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Evaluation the salivary *anti-Porphyromonas gingivalis* (IgA and IgG) response in relation to sera levels of Ferritin and Vitamin D in Patients with Beta-Thalassemia Major

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Abstract:

Beta-thalassemia major is a severe hereditary anemia that necessitates regular blood transfusions, leading to iron overload. This condition also affects various biological markers, including ferritin and vitamin D levels, and can influence immune responses and oral health.

The Aim of Study To examine the relationship between salivary *Porphyromonas gingivalis* IgA and IgG levels with the concentrations of ferritin and vitamin D in adolescent affected by Beta-Thalassemia Major. A case-control study was conducted from November 2023 to June 2024, involving 45 beta-thalassemia major adolescents and 45 healthy controls. All subjects were within ages ranged between 12-17 years. Saliva sample was collected and analyzed for estimation of salivary anti-*Porphyromonas gingivalis* IgA and IgG by using ELISA and blood collected and analyzed to measures s.ferritin and s.vitamin D by Cobas E411 system. The study group exhibited significantly lower mean salivary anti-*Porphyromonas gingivalis* IgA and IgG levels (40.59± 16.71 pg/ml), (15.23±3.21 pg/ml) compared to the control group (93.06± 55.25pg/ml), (18.92±3.32 pg/ml) (p=0.000) respectively. Serum ferritin (4158.37±542.64 ng/ml vs. 25.11±10.93 ng/ml), and vitamin D levels (9.83±4.69 ng/ml vs. 16.40±12.93 ng/ml) showed significant differences (p<0.05) between study and control groups. Beta-thalassemia major significantly impacts salivary and serum biochemical markers, highlighting the importance of regular monitoring and potential targeted therapies to manage iron overload and associated complications. The reduced immune response against *Porphyromonas gingivalis* in these patients suggests a need for enhanced oral health care and possibly prophylactic measures.

Keywords: Beta-thalassemia major, *Porphyromonas gingivalis*, iron overload, ferritin, vitamin D, immune response, oral health.

Introduction:

Beta-thalassemia, a hereditary anemia resulting from defects in globin genes inherited in an autosomal recessive pattern, manifests in severe forms such as β-thalassemia major, which requires regular blood transfusions or is classified as transfusion-dependent thalassemia (TDT) (Taher, Musallam, and Cappellini 2021; Hassan and Athab 2024). This condition is marked by an imbalance that causes early death of red blood cells and a spectrum of symptoms associated with inadequate red blood cell production, collectively termed ineffective erythropoiesis. Clinical presentations of ineffective erythropoiesis range from asymptomatic to severely anemic states (Taher and Cappellini 2014; Kadhim, Baldawi, and Lami 2017).

Ferritin, a protein crucial for iron storage, has been found essential for *P. gingivalis* to survive in iron-restricted environments under both hemin and transferrin starvation. The ability of *P. gingivalis* to accumulate iron on its cell surface provides a significant nutritional advantage under iron-limited conditions (Smalley and Olczak 2017).

In β-thalassemia major patients, vitamin D deficiency is critical because it stimulates trans-membrane calcium transport through the left ventricular dependent calcium channel (LVDCC) (Shamsah and Zaidan 2015). This process facilitates the transport of non-transferrin-bound iron (NTBI) into the myocardium, contributing to cardiac iron overload. Consequently, this leads to increased oxidative stress in myocytes and disrupts the function of sodium, potassium, and calcium ion channels, causing depolarization and repolarization disturbances, arrhythmias, and both systolic and diastolic dysfunctions (Baroni et al. 2007).

The oral cavity, with its unique environment characterized by continuous exposure to saliva, a narrow temperature range (34 to 36°C), and a pH close to neutrality, supports a diverse yet stable microbial community known as the climax community (Marcotte and Lavoie 1998). However, disruptions in the balance of oral microbiota can lead to diseases such as dental caries and periodontal diseases. Notably, these imbalances often involve a shift from Gram-positive, facultative, fermentative bacteria to predominantly Gram-negative, anaerobic, chemoorganotrophic, and proteolytic organisms, which are significantly associated with periodontal tissue destruction (Kazem, Abdulkareem, and Milward 2023; Alwaheb and Alhuwaizi 2018; Maddah and Taha, (2024).

