INVESTIGATION OF PERIODONTAL PARAMETERS, TOTAL ANTIOXIDANT STATUS AND FERRITIN LEVELS IN PATIENTS WITH THALASSEMIA MAJOR

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Abstract

Our aim was to investigate periodontal status and total antioxidant status (TAS), ferritin levels of serum and gingival crevicular fluid (GCF) in patients with thalassemia major.

This study comprised 73 individuals aged between 11 and 16 years, divided into 3 groups; Group TMS: periodontally healthy 23 patients with thalassemia major, Group TMG: 25 patients with gingivitis and thalassemia major, and Group C: gingival and systemically healthy 25 individuals. Firstly, Plaque Index (PI), Gingival Index (GI) and Probing Depth (PD) data of all individuals in the study were recorded. For serum samples, 5 cc blood was taken from the antecubital region of the forearm of every individual. At the same time 4 samples of GCF were taken from the maxillar incisive of every individual, collected by standard Periopaper strips.

In the comparison of total mouth of the TMG group with the C and TMS groups, GI, PI, PD and GCF volume values were higher in the TMG group as expected ($p<0.001$). In the evaluation of the TMS and TMG groups in comparison with the C group, statistically significant differences were determined in the ferritin and TAS values of both GCF and serum ($P<0.05$). No correlation was found in the clinical and biochemical parameters investigated in serum and GCF of the TMG and TMS groups ($p>0.05$).

In this study of relatively few patients, the conclusion was reached that thalassemia major was not a direct risk factor in the formation of gingivitis. Further studies are needed to investigate the relationship between more severe periodontal disease and thalassemia major in different age groups.

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Introduction

Periodontal disease is evaluated as a chronic localised oral infection, which may originate from bacteria in the wide epithelial surfaces and which may trigger both a local and systemic immune-inflammatory response and ulcers in the periodontal pockets. Due to these characteristics, periodontal disease status may be affected by association with several diseases[1].

Thalassemia is a group of inherited autosomal recessive blood disorders that originated in the Mediterranean region[2]. In thalassemia the genetic defect results in a reduced rate of synthesis or no synthesis of one of the globin chains that make up hemoglobin. This can cause the formation of abnormal hemoglobin molecules, thus causing anemia, the characteristic presenting symptom of thalassemia[3].

In thalassemia major, oxidative stress is caused by increased iron in the plasma together with iron not associated with hemoglobin within the cell and impairment of the globin chain structure. It is also defined by the increased

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sensitivity of thalassemic erythrocytes to oxidative stress, oxidative damage created by free radicals and iron toxicity [4,5].

As an essential for life, oxygen is normally used by the defence system cells (primarily the monocyte/macrophage system) to render foreign substances harmless to the body. The products formed through the reduction of molecular oxygen are called 'reactive oxygen species' (ROS). The rate of formation of these free molecules (oxidant capacity) and their rate of removal (antioxidant capacity) is in balance and this is known as the 'oxidative balance'. If the rate of formation of these radicals increases or the rate of removal decreases, the oxidative balance is impaired and this status is known as 'oxidative stress' [6,7].

So our aim of this study to investigate periodontal status and total antioxidant status (TAS), ferritin levels of serum and gingival crevicular fluid (GCF) in patients with thalassemia major.

Material and Method

Study Groups

The study comprised 73 volunteer subjects aged between 11 and 16 years who were allocated to 3 groups; Group TMS, 23 thalassemia major patients who were periodontally healthy, Group TMG, 25 thalassemia major patients with gingivitis, GROUP C, 25 gingival and systemically healthy individuals as the control group. The two TM groups comprised patients who had been diagnosed with thalassemia major from clinical and blood test results at the Paediatric Health and Diseases Department of Dicle University Medical Faculty. These patients were referred to the Periodontology Clinic of Dicle University Dental Faculty and were separated into two groups; 23 periodontally healthy patients (Group TMS) and 25 patients with gingivitis (Group TMG).

The control group (GROUP C) individuals who were periodontally and systemically healthy were selected from patients presenting at the Paediatric Dentistry Department of Dicle University Dental Faculty and who met the study criteria. All the subjects included in the study underwent a detailed periodontal examination. In the periodontal evaluation, the Gingival Index (GI), Plaque Index (PI) and Probing Depth (PD) measurements were made. Serum and GCF samples were taken for biochemical measurements from TAS and ferritin analyses.

