

# Oro-facial manifestations, oxidative stress marker and antioxidant in serum and saliva of patients with Beta thalassemia major

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## Abstract

**Background:** Beta thalassemia is a typically autosomal recessive form of severe anemia which is caused by an imbalance of two types of protein (alpha and beta) subunits of hemoglobin. Oxidative stress imbalance is the equilibrium between pro-oxidant\antioxidant statuses in cellular system, which results in damaging the cells. Antioxidant is a chemical that delays the start or slows the rate of lipid oxidation reaction and it play a very important role in the body defense system against reactive oxygen species. The aims of this study were to recorded the oro-facial manifestations in beta thalassemic patients and assess the oxidative stress marker malondialdehyde in serum and saliv and their role in the pathogenesis of beta thalassemia and evaluation the antioxidant uric acid in serum and saliva of those patients.

**Methods:** The study included fifty eight beta thalassemic major patients, twenty eight patients with periodontitis and thirty patients without periodontitis and twenty nine healthy subjects that were age matched with the patients. Oro-facial manifestations recorded clinically, serum and saliva malondialdehyde and uric acid were measured in all subjects.

**Results :** The main oro-facial manifestations were malocclusion ,rodent face, brown pigmentation of oral mucosa and incompetent lip.The mean serum and saliva malondialdehyde was significantly higher in thalassemic patients with periodontitis ( $p<0.001$ ). Serum and saliva uric acid was significantly higher in thalassemic patients without periodontitis ( $p<0.001$ ).

**Conclusions:** Malocclusion was the most prevalent oro-facial manifestations in beta thalassemic patients, increased serum and saliva malondialdehde refer to the role of oxidative stress in the pathogenesis of beta thalassemia. Uric acid increased to counteract the elevation in the oxidative stress process.

**Key words:** Beta thalassemia, malondialdehyde, malocclusion, uric acid. (J Bagh Coll Dentistry 2015; 27(2):93-97).

## INTRODUCTION

The  $\beta$ -thalassemias are genetic disorders of hemoglobin synthesis characterized by deficient ( $\beta^+$ ) or absent ( $\beta^0$ ) synthesis of the beta-globin subunit of hemoglobin molecule <sup>(1)</sup>.

Beta thalassemia is prevalent in Mediterranean countries, the Middle East, Central Asia, India, Southern China, and the Far East as well as countries along the north coast of Africa and in South America. The highest carrier frequency is reported in Cyprus (14%), Sardinia (10.3%), and Southeast Asia <sup>(2)</sup>.Clinically divided into 3types; Thalassemia minor is most common form of  $\beta$ -thalassemia which lack of beta protein causes no problems in the normal functioning of the hemoglobin <sup>(3)</sup>, $\beta$ -thalassemia intermedia have mild to moderate anemia. The clinical phenotype of thalassemia intermedia is roughly intermediate between thalassemia major and minor <sup>(4)</sup>.

Thalassemia major, also known as Cooley's anemia and Mediterranean anemia, is the most severe form of  $\beta$ -thalassemia, since both mutations of both  $\beta$ -globin alleles results in severely impaired  $\beta$ -globin chain production <sup>(5)</sup>.

Beta thalassemia major characteristic by infancy onset severe anemia and required life-long blood transfusion for survival. Also untreated beta thalassemia major are hepatosplenomegaly, jaundice, growth retardation , poor musculature, leg ulcer, development of masses from extra-medullary hematopoiesis and bone deformities that result from expansion of the bone marrow <sup>(6,7)</sup>. Orofacial manifestations were prevalent in beta thalassemia, which included prominent frontal and check bossing, depression of the bridge of the nose, protrusive premaxilla (rodent face), flaring of the maxillary anterior teeth, spacing teeth, lip retraction and varying degree of malocclusion <sup>(8)</sup>

Free radicals can be defined as molecules or molecular fragments containing one or more unpaired electrons in atomic or molecular orbitals, which was steal electrons from cells, DNA, enzymes and cell membranes and lead to cells are damaged, Enzymes cannot do their jobs and Compromising the integrity of cellular membranes leaves them vulnerable to attack by viruses, bacteria and other invaders.

