

Evaluation of Immunological and Biochemical Background for the Occurrence of Dental Caries in B-Thalassemic Patients

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ABSTRACT

Mohammed et al / evaluation of immunological and biochemical background

Thalassemia is a hereditary disorder. It leads to decrease production and increase destruction of red blood cells. Several biochemical and immunological alterations seen in those patients that enhance dental caries occurrence and determine severity. To evaluate immunological & biochemical background in thalassemic patients, in relation to dental caries. This is a case control study, that's done by descriptive-analytic method, in a period between (October 2016 till June 2017), which includes; a total of 56 patients suffering from β -thalassemia major (age rang 4-22) years, referring to Maternity and Children Hospital in Babylon province, AL-Hilla city and 32 healthy control (age range 3-22) year., DMFT/dmft indicator examined in both groups and serum " IL-6, IL-8, IL-17, Ca⁺⁺, Mg⁺⁺, P, Vit.D3" & salivary IgA was assessed in both groups. Caries Experience Examination shows dmft value is higher in study group (6.82 ± 3.24) than in the control group is (2.43 ± 0.85) with statistically significant differences ($p < 0.05$), DMFT is higher in study group (5.57 ± 2.51) compared to control group (2.79 ± 1.06) which is significant differences statistically ($p < 0.05$). Immunological evaluation of salivary IgA value shows Mean \pm S.D of study group lower than control group and the differences is highly statistically significant ($P < 0.001$). Also, immunological evaluation of serum proteins including "IL-6, IL-8, and IL-17." Mean \pm S.D of IL-6 value for study group is higher than for control group, and there is difference between two groups which is significant statistically ($P < 0.05$), while Mean \pm S.D of IL-8 and IL-17 is higher in study group compared to control group, but not significant statistically. While biochemical evaluation of serum minerals constituents include, " Ca⁺⁺, Mg⁺⁺, P, and Vit.D3" Mean \pm S.D of Vit.D3 & Mg⁺⁺ in study group lower than control group with significant statistically differences between them ($P < 0.05$). Concerning P, Mean \pm S.D for study group lower than control group with highly significant differences ($P < 0.001$). Regarding Ca⁺⁺ Mean \pm S.D for study group lower than control group but not significant statistically ($P > 0.05$). There is considerable increment in incidence of dental caries in Thalassemic patients. This increase incidence of dental caries might be due to alterations in the salivary level of IgA, changes in the serum values of minerals " Ca⁺⁺, Mg⁺⁺, P, and Vit.D3", while changes in the serum values of "IL-6, IL-8, and IL-17" might due to immunological alterations in thalassemic patients.

INTRODUCTION

Thalassemia is a type of hemolytic anemia, which includes defects in the synthesis of hemoglobin - or - polypeptide chains (- and -thalassemia, respectively). It causes decrease in the production of hemoglobin, which results in anemia of hypochromic microcytic type associated with dysplasia and damage of erythrocyte. Thalassemia major commonly appears during early infancy and is a life threatening disease, which results in severe anemia, which is progressive pallor and failure to thrive, are common. The manifestations of thalassemia major are skeletal and craniofacial deformities". (Hattab, 2013). In relation to oral health, Thalassemia is associated with increased dental caries (Al-Jobouri, 2011). Is a multifactorial human disease (Devi, 2009), in which acids produced by bacteria dissolve the enamel surface of a tooth, that result in localized demineralization of the inorganic components and destruction of organic constituents of the tooth, beginning on the external surfaces. (Hiratsuka, 2013).

There are few studies concerning occurrence of dental caries in patients with - Thalassemia major, their conclusion is the occurrence and rigorosity of this disease is greater in those patients than in standard controls (Al-Jobouri, 2007; Al-Raheem, et al., 2009). Previous researches found higher incidence of tooth caries and periodontal diseases in thalassemia patients. (Al-Wahadni, et al., 2002). The superiority of life for thalassemic patients' improved by blood transfusion and iron chelation therapy. However, this has led to development of many problems such as immunological changes triggering high infection level. (Kadam, et al., 2014). Therefore, this study designed to study the immunological background and concentration of mineral constituents of the blood to evaluate their role in the development of dental caries in Thalassemic patients.

