

Evaluation of salivary immunoglobulin A level in thalassemic patients with periodontitis in comparison with thalassemic patients with healthy periodontium

Mohadeseh Arabsolghar MD¹, Parvin Arbabi MD², Mohammad Mohammadi MD¹, Alireza Farsinejad PhD³

Original Article

Abstract

BACKGROUND AND AIM: This study was conducted to evaluate salivary immunoglobulin A (IgA) level in thalassemic patients with periodontitis in comparison to thalassemic patients with healthy periodontium.

METHODS: Seventy-five patients were included in this study and were divided into three groups, group A: 25 major thalassemic patients with mild to moderate periodontitis, group B: 25 thalassemic patients with healthy periodontium, and group C: 25 systemically healthy people with normal periodontium. To measure salivary IgA levels, stimulated saliva was collected and analyzed by enzyme-linked immunosorbent assay (ELISA). The data were analyzed by t-test, ANOVA, and chi-square.

RESULTS: Salivary IgA was significantly different in major thalassemia patients with periodontitis (69 $\mu\text{m}/\text{ml}$) in comparison to major thalassemia patients with healthy periodontium (81 $\mu\text{m}/\text{ml}$) ($P < 0.05$). The highest level of salivary IgA was observed in the systemically healthy people with normal periodontium (88 $\mu\text{m}/\text{ml}$).

CONCLUSION: The levels of salivary IgA were decreased in major thalassemia patients with periodontitis and healthy periodontium in comparison to systemically healthy people with normal periodontium.

KEYWORDS: Major Thalassemia; Periodontitis; Immunoglobulin A

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Thalassemia is a congenital disorder in which protruded maxilla, severe malocclusion, open bite, flattened bridge of the nose, upper lip protrusion and glossitis have been observed in these patients.^{1,2} In thalassemic patients, T cell response is diminished and cellular immunity impairment is seen.³ So immune system of these patients cannot control infection well and preventive dentistry is necessary because of anemia, iron overload, and splenectomy.

Although the prevalence and severity of periodontal diseases have been demonstrated in association with a number of systemic

diseases,^{4,5} but little information exists on the relationship between periodontal disease and thalassemia and there are contradictory results in this area.⁶

In addition to the effect of thalassemia on systemic immunity, local defence system is also affected. Therefore, no changes in salivary immunoglobulin levels in thalassemia patients with gingivitis is due to lack of response of B-lymphocytes to gingival inflammation. It was shown that burning sensation and dry mouth are the most frequent oral manifestation in thalassemia patients. One of the causes of local immune deficiency is dry mouth that reduces salivary

1- Assistant Professor, Oral and Dental Diseases Research Center AND Kerman Social Determinants on Oral Health Research Center AND Department of Periodontics, School of Dentistry, Kerman University of Medical Sciences, Kerman, Iran

2- Assistant Professor, Department of Periodontics, School of Dentistry, Zahedan University of Medical Sciences, Zahedan, Iran

3- Pathology and Stem Cell Research Center, Kerman University of Medical Sciences, Kerman, Iran

Correspondence to: Mohadeseh Arabsolghar, MD

Email: m.a.solghar@kmu.ac.ir

immune function. So, local salivary immunity of thalassemia patients is not responsive well against the gingivitis.⁷

To date, few studies evaluated the level of salivary immunoglobulin A (IgA) in thalassemia patients, and the most studies have focused on serum immunoglobulin levels,⁸⁻¹⁰ that are inconsistent with salivary immunoglobulins titer results.⁷ Since there is little information about the relationship between periodontal disease and thalassemia, and the results of studies are contradictory, we compared salivary IgA titer in thalassemic patients with mild to moderate periodontitis and thalassemic patients with normal periodontium.

Methods

In this descriptive study, a total of 75 patients aged 10-25 years old were enrolled in three groups, group A: 25 major thalassemia patients with mild to moderate periodontitis, group B: 25 thalassemic patients with healthy periodontium, and group C: 25 systemically healthy people with normal periodontium. The groups were matched in terms of gender and age. This study was approved by Ethics Committee of Kerman University of Medical Sciences, Iran, and the ethical code was k/92/479. Exclusion criteria was any antibiotic use during the last 2 weeks. Before examination, the information of patients such as age, gender, and splenectomy was recorded. Diagnosis of periodontitis was based on clinical attachment level. To measure the clinical attachment level, a Williams probe was used to determine the distance between the cemento enamel junction (CEJ) and the base of pocket. In group A, amount of loss of attachment was between 1-4 mm while there was not any loss of attachment in groups B and C. Selection of patients was performed by an experienced periodontist. The patients were selected by convenience sampling.

Collection of saliva sample

Sampling of saliva was performed at the same time between 11 to 12 am in all patients.

A few drops of 1% solution of citric acid was poured on the back of patient's tongue to stimulate salivary secretion and after 1 minute, saliva was collected in sterilized tube and was sent to the laboratory in ice pack.

