



**Biochemical and Immunological Study of Saliva in
Relation to oral health status in Thalassemia
Major Patients in Mosul**

ABSTRACT

Aims: The present study aimed to investigate some biochemical (Lysozyme and Peroxidase enzymes) and immunological (S-IgA) changes in saliva of thalassemia major patients and the correlation of these changes with the oral health status measured by the dmft/DMFT (decay, missing, filling tooth index for deciduous and permanent dentition), plaque and gingival indices. **Materials and Methods:** The study was carried out in Ibn Al-Atheer Teaching Hospital in Mosul and involved (91) subjects. The study group (70 thalassemia major patients) and the control group (21 normal non-thalassemic subjects). The study group was divided into two subgroups (35 each) according to the history of disease. Data was collected from each patient including medical status and oral health status indices and saliva samples were collected from each patient and stored at -20°C to be analyzed for salivary Lysozyme by lysoplate method, salivary Peroxidase activity and salivary secretary S-IgA by ELISA. **Results:** Showed that the dmft/DMFT plaque and gingival indices in thalassemia major patients were significantly higher ($P < 0.001$) than normal subjects. The salivary lysozyme in thalassemia major patients was significantly lower ($P < 0.001$) than normal subjects. The salivary peroxidase activity was nearly higher in thalassemic patients than normal subjects. The salivary S-IgA in thalassemic patients was significantly lower ($P < 0.001$) than normal subjects. **Conclusions:** The study showed a significant correlation between the changes in some of the salivary constituents and the high prevalence of dental caries, plaque and gingivitis in thalassemia major patients.

الخلاصة

أهداف الدراسة: يهدف هذا البحث لدراسة بعض التغيرات الكيميوحيوية والمناعية للعاب مرضى الثلاسيميا الكبرى مع تقييم صحة الفم والأسنان لديهم إضافة إلى دراسة العلاقة بين هذه التغيرات ومقاييس صحة الفم والأسنان. شملت الدراسة الكلوبولين المناعي A فضلا عن الأنزيم الحالّ و أنزيم البيروكسيداز. **المواد وطريقة العمل:** أجريت الدراسة في مركز الثلاسيميا التابع لمستشفى ابن الأثير التعليمي في مدينة الموصل وشملت (91) شخصا تم تقسيمهم إلى مجموعتين رئيسيتين ، عينة الدراسة (مرضى الثلاسيميا الكبرى) والتي كانت مكونة من (70) مريض ثلاسيميا كبرى من الذين يراجعون المركز لغرض العلاج ، وعينة السيطرة (غير المصابين) والتي كانت مكونة من (21) شخصا. قسمت عينة الدراسة إلى

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مجموعتين طبقا لتاريخ المرض وكل مجموعة كانت مكونة من (٣٥) مريضا. التحليل الكيميوحيوي لللعاب شمل طريقة لونية لقياس فعالية أنزيم البيروكسيداز في اللعاب، وطريقة Iysoplate للأنزيم الحال في اللعاب. التحليل المناعي بواسطة فحص اليزا (ELISA) لقياس الكلوبولين المناعي A في اللعاب (S-IgA).
النتائج: أظهرت الدراسة ان هناك فرق معنوي كبير في مقاييس صحة الفم (التسوس والبلاك والتهاب اللثة) بين مرضى الثلاسيميا الكبرى والأشخاص الأصحاء إذ كانت هذه المقاييس أعلى بكثير لدى مرضى الثلاسيميا الكبرى. اظهر تحليل الأنزيم الحال في لعاب مرضى الثلاسيميا الكبرى انخفاضا معنويا ملحوظا مقارنة مع الأشخاص الأصحاء. كانت فعالية إنزيم البيروكسيداز في لعاب مرضى الثلاسيميا الكبرى أعلى من الأشخاص الأصحاء. اظهر التحليل المناعي للكلوبولين المناعي A في اللعاب لدى مرضى الثلاسيميا الكبرى انخفاضا معنويا ملحوظا مقارنة مع الأشخاص الأصحاء. أظهرت الدراسة وجود علاقة معنوية بين التغييرات في بعض مكونات اللعاب والارتفاع الكبير في مقاييس صحة الفم والأسنان لدى مرضى الثلاسيميا الكبرى.
الاستنتاجات: أظهرت النتائج وجود ارتفاع ملحوظ في مقاييس تسوس الأسنان والبلاك والتهاب اللثة لدى مرضى الثلاسيميا الكبرى. كما وأظهرت النتائج وجود تغييرات معنوية ملحوظة في بعض المكونات الكيميوحيوية والمناعية في لعاب المرضى. إضافة إلى وجود علاقة معنوية بين هذه