Objectives

- To evaluate the incidence of dental caries, express the role of salivary IgA & serum (IL-6, IL-8,

IL-17) in relation to dental caries, and evaluate the alteration of serum minerals (Vitamin D, Phosphorous, Calcium, Magnesium) and their effect on the occurrence of dental caries in -Thalassemia major patients.

Materials and Methods

Patients' selection

The present study directed in Maternity and Children Hospital in Babylon province, AL - Hilla city from (November 2016 - April 2017). This study included (56) Thalassemic patients and (32) control group. Institutional ethical board of the hospital permitted, and informed consents took from the participants and their parents before their testing. Dental caries is registered by using (Decayed, Missing and Filled Index) (DMFT, dmft) rendering to the described criteria by the world health organization (WHO), 1987, and (2ml) of un stimulated saliva Samples were stored at (-20°C) in a deep freeze for subsequent analysis which was carried out in maximum period of three weeks. Before analyzing saliva, sample centrifuged at 4000 rpm for (15)

minutes; the clear supernatant solution separated by a micropipette and pouted in plain tubes unit used. Then drawing (5ml) of venous blood and putting in gel separated tube and let blood to clot at ambient temperature for (20-30) minutes, then Centrifugation for (10) minutes to obtain serum that pour off into plastic screw-cap vial and freeze (WHO, 2013).

To evaluate:

salivary IgA ELISA Kit catalog No::E-EL-H1355/96T
 serum IL - 6(Interleukin 6)ELISA Kit Catalog No:E-EL-H0102 / 96T

serum IL - 8(Interleukin 8)ELISA Kit Catalog No:E-EL-H0048/ 96T

serum IL-17 (Interleukin 17) ELISA Kit Catalog No:E-EL-H0105 / 96T

Vitamin. D3 according to ELFA Kit by VIDAS

P according to Phosphorus Kit(Phosphomolybdate method) by Mindray Ca according to Calcium Kit (Arsenazo III Method) by used Mindray

Mg according to Magnesium Kit(Xylidyl Blue Method) by Mindray

Table (1): Caries experience for primary and permanent teeth among study and control group.

Characteristic	Thalassemia	Control	P. value	Degree of Significance
	Mean ± S.D	Mean ± S.D		
Dmft	6.82 ± 3.24	2.43 ± 0.85	<0.05	S
DMFT	5.57 ± 2.51	2.79 ± 1.06	<0.05	S

Table (2): Mean and Standard Deviation for Thalassemia and Control groups regarding salivary IgA.

Characteristic	Thalassemia	Control	P	Significance
	Mean ± S.D	Mean ± S.D		
IgA	17.09 ± 5.56	143.87 ± 99.74	<0.001	HS

*high significant P<0.001

Table (3): Mean and S.D for Thalassemia and Control groups, interaction to IL-6, IL-8, & IL-17.

Characteristic	Thalassemia	Control	P value	T Test	Significance
	Mean ± S.D	Mean ± S.D			
IL-6	14.34 ± 15.00	7.43 ± 3.89	<0.05	2.551	S
IL-8	86.16 ± 89.71	72.17 ± 51.97	>0.05	0.807	NS
IL-17	65.80 ± 49.13	50.41 ± 43.65	>0.05	1.470	NS

*P< 0.05 was significant.*P> 0.05 was non-significant.

Statistics

Statistical analysis were done by (statistical package for social science; SPSS). SPSS version 17 for windows. T-test used to analyze the DMFT index, Immunological and Biochemical parameters to compare between Thalassemic and control groups.

P-value equal or less than 0.05 was considered significant for all analysis. Person's correlation used to examine the relation between (Immunological, Biochemical parameters and DMFT index) in both groups. (Daniel, 1999).

Table (4): Mean and Standard Deviation for Thalassemia and Control groups, interaction to Vit. D3, P, Mg++ & Ca++.