Approval for the study was granted by the Ethics Committee of Dicle University, Faculty of Dentistry (2009/22), and informed consent was obtained from the parents of all the study participants.

Periodontal Evaluation

To determine gingival inflammation, the GI defined by Löe and Silness was used. For plaque measurements, the PI defined by Löe and Silness was used.

Probing depth was measured with the Williams periodontal probe (Hu Friedy, Chicago, Illinois, USA) from six points (mesiobuccal, buccal, distobuccal, mesiolingual, lingual and distolingual).

GCF Collection

GCF samples were collected using 4 standard paper strips (Periopaper, Proflow Inc, New York, USA) from the mesial and distal regions of the maxillary centrals. The obtained samples were recorded using the Periotron 8000 (Oraflow Inc, Plainview, New York, USA) as Periotron units. Before the procedure, supragingival plaque was removed with a sterile curette, the sample area was dried with pressurised air and saliva was removed with a cotton swab. Using slight pressure until it was felt, the paper strips were placed into the periodontal pocket and left for 30 seconds. Each patient had previously been assigned a number and for each one, 4 samples were placed in a 1.5ml Eppendorf tube containing 400 µl pH: 7.4 PBS.

Sample Collection and Preparation for Biochemical Analysis

The samples were collected from all the study participants in the morning. 5cc blood samples were taken from the antecubital area into a vacuum biochemical tube (Vacutainer). Attention was paid that the subjects were fasted for this procedure. The obtained blood samples were centrifuged at 4000 rpm for 5 mins (Nüve 1200 R). The serum samples were transferred to Eppendorf tubes. All samples were stored at -80°C until ferritin, TAS levels were investigated in the laboratory of the Biochemistry Department of the Medical Faculty, Dicle University.

Biochemical Evaluation

All the samples were brought to room temperature on the day of biochemical analysis. After thawing, the samples were centrifuged at +40°C at 10,000 rpm for 10 mins in a refrigerated centrifuge (Eppendorf MR5415) then transferred to new Eppendorf tubes.

Total Antioxidant Status (TAS) and Ferritin Analysis

The fully automatic method developed by Erel, is a powerful method of measuring total antioxidant status of the body against free radicals. The ferritin levels of all the samples were determined by COBAS e601 analyser.

Statistical Analysis

The statistics programme SPSS 17.0 for Windows® was used. Data were stated as mean ± standard deviation. In the triple comparisons between the groups, one-way ANOVA test was used for data showing normal distribution and for data not showing normal distribution, Mann Whitney U test was applied with Bonferroni corrections by Kruskal-Wallis test method. For paired comparisons, independent t-test and Mann Whitney U test were applied. All laboratory values were based on concentration.

Results

The study participants were 30 female (41.1%) and 43 male (58.9%) with a mean age of 12.89±1.56 years (range 11-16 years).

DOS	Group C	Group TMS	Group TMG	Kruskal Wallis P	Mann Whitney U test with Bonferroni correction
Ferritin (ng/mL)	3.61±2.45	16.1±10.23	19.47±9.28	0.000***	^{x}P<0.017
					^{y}P<0.017
					^{z}P>0.017^{ns}
TAS (mmol T. eq/L)	0.24±0.06	0.20±0.02	0.19±0.02	0.003**	^{x}P<0.017
					^{y}P<0.017
					^{z}P>0.017^{ns}

ns= Not significant, *= Significantly (p<0.05), **= Very significant (p<0.01), ***= advanced significantly (p<0.001)
x = C- TMS, y = K- TMG, z = TMS- TMG

Table 1. Comparison of GCF: Ferritin and TAS levels of all Groups

In comparison among the all groups of GCF, statistically highly significant differences were determined in Ferritin (p<0.001). Statistically very significant differences were determined in the TAS levels of GCF (p<0.01). (Table 1)

In comparison among the all groups of serum, statistically highly significant differences were determined in Ferritin levels (p<0.001). Statistically significant differences were determined in the TAS levels of serum (p<0.05). (Table 2)