Enzyme-linked immunosorbent assay (ELISA) protocol

In this experiment, specific kit to measure salivary IgA (SigA, Salimertics, UK) has been used. When performing the test, the saliva samples which were frozen at -70 °C were first melted and after vortex they were centrifuged at 1500 g for 15 minutes in order to remove any mucins and other insoluble particles. Simultaneously, along with preparing the saliva samples, serial dilution (with the concentrations of 600, 200, 66.7, 22.2, 7.4 , and 2.5 mg/ml) of the standard solution was made by using standard solution available in the kit. Next, by use of the diluent of the kit, the saliva samples were diluted to a ratio of 1:5 and then 50 μ l of anti-SigA antibody which been conjugated with horseradish peroxidase (HRP) enzyme were added to the tubes containing the standard solutions and aslo the saliva. Accordingly, in such condition, the amount of extra antibody which was not attached to SigA was conversely proportional to the amount of SigA that was available in the samples. After 90 minutes incubation in room temperature and mixing, 50 μ l of the material inside the tubes were duplicated within the ELISA well plates whose surfaces were coated with human SigA. All of the free conjugated anti-SigA antibodies were connected to the SigA on the surface of ELISA wells. After 90 minutes of incubation and six times washing with washing buffer, conjugated antibody attached to the SigA on the surface was measured after adding 50 μ l substrate 3,3',5,5'-Tetramethylbenzidine (TMB), 45 min incubation in darkness and adding Stop solution (1 normal sulfuric acid). The absorbance rate in each of the wells was measured by ELISA reader machine (Biotech, USA) at a wavelength of 450 nm. Using the obtained standard curve, the concentration of

IgA dissolved in saliva in each of the samples was determined. Chi-square, t-test, analysis of variance and post-hoc Tukey test were used for statistical analysis.

Results

The age of all patients entered in this study was in the range of 10-25 years. Forty-six percent of patients were male and 54% were female. There was no significant relationship between age and gender and level of salivary IgA. IgA titer in group A was 69 $\mu\text{m}/\text{ml}$ and it was 81 $\mu\text{m}/\text{ml}$ and 88 $\mu\text{m}/\text{ml}$ in groups B and C, respectively.

Salivary IgA level is demonstrated in table 1 which expressed as mean \pm standard deviation (SD) in these three groups. The maximum level of salivary IgA was reported in group C, and the least titer was seen in group A. The difference between these three groups was statistically significant ($P < 0.05$). Prevalence of splenectomy was 12.5% in group A and 16.0% in group B. There was no statistically difference between splenectomy and level of IgA in these patients ($P > 0.05$).

Table 1. Comparison of salivary immunoglobulin A (IgA) levels in groups A, B, and C

Groups	Mean \pm SD ($\mu\text{m}/\text{ml}$)	P
Group A	69	8.5
Group B	81	6.8
Group B	81	6.8
Group C	88	5.9
Group A	69	8.5
Group C	88	5.9

SD: Standard deviation

Discussion

In thalassemic patients both local and systemic immunity systems are affected. Until now, a few studies evaluated the level of salivary IgA in thalassemic patients and most of them focused on serum immunoglobulins.⁸⁻¹⁰

In our study, there was significant difference between thalassemic patients with periodontitis in comparison with thalassemic ones with healthy periodontium. The level of IgA in group A was lower than the group B. Furthermore, IgA level was lower in

thalassemic groups in comparison with systemically healthy patients.

In Siamopoulou-Mavridou et al.⁷ study, the levels of stimulated salivary IgA in thalassemic patients with gingivitis was lower than healthy people. This result is similar to our study that was performed on periodontitis patients. In Motaleb Nejad et al. study,¹¹ the levels of salivary antibodies in thalassemic patients with gingivitis was evaluated and although mean antibody level in gingivitis group was higher than control group but this difference was not significant. They justified this slight increase of antibody by immune system dysfunction.¹¹ Their results were inconsistent with the results of our study.

Apart from the two above-mentioned study,⁷⁻¹¹ other studies have focused on the level of serum immunoglobulin and their results have been contradictory, so that some of them showed decrease while in other studies increase or no change was reported.

In Macdougall et al. study,¹² humoral immune system of children with iron deficiency was evaluated and the results demonstrated that the average concentration of serum IgA was normal and comparable with healthy adults. In another study that humoral immune system of thalassemic patients was investigated by Ghaffari et al.,¹³ none of humoral immunity markers such as IgG, IgM, IgE was significantly different compared to healthy controls but there was a modest rise in antibody levels of IgA. They concluded that there was no need to evaluate humoral immune system in routine immune tests in thalassemic patients. In another study, serum levels of IgM and IgG were significantly higher in these patients and with increasing age, the amount of these antibodies increased.¹⁴

In our study, IgA level was lower in thalassemic patients with periodontitis in comparison with thalassemic patients with healthy periodontium and both had lower values than systemically healthy people with normal periodontium. These differences between studies may be due to various

factors that affect the level of antibodies in saliva such as methodological problems and standardization of preparation, collection and storage of saliva, loss of protein during sample preparation and technical methods such as particle-enhanced nephelometric immunoassay and ELISA, flow rate of saliva, acute or chronic stress factors, age, gender, and geographic variables.¹⁵

In the studies that collected the unstimulated saliva, amounts of IgA were at least 3 times more than studies in which the stimulated saliva was used. Stimulated saliva sampling is preferred, because the method is easier and not affected by saliva storage in salivary glands. Salivary IgA is a part of adoptive immune system. Therefore, daily and seasonal variations, type of food, smoking, level and type of male and female hormones can influence on IgA levels in different times.¹⁵⁻¹⁸ In thalassemic patients that undergo splenectomy surgery, systemic as well as local immune response is reduced, so that in studies on these patients gingival inflammation was higher than thalassemic patients with no history of this surgery.¹⁹ Tovo et al.⁸ demonstrated that in the splenectomized patients in comparison to the non-splenectomized ones, a significant increase was observed in IgG and IgA in the

elder ones while a significant reduction was observed in IgM. Because of the low sample size of our study for patients underwent splenectomy, the data did not show any association between splenectomy and IgA level.

Our results showed that saliva of thalassemic patients cannot response well against infection because in these patients, function of T-cells is impaired and B-cells activity is influenced consequently and cannot defend well.

Conclusion

The results showed that salivary IgA in thalassemic patients with periodontist was lower than patients with normal periodontium. Because of impaired local immune systems in thalassemic patients, dental preventive and therapeutic consideration would be necessary.

Conflict of Interests

Authors have no conflict of interest.

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