Characteristic	Thalassemia	Control	P value	T test	Significance
	Mean ± S.D	Mean ± S.D			
Vit. D3	18.40 ± 4.370	20.21 ± 3.55	<0.05	-1.986-	S
P	4.67 ± 1.07	7.03 ± 1.88	<0.001	-7.477-	HS
Mg++	1.92 ± 1.04	2.36 ± 0.50	<0.05	-2.226-	S
Ca++	1.91 ± 0.29	1.87 ± 0.17	>0.05	0.683	NS

*significant (P<0.05)**high significant (P<0.001).

Table(5): Correlation coefficient between IgA and (dmft, DMFT) in Thalassemia patients and Control subjects.

Thalassemia				Control			
Variable	Correlation parameter	dmft	DMFT	Variable	Correlation parameter	dmft	DMFT
IgA	r	0.416*	0.317*	IgA	r	0.295	0.004
	P	0.028	0.036		p	0.307	0.986

*P< 0.05 was significant.

Table(6):Correlation Coefficient between dmft, DMFT and serum proteins.

Thalassemia				Control			
Variable	Correlation parameter	dmft	DMFT	Variable	Correlation parameter	dmft	DMFT
IL-6	R	-0.097-	-0.016-	IL-6	r	0.063	0.203
	P	0.623	0.917		p	0.832	0.341
IL-8	R	0.015	0.163	IL-8	r	0.084	0.010
	P	0.938	0.290		p	0.776	0.963
IL-17	R	-0.094-	-0.179-	IL-17	r	-0.154-	-0.164-
	P	0.634	0.244		p	0.600	0.445

*P> 0.05 was not significant(chi-square test)

Results

Clinical examination show that all patients influenced by dental decay. Mean ± S.D of dmft value and

DMFT value in study and control groups, demonstrated in Table (1). dmft value is higher in study group (6.82 ± 3.24)

than in the control group is (2.43 ± 0.85) with statistically significant difference (p<0.05). Table (1).

Immunological examination of salivary IgA

The results of the study show that the Mean ± S.D of salivary IgA value for study group is (17.09 ± 5.56) which is lower than Mean ± S.D of control group is (143.87 ± 99.74) and the differences is highly significant statistically (P<0.001) .

Immunological evaluation of serum proteins

Results show that Mean ± S.D of IL-6 of serum protein study group is (14.34 ± 15.00), while for control group is (7.43 ± 3.89), which is significant statistically between the two groups (P<0.05), as shown in table (3). Concerning to IL-8 of serum protein Mean ± S.D of study group is (86.16 ± 89.71) comparing to mean ± S.D of control group is (72.17 ± 51.97) which is higher than control group but not significant statistically, as shown in table (3). Regarding to IL-17 of serum protein Mean ± S.D of study group is (65.80 ± 49.13) while for control group is (50.41 ± 43.65) which is higher but not significant statistically (P>0.05), as shown in table (3).

Biochemical evaluation of serum minerals constituents

Table (4) shows Mean & S.D of Vitamin D of serum mineral constituents for study group (18.40 ± 4.37) which is lower than control group (20.21 ± 3.55) with statistically significant differences between them (P<0.05). Concerning Phosphorous of serum mineral constituents, the Mean & S.D for study group is (4.67 ± 1.07) while for control group is (7.03 ± 1.88), which is highly significant (P<0.001). About Mg++ of serum mineral constituents, Mean ± S.D of study group is (1.92 ± 1.04) which is lower than control group (2.36 ± 0.50), and the difference is significant statistically. (P<0.05). (Table 4). Regarding Ca++ of serum mineral constituents Mean ± S.D of study, group and control group are (1.91 ± 0.29) (1.87 ± 0.17) respectively, with no significant differences statistically. (Table 4)

Correlation coefficient of salivary IgA with caries experience (dmft, DMFT)

Table (5) shows the correlation between IgA with (dmft, DMFT). In study group, there is significant positive correlation between IgA with (dmft, DMFT) (r=0.416), (P<0.05), (r=0.317), (P<0.05), respectively, while for control group the correlation is not significant. Table (3.7) show the correlation between IgA with (dmft, DMFT) . In study group, there is significant positive correlation between IgA with (dmft, DMFT) (r=0.416), (p<0.05), (r=0.317), (p<0.05) respectively .while for control group there is in significant correlation.

Correlation Coefficient between dmft, DMFT and serum proteins (IL-6, IL-8, IL-17).