Serum	Group C	Group TMS	Group TMG	One way ANOVA P	t-test p
Ferritin (ng/mL)	33.61±22.30	3128.59±2162.18	3940.47±2095.12	0.000***	^{x}P<0.001
					^{y}P<0.001
					^{z}P>0.05^{ns}
TAS (mmol T. eq/L)	1.64±0.20	1.51±0.18	1.50±0.21	0.031*	^{x}P<0.05
					^{y}P<0.05
					^{z}P>0.05^{ns}

ns= Not significant, *= Significantly (p<0.05), **= Very significant (p<0.01), ***= advanced significantly (p<0.001)
x = C- TMS, y = K- TMG, z = TMS- TMG

Table 2. Comparison of Serum: Ferritin and TAS levels of all Groups

Comparison of GCF Ferritin and TAS levels between the TMS Groups and the Control Group.

In the paired comparison between the study groups of GCF, statistically highly significant differences were determined in Ferritin levels (p<0.001). Significant differences were determined in the TAS levels of GCF (p<0.05). (Table 1)

Comparison of Serum Ferritin and TAS levels between the TMS Groups and the Control Group.

In the paired comparison between the study groups of serum, statistically highly significant differences were determined in Ferritin levels (p<0.001). Significant differences were determined in the TAS levels of serum (p<0.05). (Table 2)

Comparison of Periodontal Status Betweeen the TMS Group and TMG Group

In the paired comparison between the study groups in terms of GI, PI, PD and GCF volume values, on the whole mouth basis and the GCF sampling area basis, statistically highly significant differences were determined (p<0.001). (Table 3)

Serum	Group C	Group TMS	Group TMG	Kruskal Wallis P	Mann Whitney U test with Bonferroni correction
PI	0.18±0.15	0.15±0.12	1.46±0.33	0.000***	^{x}P>0.017^{ns}
					^{y}P<0.017
					^{z}P<0.017
GI	0.06±0.08	0.07±0.09	1.22±0.28	0.000***	^{x}P>0.017^{ns}
					^{y}P<0.017
					^{z}P<0.017
CD (mm)	1.05±0.11	1.07±0.09	1.72±0.28	0.000***	^{x}P>0.017^{ns}
					^{y}P<0.017
					^{z}P<0.017
DOS H. (µl)	0.40±0.02	0.41±0.02	0.67±0.08	0.000***	^{x}P>0.017^{ns}
					^{y}P<0.017
					^{z}P<0.017

ns= Not significant, *= Significantly (p<0.05), **= Very significant (p<0.01), ***= advanced significantly (p<0.001)
x = C- TMS, y = K- TMG, z = TMS- TMG

Table 3. Comparison of Periodontal Status Betweeen the TMS Group and TMG Group

Discussion

The factor leading to the destruction of periodontal tissue is a complex interaction between microbial pathogenic bacteria in dental plaque and the defence mechanism of the host tissue. In addition to the direct pathological effects of the bacteria, destruction of periodontal tissue occurs to a great extent by means of indirect mechanisms of bacterial host interaction. Therefore, nowadays studies oriented towards the physiopathology and etiopathology of periodontal diseases, have put into place the understanding of the role played by indirect mechanisms in the pathogenesis of periodontal diseases [8]. In a clearer statement, proteolytic enzymes and their inhibitors and the homeostatic balance between ROS and the antioxidant defence system are responsible for the protection and repair of cellular and molecular components of live tissue. This irregularity is 32 -82% genetic in origin [9].

Thus data obtained from studies have revealed a strong direct association between periodontal diseases with genetic diseases and systemic diseases [10,11]. Thalassemia is both a genetic and haemotological disease showing autosomal recessive transfer in the heterozygote form and in the homozygote form leading to chronic haemolytic anaemia [12]. The oxidative damage created by free radicals to the thalassemic erythrocytes is determined by iron toxicity [13, 14].

The TAS level only showed only the total of the independent antioxidant concentrations present in that environment. This is a more economical determination of TAS level rather than individual evaluations of rates of antioxidants [15]. Therefore, in this study the determination of TAS level was selected.