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INTRODUCTION

The term thalassemia is derived from the Greek term 'Thalassa' which means "the sea" (the Mediterranean) and -emia in the blood. This term was first used by Whipple and Bradford⁽¹⁾. Thalassemias are group of congenital anemias that have in common deficient synthesis of one or more of the globin subunits of the normal human hemoglobins⁽²⁾. There may be homozygous or heterozygous factors. This has a bearing on the severity of the disease⁽³⁾. Person having heterozygous gene (Thalassemia minor) usually has little or no manifestation of the disease, whereas a person having a homozygous gene (thalassemia major) is severely affected⁽⁴⁾. Oral manifestations may be more common in thalassemias than the literatures would indicate. The rapid changes in the alveolar and facial bones corresponding to the deterioration of the patient's general condition⁽⁵⁾.

There have been several attempts over years to establish methods for diagnosis and prognosis of oral diseases by compositional analysis of saliva. Thus salivary diagnosis is anticipated to be particularly useful in cases where repeated samples of body fluids are needed but where drawing blood is impractical, unethical or both^(6; 7; 8; 9; 10; 11; 12).

This study was conducted to investigate some of the biochemical and immunological changes in saliva of thalassemia major patient and correlate these changes with the oral health status measured by the dmft/DMFT, plaque and gingival indices.

MATERIALS AND METHODS

Subject Group : The study was carried out on total number of 91 subjects attended Ibn Al-Atheer Teaching Hospital in Mosul with age range between 8-18 years old.

Control Group : This group consisted of 21 healthy looking subjects (11 males and 10 females) with mean age 13 ± 2.18 years.

Study Group : This group consisted of 70 Thalassemia major patients (35 males and 35 females) with age range between 8-18 years old. This group was subdivided into two subgroups according diagnosis, severity, attendance or number of visits to the center and commitment with the treatment especially the chelating therapy.

Subgroup 1 : Consisted of 35 subjects (18 female and 17 male) with mean age 11.69 ± 1.02 years. They were diagnosed at early stage of the disease and attended the center mainly once every month and more committed with treatment especially the chelating therapy.

Subgroup 2 : Consisted of 35 subjects (18 male and 17 female) with mean age 13.5 ± 1.69 . They were diagnosed later than the first group and attended the center 2-3 times every month and less committed with the treatment especially the chelating therapy.

Permission to examine the subjects and data source were obtained from the concerned authority in Ministry of Health and Thalassemia Center in Ibn Al-Atheer Teaching Hospital. The basic methods for examination of each subject and determine dmft/DMFT, PI, GI were carried as describe previously

Collection of Saliva: Unstimulated whole mixed saliva was collected from each subject as described by Dittmer⁽¹⁸⁾. Salivary samples were centrifuged at 3500 rpm for 10 minutes then clear supernatant separated and placed in sterile plastic Eppendorff tubes (0.5 ml) and stored at -20°C until used.

Biochemical Analysis: *a. Salivary peroxidase:* Salivary peroxidase activity was measured by colorimetric method⁽¹⁹⁾. This method depends on the formation of a color product with measurable photo absorption at a particular wavelength by visible spectrophotometer.

b. Salivary Lysozyme Activity: Lysoplate assay method was used as described previously⁽²⁰⁾. This assay depends on the ability of the lysozyme to break the bond between N-acetyl glucosamine (NAG) and N-acetylmuramic (NAM) which is the basic constituent of the bacterial cell wall. The bacteria used in the assay were *Micrococcus lysodeikticus*.

Immunological Analysis: Salivary secretary IgA level was measured by ELISA using ELISA kit (IMMUCHROM GMBH, Germany) for the determination of s-IgA in stool and saliva. The test was performed according to the manufacturer instructions. ELISA reader with filter 450 nm (reference filter 620 nm) was used (DNM-9602 microplate reader (BEIJING PROLONG NEWTECHNOLOGY CO. LTD).

Statistical Analysis of the Data : Data analysis was carried out using unpaired student t-test for all comparisons. And Pearson correlation coefficient to find the relationship among the measured variables.