Table (6) shows the correlation between "dmft, DMFT" with " IL-6, IL-8, IL-17" in both study and control groups which is not significant (P>0.05).

Discussion

According to the necessity of dental and oral health and their effect on the superiority of individual's life, specifically in thalassemic patients. Moreover, because of the variations in findings among other researches, on the status of dental caries in those patients, the "DMFT, dmft indicators are studied in a collection of patients complaining from - Thalassemia-major, compared with age matched control group".

Data of this study showed that all the participants had dental caries; this may signaled that the two groups might have some susceptibility to dental decay. However, current data show differences in the intensity of caries occurrence between those groups; this includes both deciduous and permanent teeth. This study found that, the B-Thalassemia patients had greater caries occurrence for both dentitions than control group. That in agreement with (Al-Hadithi, 2011; Kaur, 2012; Arora, et al., 2014; Pedullà, et al., 2015; Shooriabi, et al., 2016). The chronic nature of thalassemia may explain this level of dental decay in those patients. Because Thalassemia is a life-threatening problem, so, patients care less about oral and dental health care, in addition to poor motivation of these patients. Mostly their demands for dental treatments not done because of economic status and other limitations. However, are disagreement with (Scutellari, et al., 1994; Luglie, et al., 2002; Jahangirnezhad, et al., 2014). Who showed that caries-experiences in both study and control group was similar.

Salivary IgA is a part of immune system, considered as the first-line of defense against pathogens, limiting the adhesion of microbes. These antibodies react to the formation of dental plaque; therefore interfere with the pathogenesis of dental plaque, including dental caries and periodontal diseases. (Biesbrock, et al., 1991; Bokor-Bratić, 2000; Dodds, et al., 2005). In addition to that, play a role in neutralizing toxins, enzymes, and viruses; or by cooperation with other factors like lactoferrin and lysozyme. (Marcotte, 1998). In present study found low concentration of salivary IgA in thalassemic group as compared to control group, that explain the reason of high caries occurrence in those patients, through the reduction ability of this antibody to selectively connection with microorganisms in plaque and thus excess their colonization on the enamel tooth surface for those patients. That in agreement with (siamopoulou-mavridou, et al., 1992; Gomber, et al., 2006; Al-Hadithi, 2011). "Different immunological defects found in patients with - Thalassemia (Joob. 2017). Which include defective activity of the complement alternative pathway, reduced activity of monocytes and neutrophils, numerical or functional variation of different peripheral lymphocytes, increased serum immunoglobulin levels and blood abnormalities of serum level of cytokines". (Consolini, et al., 2001).

"Iron-overload, Desferrioxamine drug, splenectomy, immunosuppressive viruses, contact with allogeneic antigens in blood, and damaging of liver after hepatitis, those factors might lead to those immunological abnormalities". (Kadam, *et al.*, 2014). The effect of immunological variations according to the clinical course of β -Thalassemia is not determined, though they are treated according to the episodes of infections that those patients suffering from. (Consolini, *et al.*, 2001). Findings of present study revealed significant increase in the level of "IL-6" in the serum of patients with thalassemia, with increase in the serum level of "IL-8, IL-17" but not significant statistically. The increment in those cytokines level not correlated to high incidence of dental caries in thalassemic patients. These results are in contract with other studies. (Aggeli, *et al.*, 2005), who found increased expression of IL-6 in patients have β -Thalassemia major. That might be because of the raised oxidative stress which linked the iron-overload distinguished in β -Thalassemia major (Mitnick, *et al.*, 1981). El-Rasheidy, *et al.*, in (2016), found the same results and they proposed that the elevation of serum IL-6 level in β -Thalassemia major regarded as an indication for the pathogenesis of complications. "Ugucioni, *et al.*, in 1993 reported that high serum IL-8 concentrations in those patients could be due to continuous antigenic stimulation because of blood transfusion, and overload of iron which lead to stimulation of macrophages, they reached that macrophages and fibroblasts could be accountable for IL-8 production directly or indirectly via TNF- synthesis". The high level of serum IL-8 and TNF- recorded in homozygous poly transfused β -Thalassemia major. (Ugucioni *et al.*, 2011). Authors suggested the rise of these cytokines might be due to the activation of macrophage because of antigenic stimulation related to chronic transfusion therapy and iron overload, in addition, "it reported that during erythrophagocytosis, the monocytes activated, which may lead to production of multiple cytokines to improve the phagocytic function of monocytes" (Simms *et al.*, 1991). Variable immunological alterations, which, associated with β -Thalassemia Major like neutrophil impairment, macrophage phagocytic function and cytokine production, might explain the elevation of plasma IL-8 level. (Shfik, *et al.*, 2011). It known that "IL6 & IL-8" are essential factors for the pro-inflammatory response. The patho physiology of β -thalassemia are affected by these serum levels of these cytokines." (Oztürk *et al.*, 2001). Present study found that IL-17 level is more in β -Thalassemia patients as compared to the control group, but not significant. "At the site of inflammation, IL-17 may be produced locally without reaching high systemic levels," in contract with Balouchi, *et al.*, 2014. Previous studies suggested that, "the inflammation associated with the T cell immune response suppression." (Baharlou, *et al.*, 2016; Balouchi, *et al.*, 2014). "Elevation in the