Porphyromonas gingivalis, a major periodontal pathogen, is a prime etiological agent in the pathogenesis and progression of periodontal disease's inflammatory events (H. H. Kareem, Al-Ghurabi, and Albadri 2022). Iron is utilized by *P. gingivalis* primarily in the form of heme and plays an essential role in its growth and virulence. The bacterium employs specific outer membrane receptors, proteases, particularly gingipains, and lipoproteins to secure iron/heme (Smalley and Olczak 2017).

P. gingivalis has been implicated in several systemic conditions beyond periodontal disease. It can disrupt immune tolerance in susceptible individuals, potentially exacerbating rheumatoid arthritis through the enzymatic modification of host proteins (H. H. Kareem, Al-Ghurabi, and Albadri 2022). Furthermore, periodontal disease has been identified as a risk factor for adverse pregnancy outcomes, such as pre-term low birth weight (Smalley and Olczak 2017).

Materials and Methods:

The clinical examinations and laboratory biochemical tests were conducted from November 2023 to June 2024, and the case-control study received the requisite approvals from the Ministry of Health and the Ministry of Education. The control group was composed of 45 healthy school students, while the study group was composed of 45 adolescents with beta-thalassemia major, who were recruited from the Al-Sadr City Ibn Al-Baladi Hospital. All subjects were aged 12-17 years.

The inclusion criteria included participants that are medically diagnosed with beta-thalassemia major, and the study is open to both males and females aged between 12 and 17 years. To maintain consistency and avoid confounding factors, the exclusion criteria eliminate those who are on medications for other chronic diseases, those who are unwilling to participate, individuals with adverse habits such as tobacco chewing or smoking, and those who have received periodontal therapy within the past six months.

Ethical Approval:

The research protocol was approved by the Basic Science Department's scientific committee at the College of Dentistry, University of Baghdad, on January 01, 2024 (Project No. 890824).

Saliva Collection

One to three millilitres of whole, unstimulated saliva were taken from the participants between 8 and 10 a.m. The subjects were instructed not to eat or drink for three hours prior to the saliva collection procedure, to wash their mouth with distilled water for one minute, and to relax for five minutes before beginning saliva collection; subjects were then instructed to spit saliva into sterilized cups (S. J. Kareem and Al-Ghurabi 2023). Then, saliva was centrifuged at 3500 rpm for 10 minutes, and the supernatant was frozen at -20°C until the Anti *Porphyromonas gingivalis* IgA and IgG was analysed using the Enzyme immunoassay (ELISA) method.

Blood collection:

Each patient provided five millilitres of venous blood, which were collected aseptically. Blood was transferred to a sterile gel tube, and serum was separated by centrifugation at 3000 rpm for 10 minutes before being split into tiny aliquots and stored at -20°C until required to analyse serum vitamin D and ferritin using Cobas machine.

Statistical analysis:

In this investigation, SPSS version 26 and Microsoft Excel 2010 were employed to evaluate the difference between groups and by using normality tests, for determination whether the study's data was parametric or non-parametric test. Statistical tests were thus used. T-test and ANOVA test to evaluated differences.

Results:

The comparison age (years) of participants in the study group (Beta Thalassemia major adolescents) and the control group (healthy adolescents) showed minimum age in the study group is 12 years, while in the control group is 15 years. The maximum age in both groups is 17 years. The mean ± SD value is 14.89 ± 1.51 years for the study group and 16.38 ± 0.72 years for the control group. Although the control group is slightly older than the study group, the difference is not statistically significant ($p = 0.07$) using t-test.

The comparison of salivary anti- *Porphyromonas gingivalis* IgA levels between the study and control groups is demonstrated that mean anti-PG IgA level is significantly lower in the study group (40.59± 16.71 pg/ml) compared to the control group (93.06± 55.25pg/ml), (p=0.000) using t-test.

The differences in levels of salivary anti-*Porphyromonas gingivalis* IgG between the study and control groups showed the mean anti-PG IgG level is significantly lower in the study group (15.23±3.21 pg/ml) compared to the control group (18.92±3.32 pg/ml), (p= 0.000) using t-test.