RESULTS

Oral Health Status :

A. Subgroups and Control Group : The mean values of dmft/DMFT, plaque and gingival indices (10.23, 2.01 and 2.03 respectively) in subgroup 1 were higher than in control group (6.55, 1.59 and 1.6 respectively) and the difference was highly significant ($P < 0.001$). Also the same indices (11.37, 2.15 and 2.25) were higher in subgroup 2 than control group with high significant difference ($P < 0.001$). Subgroup 2 showed higher dmft/DMFT and plaque indices than subgroup 1 and the difference was significant ($P < 0.05$), while gingival index was significant at $P < 0.01$ (Figure 1)

B. Total Thalassemic and Control Groups : All indices measured in this study were higher in total thalassemic group than control group (Figure 2) with high significant difference ($P < 0.001$).

Biochemical Analysis : Salivary Enzymes :

A. Subgroups and Control Group : The mean values of SLZ in subgroups 1 and 2 (1.96 $\mu\text{g/ml}$ and 2.17 mg/ml respectively), were lower than control group (6.19 $\mu\text{g/ml}$) and the difference was highly significant ($P < 0.001$). Whereas the mean value of SPX activity in subgroups 1 and 2 (0.10 U/ml) was higher than control group (0.04 U/ml) but the difference was not statistically significant ($P = 0.06-0.08$). Comparison between the two subgroups showed no significant difference ($P > 0.05$) for both salivary enzymes (Fig 3).

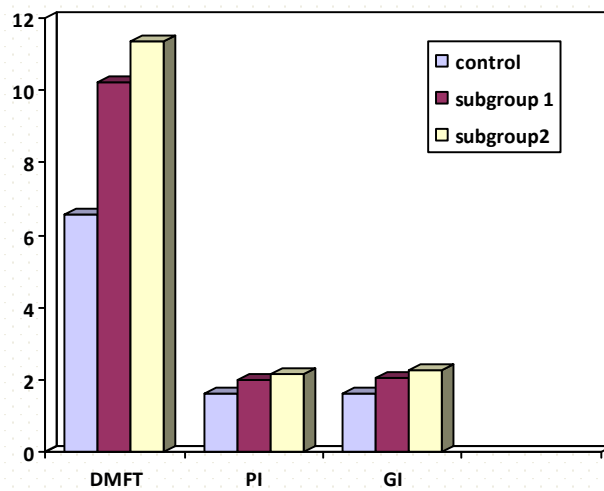


Figure 1 : Comparison of DMFT, PI and GI between subgroups 1, 2 and control groups.

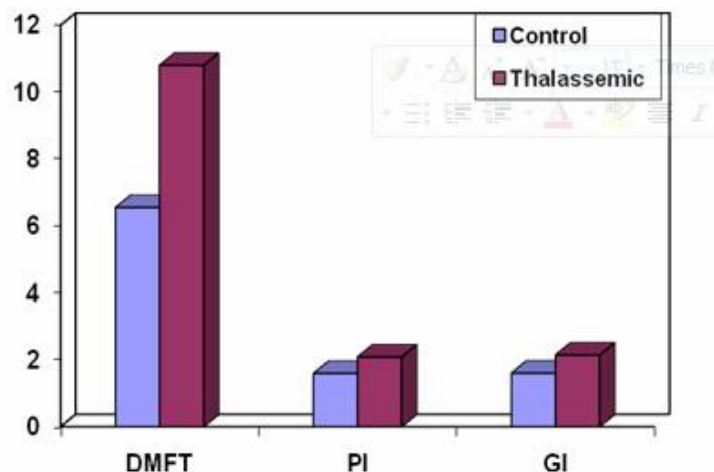


Figure 2 : Comparison of DMFT, PI and GI between total thalassemic and control groups .