Ferritin, which is a major protein store in the body, is a molecule which can centrally store iron as varying amounts of ferric hydroxyphosphate. Ferritin is found in all the cells of the body and all tissue fluid [16]. Serum ferritin is the most frequently used marker to determine the level of iron stored in the body. Therefore, serum ferritin concentration level has been defined as a diagnostic technique to show the iron store in the body in thalassemia patients who are undergoing transfusion.

The mean age ranges of the groups included in the study were similar to each other, which enabled a comparison of the health status between the groups. Moreover, heart failure in TM patients may result in early death [17]. Therefore, the patient group regularly undergoing transfusions were selected at this age range.

There are an insufficient number of studies in literature on dental and periodontal disease in thalassemia patients. It has been reported that as systemic problems are focussed on in TM patients, a low level of interest is shown in oral hygiene [18]. However, these factors are predisposing factors for the formation of dental plaque. Nonetheless, in a study of 50 patients to determine a relationship between TM oral findings and periodontal disease, Kaplan RI et al [19] reported malocclusion and dryness of the mouth were caused by the patient not being able to close the mouth due to maxillary growth. In the current study, as expected, the PI scores of the C and TMS groups for both the whole mouth and the sample area were very low compared to the TMG group. If the plaque scores of the TMG group had been close to those of the two periodontally healthy groups, it would have strengthened the idea that haemotological problems come to the fore in the formation of gingivitis. However, the results obtained support the idea that thalassemia may have no effect in the development of gingivitis. In the same study, gingival inflammation was observed in 16 (32%) patients, one or more periodontal pockets in 5 (10%) patients, supra or sub-gingival calculus in 5(10%) patients and only supra-gingival calculus in 2 (4%) patients. In conclusion, there were findings of gingivitis in 28 (56%) patients. These findings were declared to be similar to the rate of gingivitis patients seen in the general population.

Parallel to these findings, in the current study of 48 TM patients, 25 (52%) patients were determined to be diseased and 23 (48%) patients were determined to be healthy from the periodontal examination. This also shows the incidence of gingivitis in TM patients to be similar to that of the general population. Study of Al-Wahadni et al supporting these results, to determine a relationship between TM and the progression of periodontal disease, the GI and PD parameters 61 TM patients and 63 healthy controls were defined. The conclusion was reached that there was no relationship between TM and the progression of periodontal disease[20].

While clinical parameters such as probing depth, plaque index, gingival index and radiographic measurement of alveolar bone loss provide sufficient information about the severity of periodontal disease, they cannot be used to measure the disease activity [21]. Objective parameters such as saliva, blood, bacteria plaque and GCF are useful for this. Therefore, in this study, importance was given to the examination of oral biological fluids such as GCF and systemic biological fluids such as serum as biochemical markers of periodontal diseases.

There are few studies in literature investigating the GCF ferritin level in periodontal disease patients. In a study by Enhoş et al [22] of iron-deficient anaemic subjects and a control group, the GCF ferritin concentrations were seen to have significantly reduced following periodontal treatment, but reduced local ferritin did not cause any change in systemic ferritin values. The results of the current study conformed with those of the above-mentioned study in that GCF ferritin levels were determined to increase relative to inflammation, which is one of the causes of periodontal disease.

In research of the study by Naithani R et al [23] examining serum ferritin in paediatric TM patients and serum studies in literature of GCF parameters of TM patients, it was seen that together with an increase in oxidative stress, the antioxidant defence effect decreased. When GCF was evaluated locally, the reason for no correlation between TAS and ferritin may be that different antioxidants were present rather than those we examined. It is also thought that it may originate from the presence of different local or systemic defence mechanisms, which show biological activity, although current knowledge is not as yet complete.

Conclusion

When the blood and GCF, TAS levels of the TMS and TMG groups were compared, there was no statistical significance. Although the systemic and local ferritin level of the TM patient groups was seen to be high compared to the control group, no relationship was determined between periodontal parameters, which leads to the conclusion that TM is not a risk factor associated with periodontal disease. There is a need for further extensive epidemiological studies which would include subjects in a greater age range, with a more severe level of periodontal disease, which would determine local variables of periodontal treatment and evaluate a greater number of individuals.

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