlukin-10 (IL-10) pg/ml

The statistical analysis of salivary TNF-α levels for both the patient and control groups demonstrates in Table 4 and Figure 3. The data indicates that the mean salivary TNF-α level was elevated in the patient group compared to the controls, and this difference was statistically significant ($P < 0.05$).

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Table 4: Descriptive and statistical test of TNF-α pg/ml among groups.

Statistics	Groups	
	C	P
Minimum	1.200	5.200
Maximum	26.000	29.600
Mean	11.060	17.808
±SD	5.805	5.324
±SE	0.918	0.768
T test	5.682	
P value	0.000 Sig.	

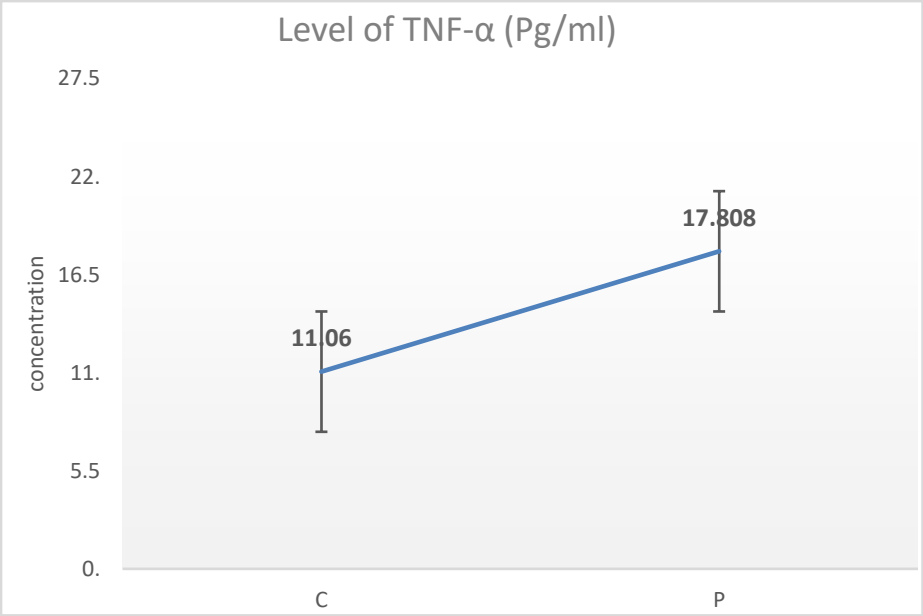

Figure 3: mean value of salivary TNF-α pg/ml

Table 5 displays the correlation coefficients of biomarkers with the dental caries experience in primary dentition (dmfs) among patients and control groups. The correlation of IL-10 and TNFα the result recorded a non-significant correlation with all dmfs index in both groups.

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Table 5: Correlation between caries experience, its severity of primary teeth and biomarkers

Groups		IL10		TNFa	
		r	p	r	p
C	d1	-0.014	0.935	-0.104	0.552
	d2	-0.033	0.851	-0.070	0.690
	d3	0.244	0.158	0.030	0.863
	d4				
	ds	0.128	0.465	-0.054	0.759
	ms	0.017	0.920	-0.119	0.483
	fs	0.026	0.882	-0.159	0.361
	dmfs	0.175	0.301	-0.094	0.581
P	d1				
	d2	0.164	0.317	-0.086	0.603
	d3	0.283	0.081	-0.016	0.923
	d4	-0.135	0.413	0.033	0.840
	ds	0.010	0.950	0.016	0.921
	ms	0.118	0.440	-0.043	0.777
	fs				
	dmfs	0.016	0.918	0.052	0.737

The correlation between IL-10 and TNFα was positive significant in control group. In contrast, there was a non-significant negative correlation between IL-10 and TNFα within the patient group, as recorded in Table 6.

Table 6: Correlation between biomarkers among groups.