The differences in sera levels of ferritin (ng/ml) and vitamin D (ng/ml) between the study and control groups shown, the mean value of ferritin level is significantly higher in the study group (4158.37±542.64 ng/ml) compared to the control group (25.11± 10.93ng/ml), (p=0.000), whereas the mean vitamin D level is lower in the study group (9.83±4.69 ng/ml) compared to the control group (16.40±12.93 ng/ml), with a statistical analysis of a significant difference (p=0.002) using t-test.

The correlation coefficients (r) test between both IgA, IgG and various studied variables (Ferritin and VitD) in the control and study groups revealed no significant correlation with each of ferritin and vitamin D in study and control groups.

Table 1: Correlation between IgG and studied variables.

Groups		IgA		IgG	
		r	p	r	p
Control	Ferritin	-0.240	0.112	-0.073	0.635
	VitD	0.151	0.323	-0.002	0.989
Study	Ferritin	-0.188	0.217	0.101	0.510
	VitD	-0.125	0.415	-0.288	0.055

Discussion:

The primary objective of this study was to evaluate the relationship of immunological markers, focusing on the levels of salivary anti-*Porphyromonas gingivalis* IgA and IgG, particularly with serum ferritin and vitamin D in individuals with Beta Thalassemia Major compared to healthy controls.

The present study revealed a significant decrease in mean level of salivary anti-*Pg* IgA in the Thalassemic group when compared to healthy control. This finding is correspnds with a study conducted by Hallikainen et al. (2021), that revealed that the presence of serum IgG antibodies against *P. gingivalis* was linked to systemic conditions. This suggests that the immune response may be not solely reliant on levels of antibodies in the mucous membranes.

The beta-thalassemia major group in the current study had showed significantly higher ferritin levels in sera than the control group.These findings align with studies by done by De Domenico et al. (2008) (De Domenico, McVey Ward, and Kaplan 2008) who found a significant increase levels of ferritin in beta-thalassemia patients compared to controls. The study presented by Zekavat et al. (2014) (Zekavat et al. 2014), also found a significant ferritin increase in beta-thalassemia patients compared to controls. Hussein et al. (2022) found that beta-thalassemia patients have more ferritin than healthy persons and eliminate it via the kidneys when severe anaemia develops (Hussein 2022).

This may be due to a genetic cause like a mutation or deficiency in the haemoglobin gene that hinders haemoglobin formation and red blood cell breakdown, causing severe anaemia and iron accumulation in tissues, glands, and organs. It increases iron toxicity and may be addressed by treating the patient with iron chelating agent which is transported by the kidneys with severe anaemia. In order to compensate for the lack of red blood cells needed for cell function, blood must be delivered to the patient regularly which resulted in ferritin increase that causes iron level rises, indicating illness (Mettananda and Higgs 2018).

The current results showed that study group had decreased mean vitamin D levels compared to the control group with a statistically significant change (p=0.002). Vitamin D, a prohormone, regulates calcium homeostasis and increases intestinal calcium absorption (Holick 2007). Without vitamin D, the body absorbs and selects 60 percent of dietary phosphorus and 10 to 15 percent of calcium. Sufficiency of VD improves 30–40% and 80% of phosphate and calcium absorption respectively(LIPS et al. 2006). Many researches proceeded by Dandona et al. (1987) (Dandona et al. 1987), Napoli et al. (2006) (Napoli et al. 2006) linked vitamin D insufficiency to thalassemia bone density loss.

The results of current study show that IgG does not have any significant correlations with the studied variables (Ferritin and VitD) in both the study and control groups. This suggests that *Porphyromonas gingivalis* IgA and IgG respnse are not directly affected by the levels of the ferritin and vitamin D in sera of either study or control populations. The absence of significant correlations between *Porphyromonas gingivalis* (IgA and IgG) and aforementioned variables indicates that the production and activation of anti-Pg IgA and IgG may be independent on the specific regulation mechanisms of Ferritin and VitD in these groups.

Conclusion:

In conclusion, beta-thalassemia major significantly affects salivary and serum biochemical markers, such as reduced levels of anti-*Porphyromonas gingivalis* IgA and IgG, elevated ferritin, and decreased vitamin D. These changes underscore the need for regular monitoring and targeted therapies to manage iron overload and related complications in these patients. The diminished immune response against *Porphyromonas gingivalis* suggests an increased need for enhanced oral health care and potential prophylactic measures to mitigate oral health issues in individuals with beta-thalassemia major

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