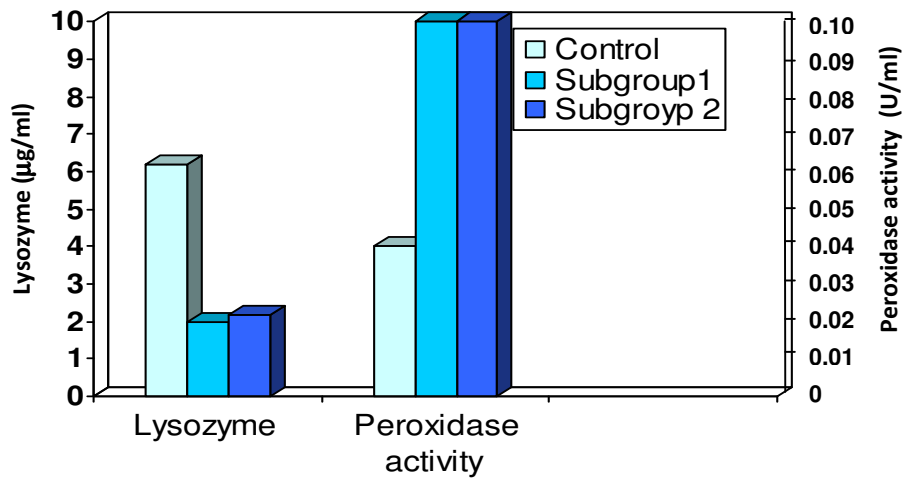


Figure 3 : Comparison of Salivary Lysozyme & Peroxidase activity between subgroups 1, 2 & control group.

B. Total Thalassemic and Control Groups : The mean value of SLZ in total thalassemic group was (2.06) µ/ml which was lower than control group and the difference was highly significant ($P < 0.001$). The mean value of SPX activity in total thalassemic group was 0.10 U/ml. which was higher than the control group but the difference was not statistically significant ($P = 0.06$) (Figure 4).

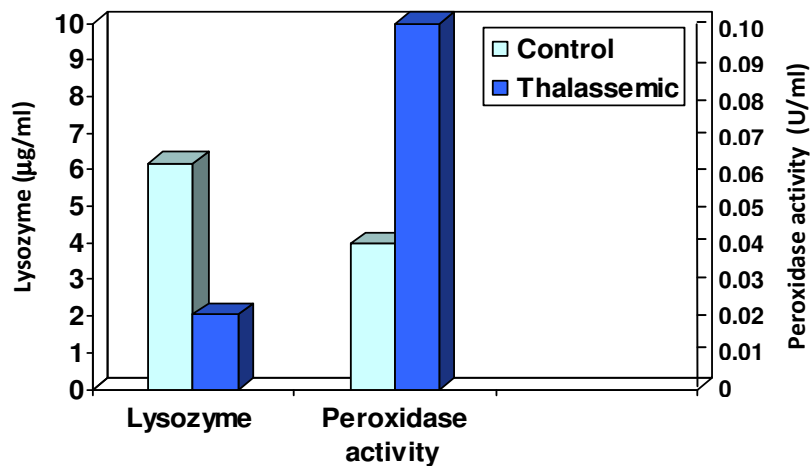


Figure 4 : Comparison of Salivary Lysozyme & Peroxidase activity between total thalassemic and control groups

Immunological Assay : Salivary Secretory IgA (S-IgA) :

A. Subgroups and Control Group : The mean value of S-IgA in subgroups 1 and 2 (318.9 and 310.45 µ/ml respectively) were lower than control group (554.74 µg/ml) with highly

significant difference ($P < 0.001$). In comparison between subgroups 1 and 2, there was no significant difference ($P > 0.05$) (Figure 5).

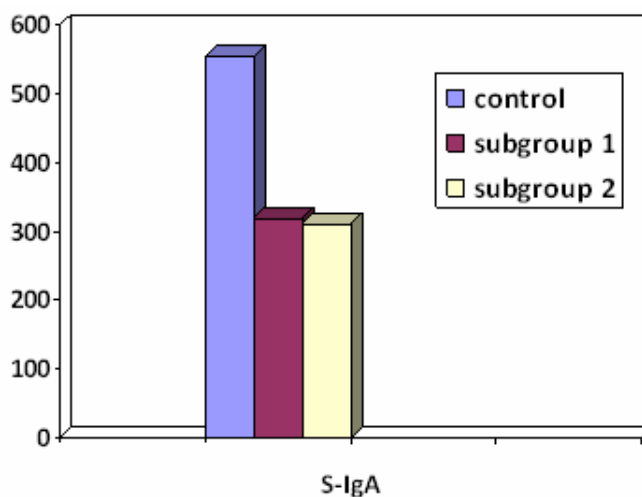


Figure 5 : Comparison of S-IgA ($\mu\text{g/ml}$) between subgroups 1, 2 & control group.

C. Total Thalassemic Group and Control Group : The mean value of S-IgA in total thalassemic group was $314.68 \mu\text{g/ml}$ which was lower than control group and the difference was highly significant ($P < 0.001$) (Figure 6).