Groups		TNFa	
		r	p value
C	IL10	0.520	**0.001**
P	IL10	-0.028	0.852

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The prevalence of dental caries in paediatric patients with β-thalassemia major is a significant concern due to their compromised immune system and increased susceptibility to infections. The potential of salivary biomarkers, particularly anti-inflammatory and pro-inflammatory cytokines, in detecting dental caries in these patients has garnered attention in recent research (Hattab et al., 2021). This study aims to explore the potential of salivary IL-10 and TNF-α levels as diagnostic indicators for dental caries in pediatric β-thalassemia major patients. Thalassemias, particularly β-thalassemia, are prevalent in various regions, including Mediterranean countries, South-East Asia, Africa, the Middle East, and the Indian subcontinent. The estimated global prevalence of thalassemia is about 1.4 per 100,000 individuals, with varying rates in different countries. For instance, the prevalence of thalassemia in Iraq was reported to be 37.1% of the population, which is notably higher than in other regions. This higher prevalence in Iraq may be attributed to factors such as a high rate of consanguineous marriages, a lack of effective prevention programmes, and poor legislation (Harteveld et al., 2010; Kadhim et al., 2017). Research has shown that paediatric patients with β-thalassemia major are at a higher risk of dental caries, and the exploration of salivary biomarkers, including IL-10 and TNF-α, holds promise for early detection and monitoring of caries in this vulnerable opulation (Aziz et al., 2022). Studies have indicated altered levels of inhibitory cytokines, such as IL-10, in patients with thalassemia major and gingival inflammation, suggesting a potential link between these cytokines and oral health conditions (Akcalı et al., 2019). Additionally, elevated levels of TNF-α have been found in the saliva of children with dental caries, further emphasizing the potential of TNF-α as a biomarker for caries detection (Spinei et al., 2023).

Regarding the severity of dental caries in primary dentition, the results of this study showed that the mean value of d1 and d2 was lower among the thalassemic group than their controls. In contrast, d3 and d4 were higher among the thalassemic group than their controls.

The β-thalassemia children's high caries experience might be ascribed to a lack of desire, poor oral care, and a misalignment of the teeth (Almaas and Diab,2020) Meanwhile, the chronicity of β-thalassemia syndrome makes the parents worried about the child's chief, life-threatening immanent disease and neglect basic oral care (Singh et al., 2013). They give less consideration to oral diseases, and they seek dental care just when their children have pain (Hayat et al., 2022).

Studies by Alwaheb et al. (2018) suggested that the increase in severity of dental caries among β-thalassemias could be correlated to the higher counts of mutans streptococci in comparison to healthy controls.

According to the findings of the present study, the level of salivary interleukin-10 (IL-10) was higher in the β-thalassemia patient group compared to the control group within dental caries. IL-10, primarily recognised for its anti-inflammatory properties, plays a critical role in curbing pro-inflammatory cytokines and thwarting T cell, macrophage, and monocyte activation (Francisco et al., 2010). On the other hand, β-thalassemia invariably results in chronic inflammation, which might elicit a compensatory surge in anti-inflammatory molecules. Regular blood transfusions in β-thalassemia could augment antigenic stimulation, further fueling the inflammatory cascade and possibly the production of IL-10 (Samba Mondonga, 2019). Furthermore, the exact cytokine profile can be heterogeneous across patients, with variations in IL-10 and TNF-α, among others. The result of the current study is in agreement with Weatherall (2010), who emphasized the chronic inflammatory state in β-thalassemia and its potential repercussions on multiple body systems, including the oral cavity. The inflammation, coupled with the potential immunosuppressive effects of elevated IL-10, might predispose these patients to dental caries. Furthermore, frequent blood transfusions in β-thalassemia could induce antigenic stimulation, potentially affecting the oral immune environment. The exact relationship between cytokine levels, especially IL-10, and clinical manifestations in β-thalassemia remains a topic of active research (Gharagozloo et al., 2009).