Malondialdehyde is one of several low-molecular-weight end products formed via the decomposition of certain primary and secondary lipid peroxidation products <sup>(9)</sup>.Antioxidants inhibit the formation of free radical and hence contributes to the stabilization of the lipid sample. Naturally,

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there is a dynamic balance between the amount of free-radicals generated in the body and antioxidants to quench and/or scavenge them and protect the body against their deleterious effects<sup>(10)</sup>. Uric acid was an important hydrophilic radical scavenger<sup>(11)</sup>. Urate is predominant salivary antioxidants. Previous studies showed estimation of the uric acid concentration in saliva might be better index of uric acid production in the body than the uric acid concentration in blood or urine<sup>(12)</sup>.

Periodontitis comprise a group of diseases, which are inflammatory in origin and generally affect the connective tissues attachment and supportive bone present around the teeth, There was direct link between oxidative mechanism and periodontitis<sup>(13,14)</sup>.

The purpose of this study is to assessment the oro-facial manifestations and evaluates the oxidative stress and its role in the pathogenesis of beta thalassemia major patients through the study of serum and saliva malondialdehyde and the status of uric acid in serum and saliva of beta thalassemic major patients.

## MATERIALS AND METHODS

Fifty eight thalassemic patients with no history of systemic diseases with age range 10-20 years, they divided into twenty eight thalassemic patients with periodontitis and thirty thalassemic patients without periodontitis and twenty nine healthy subjects with age matched with study patients. Thalassemic patients were diagnosed by hematologist according to laboratory investigations. Extra and intra-oral examination was done for each individual and also periodontitis diagnosed by assessment of clinical attachment loss.

Five ml. of blood sample was aspirated using disposable syringe from each subject which was collected in sterile disposable tubes then centrifuged and aspirated the supernatant serum and stored in frozen at -20 C° for analysis. Five

ml whole unstimulated saliva was taken from each subject and centrifuged, aspirated the clear supernatant and stored at -20C° for analysis. Malondialdehyde, lipid peroxidation end products react with thiobarbituric acid under acidic condition and heating to give a pink color that measured spectrophotometrically at 532nm. Serum and saliva uric acid was measured by oxidized uric acid by uricase to allantoin and hydrogen peroxide, which under the influence of POD, 4-aminophenaxzone and 2-4 Dichlorophenol sulfonate form a red quinoneimine compound that measured spectrophotometrically at 520nm.

## RESULTS

The mean age of thalassemic patients with periodontitis was 15.10 years, the mean age of thalassemic patients without periodontitis was 14.83 years and the mean age of healthy subjects was 15.83 years. The study showed that the main oro-facial manifestations were malocclusion (60%) followed by rodent face (35%) , brown pigmentation of oral mucosa (23%) then incompetent lip (8%).

The present study showed that serum and saliva malodialdehyde was highly significantly higher in thalassemic patients with periodontitis than that in other study groups using F-test ( $p < 0.001$ ). The study showed that there was no significant correlation between serum and saliva malondialdehyde in any of study groups ( $p > 0.05$ ).

This study also showed that the serum and saliva uric acid were highly significantly higher in thalassemic patients without periodontitis using F-test ( $p < 0.001$ ), there was a positive significant linear correlation between serum and saliva uric acid in thalassemic patients without periodontitis ( $p < 0.05$ ), also a positive highly significantly linear correlation between serum and saliva uric acid in thalassemic patients with periodontitis ( $p < 0.001$ ) was found.

**Table 1: The mean, SD and ANOVA test of malondialdehyde between study groups**

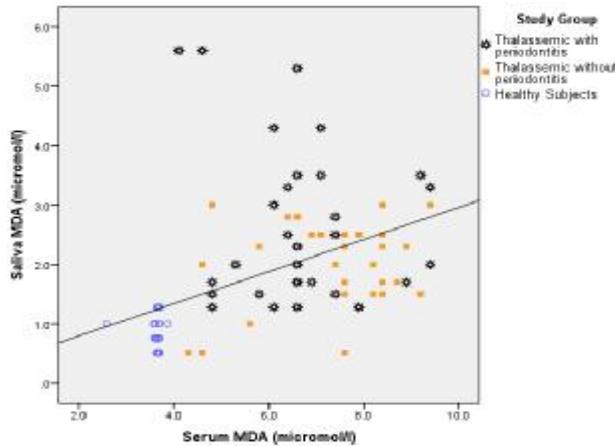
Variable & Study Groups	Mean	SD	F	P
<b>Serum MDA (µmol/l)</b>			87.125	<0.001**
Thalassemic with periodontitis	7.30	1.43		
Thalassemic without periodontitis	6.65	1.38		
Healthy subject	3.61	0.20		
<b>Saliva MDA (µmol/l)</b>			31.658	<0.001**
Thalassemic with periodontitis	2.65	1.32		
Thalassemic without periodontitis	2.01	0.73		
Healthy subject	0.85	0.26		