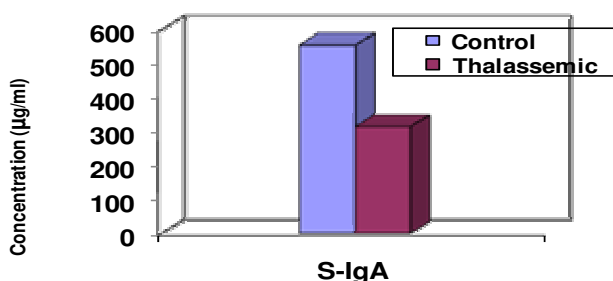


Figure 6 : Comparison of S-IgA between total thalassemic & control groups.

Correlation Between Oral Health Indices and Changes In Some Salivary Constituents:

dmft/DMFT Index : The relation between dmft/DMFT index with both salivary lysozyme and S-IgA was highly significant reverse relation as shown in Figures (7 and 8).

Plaque Index : Significant reverse relation was found between PI and Salivary lysozyme and highly significant reverse relation with S-IgA, while highly significant positive relation was found between PI and salivary peroxidase activity as shown in Figures (9, 10 and 11) .

Gingival Index : The relations between GI and salivary lysozyme was significant reverse relations and with S-IgA was highly significant reverse relation as shown in Figures (12 and 13).

Whereas highly positive relation was found between GI and salivary peroxidase activity as shown in Figure (14).

PI and GI : Highly significant positive relation was found between PI and GI as shown in Figure (15).

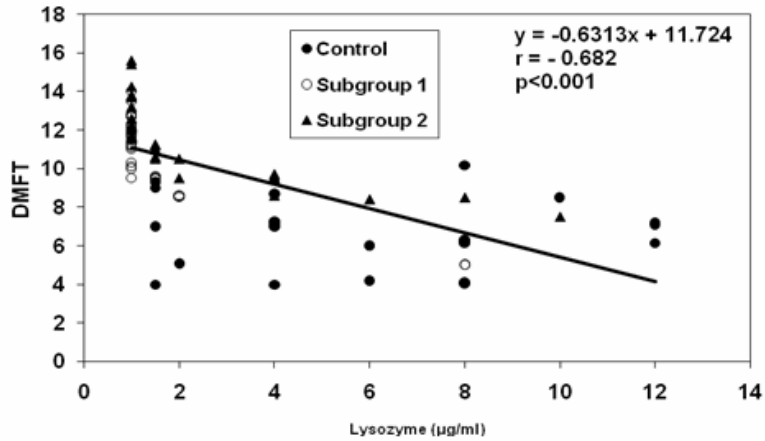


Figure 7 : Relationship between DMFT and lysozyme in the studied groups.

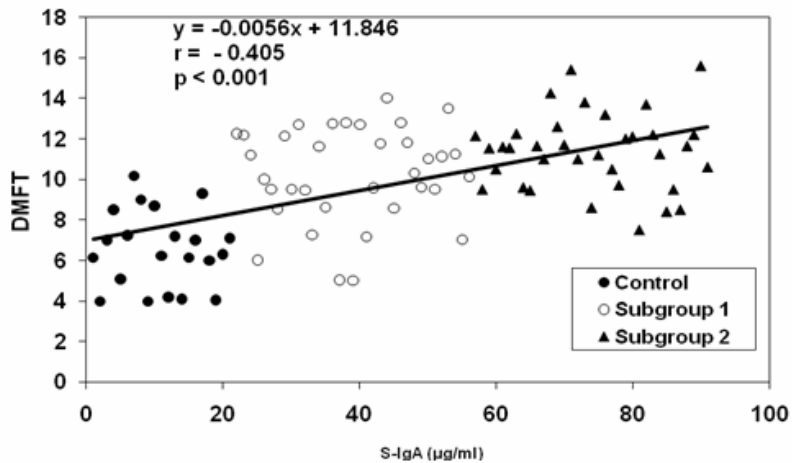


Figure 8 : Relationship between DMFT and S-IgA in the studied groups.