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The data in the present study indicates that the mean salivary TNF-α level in dental caries increased significantly in the patient in the β-thalassemia group compared to the control without the β-thalassemia group within dental caries. This may be due to the interaction between systemic infections, blood disorders, and oral infections. Further, high TNF-α concentrations have been documented among thalassemia patients in several studies. The authors assigned this rise to macrophage activation by iron overload, the antigenic stimulation promoted by chronic transfusion therapy, and accelerated erythropoiesis (Gharagozloo et al., 2009). Tselepis et al. (2010) disclosed a significant increase in TNF-α levels in the thalassemia patient group compared with the controls being higher in patients, a finding that came in accordance with current results in this regard. On the contrary, Hahalis et al. (2011) reported a non-significant difference in serum TNF-α level between their studied thalassemia adults and healthy controls.

According to Weatherall (2010), who reported that β-thalassemia major is characterised by reduced or absent beta-globin chain synthesis, leading to chronic hemolysis, This continuous destruction of red blood cells and subsequent iron overload result in a persistent state of inflammation. Actually, chronic inflammation, combined with potential alterations in the oral environment due to β-thalassemia (such as changes in salivary pH, composition, or flow rate), could lead to an imbalance in the oral microbiome. An altered microbiome might favour the proliferation of cariogenic bacteria, which, when they metabolise dietary sugars, produce acids that can erode tooth enamel, leading to caries. The host immune response to this bacterial challenge might involve the upregulation of inflammatory cytokines (Baker and Edlund,2019). According to Fibach and Rachmilewitz, (2008), β-thalassemia patients often undergo frequent blood transfusions, leading to iron overload. Excess iron can catalyse the generation of reactive oxygen species (ROS), inducing oxidative stress. Oxidative stress can further stimulate the production and release of pro-inflammatory cytokines.

Borgna-Pignatti and Gamberini (2011) suggested that β-thalassemia patients may have a dysregulated immune system with altered cytokine profiles. Elevated TNF-α levels could be a reflection of this immune dysregulation, which might also play a role in their increased susceptibility to dental caries (Leme et al., 2022).

Gorr (2009) reported that TNF-α can influence the integrity of the oral mucosal barrier, making the oral environment more susceptible to bacterial colonisation and subsequent dental caries.

Regarding IL-10 and TNFα the result recorded a non-significant correlation with all dmfs index in both groups. This may be explained by that IL-10 might modulate oral inflammation, but its levels might not directly correlate with the extent or severity of dental caries, at least when measured systemically in saliva. IL-10 plays a crucial role in downregulating pro-inflammatory responses, thereby limiting host immune responses to pathogens. This means that IL-10 might play a role in limiting the inflammatory response to cariogenic bacteria, potentially preventing excessive tissue damage (Schülke, 2018).

On the other hand, TNFα can promote the inflammatory response to bacterial invasion, elevated levels of TNFα could exacerbate tissue damage in response to cariogenic bacteria (Graves, 2008). The absence of a correlation between TNFα and the dmfs index suggests that the progression and severity of dental caries might involve a myriad of factors, and the systemic levels of TNFα in saliva might not directly reflect caries status. Actually, the dental caries susceptibility index (dmfs) serves as a measure of oral health, reflecting the presence of decay, missing teeth due to decay, and filled surfaces in primary teeth.

4. Conclusion :

The present study concluded that pediatric patients with β-thalassemia Major exhibit markedly elevated levels of salivary cytokines Interleukin-10 (IL-10) and Tumor Necrosis Factor-alpha (TNF-

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α), a notable deviation from the levels observed in healthy controls. This elevation suggests a critical indicator of the altered immunological state inherent in β-thalassemia Major, likely influenced by chronic inflammation, iron overload due to frequent blood transfusions, and oxidative stress. Additionally, the study reveals a higher severity of dental caries in the β-thalassemia patient group, this suggesting that the immune dysregulation and inflammatory response in these patients may contribute to their increased susceptibility to dental caries.

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