level of IL-17 might contributed to abnormalities in the metabolism of iron and may be due to overstimulation of Th17". (Baharlou, *et al.*, 2016). The regulation of Th17 responses in patients with thalassemia may be affected by deposition of iron in the reticuloendothelial system like epithelial cells and macrophages and that lead to increase the levels of its cytokines in the circulation. (Lombardi, *et al.*, 1994) Darvishi, *et al.*, in 2016 suggested that the fall of serum IL-17 level due to immune abnormality, inflammation, and immune suppression caused by overload of iron in β -thalassemia patients. Results of present study demonstrate the concentration of Vit.D and blood elements (Ca⁺⁺, Mg⁺⁺ and P), which found low concentration of these constituents in thalassemic patients, that explain the increase in the incidence of dental caries in patients with thalassemia. (Vit. D, Ca⁺⁺, Mg⁺⁺ and P) have a significant role in the reduction risk of caries. Enamel is the greatest mineralized tissue in the body and it contains mainly of calcium and phosphorous. (Nanci, 2012). "Vit. D act as a significant role in craniofacial area development and maintenance of good oral health. (Berdal, *et al.*, 2005), and has a role in absorption of calcium and phosphorous from the food that is consumed, this absorption helps in the improvement of the strength of tooth and bone surrounding. (Parthasarathy, *et al.*, 2016). Receptors for vitamin D, found in cells of the immune system, which binds to vitamin D and increases the production of antimicrobial protein, which helps in fighting against bacteria that cause dental caries. (Hujuel, 2013). Different studies present that increase in the vitamin D insufficiency in the general population, and in addition to increased metabolic demands, iron overload and chronic medical care, making the deficiency of vitamin D is relatively common in patients with thalassemia major. (Wood, *et al.*, 2008; Napoli *et al.*, 2006; Moulas *et al.*, 1997). That in agreement with present study, which shows deficiency of Vit. D concentration in those patients, as a reason of the important role of vitamin D in formation of enamel and dentin. Thus, deficiency of this vitamin through time of primary and secondary tooth formation can explain high occurrence of dental caries in thalassemic patients. (Berdal, *et al.*, 2005). In this study, control group are thalassemia free, but have dental caries, which is associated with Vit. D deficiency in 50% of cases, this result in agreement with (Schroth, *et al.*, 2016). In other study, the authors recommended that the peak concentrations of vitamin D (≥ 75 nmol/L) related to 39% lower odds for dental decay. This seems to verify results from a recent review proposing that peak 25(OH) D concentrations (e.g., ≥ 75 nmol/L) are protecting against dental decay (Grant, 2011). Other study also proposes that kids reaching and exceeding the threshold of IOM for vitamin D sufficiency (≥ 50 nmol/L) are at 47% lesser odds for occurrence of tooth decay. (Schroth, *et al.*,