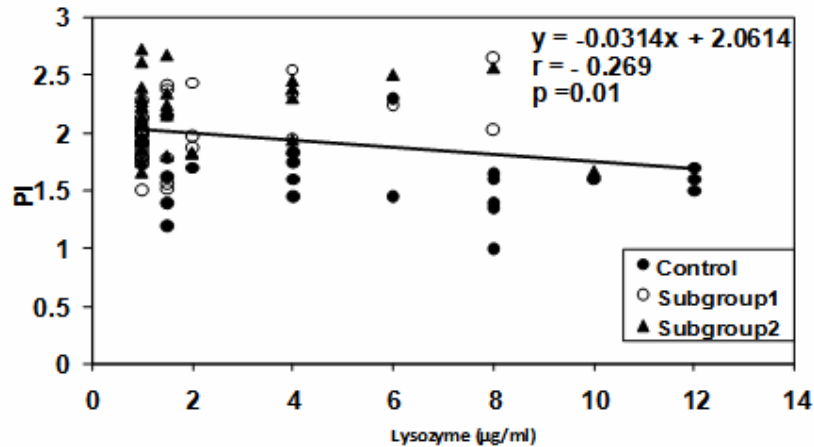


Figure 9 : Relationship between PI and SLZ in the studied groups.

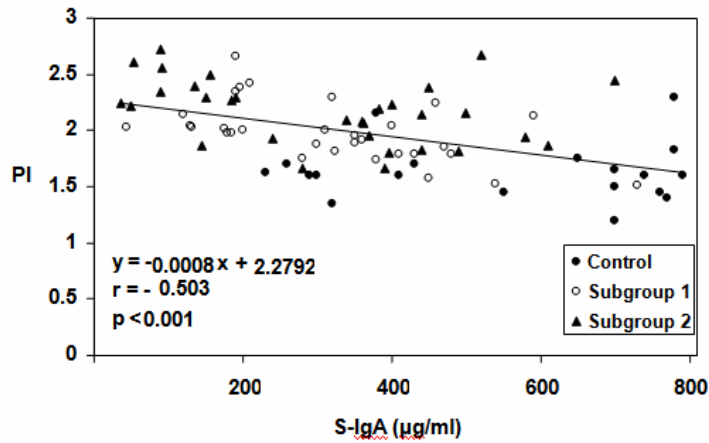


Figure 10 : Relationship between PI and S-IgA in the studied groups.

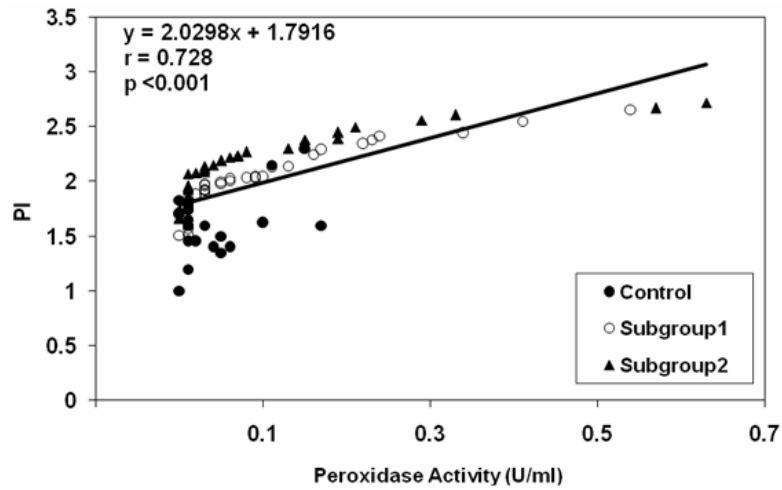


Figure 11 : Relationship between PI and SPX activity in the studied groups.

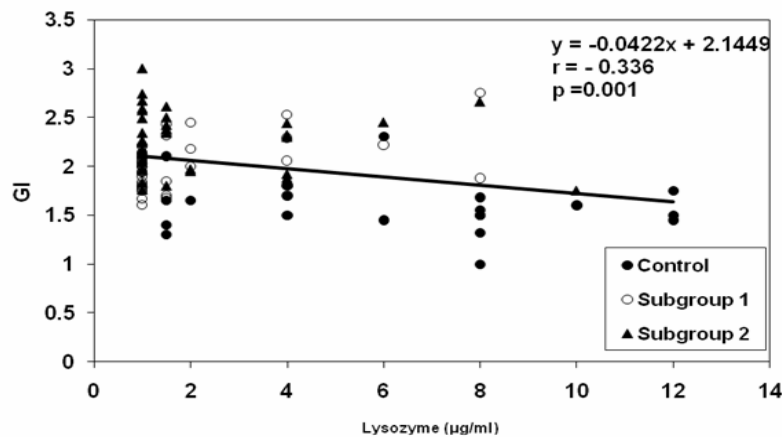


Figure 12 : Relationship between GI and SLZ in the studied groups.