\*\*highly significant ( $p < 0.001$ )

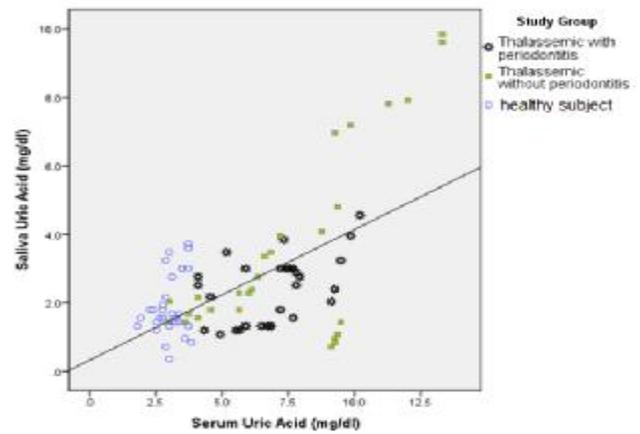
**Table 2: The mean, SD and ANOVA test of uric acid between study groups**

Study Groups		Mean	SD	F	P
Uric Acid	Serum Uric Acid (mg/dl)			45.897	<0.001**
	Thalassemic with periodontitis	6.93	1.72		
	Thalassemic without periodontitis	7.63	2.96		
	Healthy subject	3.02	0.53		
	Saliva Uric Acid (mg/dl)			5.147	0.008*
	Thalassemic with periodontitis	2.43	0.96		
	Thalassemic without periodontitis	3.34	2.72		
Healthy subject	1.91	0.90			

\*significant (p<0.05), \*\*highly significant (p<0.001)



**Figure 1: The correlation between serum and saliva malondialdehyde in each study group.**



**Figure 2: The correlation between serum and saliva uric acid in each study group.**

## DISCUSSION

The fact of orofacial complications is due to, when ineffective erythropoiesis damages the red blood cells (RBC) leading to severe anemia, the body responds by increasing the production of red blood cell, consequently causing expansion of bone marrow up to 15-30 times higher than the normal amount. The bone marrow expansion causes hyperplasia of the alveolar process of the maxilla at the expense of the sinus's normal volume. This in turn leads to the occurrence of maxillary anterior teeth protrusion, spacing between upper anteriors, anterior open bite. The skull and face deformities are closely related to the patient's age, the intensity of anemia, and the beginning time of treatment. Patients who receive inadequate blood transfusion in childhood will have more bony changes in adolescence <sup>(15)</sup>.

In the present study the results showed that the most prevalent orofacial manifestations were malocclusion (60%), then followed by rodent face (35%), oral soft tissue brown pigmentation (23%), then incompetent lip (8%) and this was agree with **Elangovan et al.** <sup>(16)</sup> who reported that the most prevalence orofacial manifestations of beta-

thalassemia major patients were malocclusion, rodent face and incompetent lip. **Norri and izdeen** <sup>(17)</sup>, **Jaafar and Al-Aswed,** <sup>(18)</sup> reported that malocclusion was the main oro-facial manifestations in beta thalassemia major patients.

Also **Ashraf et al.,** <sup>(19)</sup> reported that the most prevalent orofacial manifestation of beta thalassemic patients was malocclusion, the severity of malocclusion increased with age, also reported other features incompetent lip and gingival pigmentation with least percentage than malocclusion.

Oxidative stress is a basic mechanism in  $\beta$ -thalassemia major ( $\beta$ TM) pathological alternations. It has already been established that oxidative stress is increased in patients with iron overload. They initiate the process of autocatalytic free radical lipid peroxidation generating a large variety of potential genotoxic breakdown products, including alkoxyl radicals, peroxy radicals and aldehydes, such as malonedialdehyde. The level of thiobarbituric acid reacting substance thiobarbituric acid-reacting substance in the investigated beta thalassemia major patients was raised by more than 100% <sup>(20)</sup>.

The results showed increased in serum and saliva malondialdehyde levels in patients with beta thalassemia major and was significantly higher in thalassemic patients with periodontitis than that in the healthy subject and than that in thalassemic patients without periodontitis ( $p < 0.001$ ). The results of this study agree with **Naithani et al.**,<sup>(21)</sup> **Kattamis et al.**,<sup>(22)</sup> **Sonali et al.**<sup>(23)</sup> who reported increased level of serum malondialdehyde in beta thalassemic patients.