2016). In addition, Kühnisch *et al.*, in 2015 found that greater levels of 25(OH)D were accompanying with lesser occurrence of dental caries in secondary teeth. In addition, Hewison in 2010 and Grant in 2011 believed that improvement of vitamin D level might decrease the risk of dental decay".
 ***"Marshall and colleagues in 2003, recognized that lower intake of vitamin D during the first three years of age was significantly associated with the increase in the occurrence of caries in primary dentition, and also showed that there is strong relationship between insufficient intake of vitamin D and increase in caries occurrence". Longitudinal Iowa Fluoride Study, found very strong association between insufficient vitamin D intake and increased occurrence of caries". (Marshall, *et al.*, 2003). Different studies recognized that, high prevalence of vitamin D deficiency in general population, in addition to high metabolic needs, continuing medical care, and iron excess, that making, vitamin D deficiency is relatively common in patients with major thalassemia. (Moulas *et al.*, 1997; Napoli *et al.*, 2006; Wood, *et al.*, 2008), that in agreement with present study, which shows deficiency in Vit. D concentration in thalassemic patients. Concerning phosphorus (P), deficiency may lead to impairment in both formation and mineralization of bone matrix. (Bingham, *et al.*, 1974). The low concentration of serum P may have indirect adverse effect on bone formation. (Tanaka, *et al.*, 1973). Present study shows highly significant differences of P concentration between study and control groups due to decreased concentration of P in Thalassemic patients, that in agreement with (Choremis, *et al.*, 1965; Zamboni, *et al.*, 1986). Regarding Calcium concentration (Ca⁺⁺), in both study and control groups there is significant fall in serum Calcium concentration this explain present of dental caries in both groups. There is fall in mean concentration of Ca⁺⁺ in both control and study groups, which explain the increase in incidence of dental caries in both groups, in spite that patients group show slightly more mean concentration on Ca⁺⁺ level due to Ca supplementation in those patients, that's in contract with (Jawed, *et al.*, 2009). In addition, Hegde, *et al.*, in 2014, found a reverse association between the levels of salivary and serum calcium and dental caries confirming the importance of Calcium levels in inhibiting caries progression. Recently "several studies have recognized that the Magnesium, not Calcium, responsible for formation of hard enamel part that resists caries, therefore, without Magnesium only soft enamel formed and not important how much Calcium taken. (McKeith, 2006). It believed that high- level intakes of Calcium and Phosphorus intakes lead to hardening of enamel and prevent dental caries. However, later evidence indicates that there is no benefit from increase of these two elements without increase in Magnesium

consumption. Therefore, milk that high in Calcium and Phosphorus, but poor in Magnesium, not only affects Magnesium metabolism, but also antagonizes the mineral responsible for caries inhibition. (Rodale, *et al.*, 1978). McKeith, in 2006 showed the importance of role of Magnesium in stabilization of physical, chemical, and electro kinetic status of calcium present in the surface enamel. In addition to that, magnesium deficiency lead to enamel hypoplasia, impaired dentine mineralization and retardation of dentine formation. (Salkin, 1973; Cerklewski, 1983). Cerklewski in 1983 demonstrated that maternal dietary deficiency of Magnesium increases susceptibility for caries in offspring. A study in New Zealand showed that "the teeth resistant to caries have about twice magnesium amount as caries-prone teeth, regarding the hardness of teeth and bones consider the calcium as chalk and magnesium as superglue. The magnesium superglue binds and converts the chalk into teeth and superior bones. (Bourke, 2008). Hypomagnesaemia in -Thalassemia major patients as a result of dietary insufficiency of magnesium and the inappropriately high dose of Desferioxamine that shown in some studies (Al-Samarraietal., 2008, Rawa, *et al.*, 2015), are in agreement with present study that showed significant differences in Mg⁺⁺ concentration between study and control groups.

Conclusion

The decrease in serum level of "Vit. D and blood elements (P, Ca⁺⁺ & Mg⁺⁺) of thalassemic patients' leads to increase the incidence of dental caries in those patients than control group. In addition to that, a decrease in the salivary IgA level has a significant role in the occurrence of dental caries and increase its incidence in thalassemic patients. While elevation in the level of serum IL-6, IL-8, & IL-17 are not accompanying with the development of dental caries, and may be due to several immunological alterations that occur in such kind of patients.

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