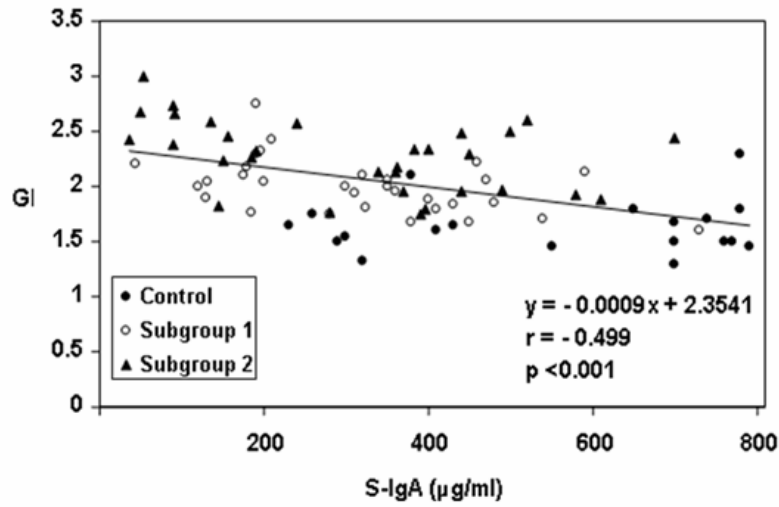


Figure 13 : Relationship between GI and S-IgA in the studied groups.

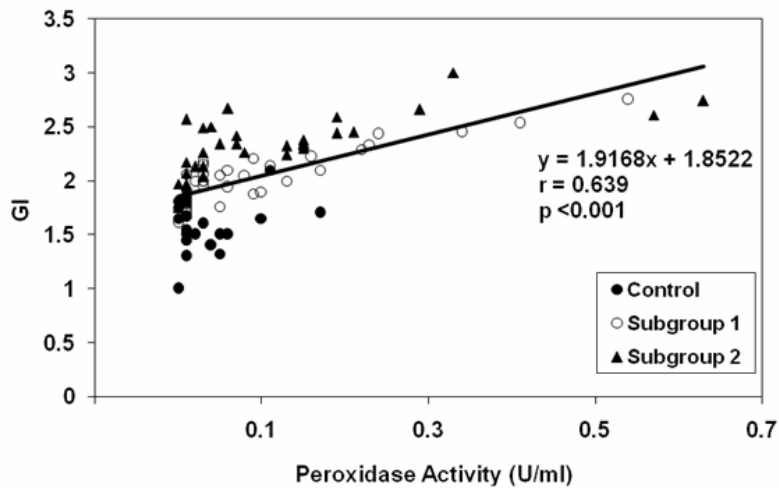


Figure 14 : Relationship between GI and SPX activity in the studied groups.

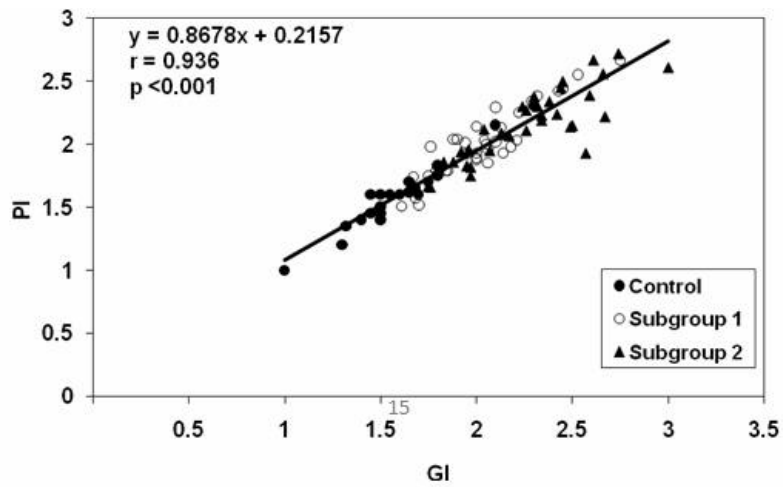


Figure 15 : Relationship between PI and GI in the studied groups.