**Rai et al.**,<sup>(24)</sup> reported increased the level of malondialdehyde level in both serum and saliva of patients with periodontitis. **Khalili and Biloklytska**,<sup>(25)</sup> also revealed increased levels of malondialdehyde in saliva of patients with periodontitis.

The polymorphonuclear leukocytes are the initial and the predominant defense cells produced during the host response to bacterial pathogens. The periodontopathogens along with their products induce the generation of free radicals. This reactive oxygen species generation may be responsible for the bone resorption, degradation of connective tissue. The over production of malondialdehyde at the inflammatory site can be related to the greater degree of oxidative stress in patients with periodontitis<sup>(26)</sup>.

The study showed increased in serum and saliva uric acid in patients with beta thalassemia major the results agree with **Aldudak et al.**,<sup>(27)</sup> reported elevated serum levels of uric acid and phosphate in  $\beta$ TM patients.

The increased level of serum and saliva uric acid in thalassemic patients (with and without periodontitis) may be due to counteract the increased oxidative stress in those patients represented by the increased level of serum and saliva oxidative stress biomarker malondialdehyde.

Also deferoxamine therapy has been proved to be nephrotoxic and induce dose-dependent proximal tubular dysfunction by an unknown mechanism<sup>(28)</sup>.

Although transfusion therapy prolongs survival in beta thalassemia major, the absence of a physiological iron excretion mechanism leads to uneven accumulation of this metal in various body organs results in death, usually during the second decade of life, so it's may be accumulated in kidney and cause renal dysfunction<sup>(29)</sup>.

The results showed that uric acid was significantly higher in thalassemic patients without periodontitis than that in other study groups. This was agree with many studies suggested decreased level of uric acid in saliva of patients with periodontitis like disease<sup>(30,31)</sup>.

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### الخلاصة

**الخلفية:** التلاسيميا بيتا هونموذج من مرض وراثي من فقر الدم الشديد والذي كان سببه وجود خلل في نوعين من البروتين (الفا و بيتا) من مكونات الهيموغلوبين. الشد التاكسدي هو اختلال التوازن بين حالة الأوكسدة/مضادات الأوكسدة في النظام الخلوي مما يؤدي الى اتلاف الخلايا. مضادات الأوكسدة هي مواد كيميائية تؤخر بدء أو تبطئ معدل تفاعل أكسدة الدهون وأنها تلعب دورا مهما في نظام دفاع الجسم ضد انواع الأوكسجينات التفاعلية. كانت اهداف هذه الدراسة هي تسجيل الظواهر الوجهية والفموية لمرضى بيتا تلاسيميا و تقييم عامل الأوكسدة malondialdehyde ودوره في مرض بيتا تلاسيميا وتقييم مضادات الأوكسدة حامض اليوريك في دم ولعاب هؤلاء المرضى.

**الطرق:** شملت الدراسة ثمانية وخمسون مريضا مصابا بمرض بيتا تلاسيميا كبرى، ثمانية وعشرون مريضا يعانون من التهاب اللثة و ثلاثون مريضا غير مصابين بالتهاب اللثة مع تسعة وعشرين شخصا سليما اعمارهم متطابقة مع مرضى بيتا تلاسيميا . الظواهر الوجهية والفموية سجلت سريريا، malondialdehyde وكذلك حامض اليوريك تم قياسها في مصل ولعاب جميع الأشخاص.

**النتائج:** الظواهر الرئيسية الوجهية والفموية هي سوء الاطباق و وجه القوارض و التصبغ البني للعشاء المخاطي و عدم اطباق الشفتين. معدل malondialdehyde في المصل واللحاح عالي جدا بكثير لدى مرضى بيتا تلاسيميا مع التهاب اللثة ( $p < 0.001$ ). معدل حامض اليوريك في المصل واللحاح عالي جدا بكثير لدى مرضى بيتا تلاسيميا بدون التهاب اللثة ( $p < 0.001$ ).

**الاستنتاج:** سوء الاطباق المظهر الأكثر انتشارا بين المظاهر الوجهية والفموية لدى مرضى بيتا تلاسيميا، الزيادة في معدل مصل ولعاب malondialdehyde يشير الى دور الأوكسدة في التسبب بمرض بيتا تلاسيميا، حامض اليوريك يزداد لمواجهة الارتفاع الحاصل في عملية الشد التاكسدي. **الكلمات الرئيسية:** بيتا تلاسيميا، malondialdehyde، سوء الاطباق، حامض اليوريك.