DISCUSSION AND CONCLUSIONS

Dental caries in thalassemic patients may be affected by changes in salivary glands, saliva flow rate and salivary constituents^(22, 23). In the present study, the dmft/DMFT Index in thalassemic patients was higher than the control group. Such variation probably due to changes in some salivary constituents such as reduced levels of salivary lysozyme and S-IgA. A significant reverse correlation was found between these salivary constituents and dental caries. Poor oral hygiene by patients as a consequence of focusing more on the thalassemia treatment rather than dental care may also give an explanation for high dental caries prevalence in TM patients. Similar findings by others were also recorded^(23, 24). Researchers found a correlation between dental caries experience and both high ferritin level and blood transfusion in TM patients⁽²⁵⁾. Such finding noticed in the present study especially for subgroup 2 subjects who were receiving more blood transfusion and showed higher dental caries experience than both subgroup 1 and control group. Others showed that there was no significant difference in dental caries prevalence between thalassemic and non-thalassemic subjects⁽²⁶⁾.

The DMFT Index was higher in thalassemic subjects than healthy subjects which is probably due to high colonization of *Streptococcus mutans* that has been found in TM patients. Such bacteria has a major role in high caries incidence among TM patients⁽²⁷⁾.

Plaque is known to be the major cause of gingivitis and they are always associated together. As the PI and GI are part of the oral health status, they were both measured in this study as well as dmft/DMFT index to express the oral health status in TM patients. PI in subgroups 1, 2 and thalassemic group as total was higher than the control group. This may be attributed to the changes in some salivary constituents in addition to the negligence of the oral health care by the patients and their parents who were concerned more with life saving therapy rather than oral health care.

The GI in subgroup 1, subgroup 2 and total thalassemic group was higher than the control group. This may be attributed to the low concentrations of salivary lysozyme and S-IgA reasons as well as the high value of PI in those patients⁽²³⁾. The current study showed a significant positive correlation between PI and GI, although others reported that thalassemia was not associated with increased levels of gingivitis or periodontitis⁽²⁴⁾.

Plaque accumulation and gingivitis were higher in TM patients than normal subjects which is probably due to malocclusion and decreased salivary flow rate in TM patients in addition to the limited oral hygiene care⁽²⁸⁾ ..

The present study showed that salivary lysozyme concentration in TM patients was lower than normal subjects. Others showed similar results and found that the excess of iron derived from destructed red blood cells deposit in the vital organs like spleen, liver, heart and glands, interfere with their functions and this may give explanation for the reduced salivary lysozyme concentration in TM patients.⁽²⁹⁾

Perera et al.⁽³⁰⁾, found that salivary lysozyme concentration is sensitive to psychological stress. and its concentration among actually stressed students before final examinations was significantly lower than the concentration observed after the completion of the examinations. Similar results found that salivary lysozyme concentration was decreased among emergency department nurses in relation to their high professional stress⁽³¹⁾. This may give another explanation for the reduced values of salivary lysozyme in TM patients who are under continual stress because of their disease.

The activity of salivary peroxidase is increased with plaque accumulation and gingivitis to overcome the high amounts of hydrogen peroxide product as a result of bacterial activity

which is harmful for the epithelial cells of the oral mucosa. Salivary peroxidase converts H_2O_2 in the presence of salivary thiocyanate into water and hypothiocyanite which is less toxic than H_2O_2 ⁽³²⁾. This may give an explanation for the increased salivary peroxidase activity in TM patients in the present study.

The same explanation for the reduced level of salivary lysozyme may be applied for the reduced salivary S-IgA in TM patients in the present study, which was the iron overload and the resultant hemosiderin deposition in vital organs and glands such as the salivary glands. Moreover it has been reported who that immunological abnormalities (such as decreased T4/T8 ratio due to reduced T4+ cells) in beta-TM patients appear to be acquired, transfusion-associated and related to iron overload which depends on the appropriate chelating therapy ⁽³³⁾.

Many strains of Streptococci and some of the bacteria colonizing the gingival pockets, in addition to suspected periodontopathogens such as *Porphyromonas gingivalis* and *Prevotella intermedia*, produce proteases that are capable of not only degrading S-IgA but also other immunoglobulins and complement factors ^(32, 34, 35). This may give another explanation for the reduced level of S-IgA in TM patients in the present study, especially they had high scores for plaque and gingivitis indices.

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