Evaluation of Oral Hygiene Status, Salivary Fluoride Concentration and Microbial Level in Thalassemic and Hemophilic Patients

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ABSTRACT

Objective: This study aimed to evaluate oral hygiene status, salivary fluoride concentration, and Streptococcus mutans and Lactobacillus levels in saliva of thalassemic, hemophilic and individuals without any other systemic disorders.

Materials and Methods: A total 162 individuals (44 healthy individuals, 86 thalassemic and 32 hemophilic patients) were selected, and randomly (n=30 in each group), the patients were allocated to Group A: individuals without any systemic condition, Group B: thalassemic patients, and Group C: hemophilic patients. Detailed case history, DMFT/DMFS, and OHI-S index were recorded. An aliquot of 5 ml of saliva was collected from each patient to determine the salivary fluoride concentration and predominant microbial colony in saliva. The data were analyzed by chi-square test of independence and nonparametric Kruskal-Wallis H test.

Results: The mean debris and calculus index among groups A, B, and C was 0.55 ± 0.43, 0.61 ± 0.46, 0.46 ± 0.47 and 0.33 ± 0.48, 0.18 ± 0.34, and 0.15 ± 0.34, respectively. The DMFT score for group A was high (1.93 ± 1.86, 1.67 ± 1.92) compared to groups B (0.40 ± 0.77, 0.67 ± 1.37) and C (0.47 ± 0.68, 0.30 ± 0.54). The fluoride concentrations among three groups (A, B, and C) were 0.06 ± 0.07, 0.12 ± 0.13, and 0.12 ± 0.13 ppm respectively. The number of colony-forming units was highest in the healthy individual>hemophilic>thalassemic and presence of predominant microorganisms showed insignificant association among the groups (p=0.323).

Conclusion: Compared to healthy individuals, thalassemic and hemophilic patients had better oral hygiene.

Keywords: Dental caries; fluorides; hemophilia; thalassemia; saliva; lactobacillus; Streptococcus mutans (Siriraj Med J 2022; 74: 314-322)

INTRODUCTION

Thalassemia is a genetic blood disorder that can result in the abnormal formation (partial or complete synthesis of α-globin or β-globin chains in hemoglobin, a tetramer of α2β2) of hemoglobin.1 The two main types are alpha and beta-thalassemia.2 The patient becomes thalassemia major if a gene defect is inherited from both parents. If the defect is inherited only from one parent,
it is known as thalassemia minor. Such individuals are carriers of the disease and most of the time remain asymptomatic. Hemophilia is a rare hereditary condition, resulting in prolonged and uncontrolled bleeding either spontaneously or subsequently after trauma. It inherits an X-linked recessive pattern, which occurs mostly in males. It occurs due to the absence of one or more clotting factors that lead to prolonged clotting time and excessive bleeding that can cause risk to life. The two most common forms are hemophilia A and hemophilia B which are caused by factors VIII and IX deficiency, respectively.

Dental caries is a disease of microbial origin. In addition to Lactobacillus and Actinomyces species, “Streptococcus mutans” (gram-positive facultative anaerobic cocci commonly found in the oral cavity of a human) is the most common pathogen associated with caries. According to literature Ora-facial abnormalities (protrusion of maxillary incisors, wide spacing of teeth, occlusion abnormalities, and nasal deformity) are common in thalassemic patient and high caries prevalence in thalassemic and hemophilic patients. Authors have observed that the prevalence of caries experienced was low in hemophiliacs. Dental practitioners should be aware of the risk associated with the procedures among the aforementioned patients. Early detection and prevention of dental caries is an effective caries-control strategy. Fluoride in saliva promotes tooth remineralization by producing less soluble fluorapatite crystals. As a result, it is essential to determine whether thalassemia and hemophilia patients are more prone to tooth decay than the general population. The purpose of this study was to investigate: a) comparing the oral hygiene status of patients with thalassemia, hemophilia, and healthy individuals, b) determining their salivary fluoride concentration, and c) identifying the predominant microorganism (S. mutans and Lactobacillus) levels in saliva. The hypothesis for this study was that the categorical variables had no association.

MATERIALS AND METHODS

The study was approved by the Ethics Committee Institute of Medical Sciences (IMS) and Sum Hospital Siksha ‘O’ Anusandhan Deemed to be University (Ref. No. DMRI IMS. SH/SOA/180319). The research was carried out between 2018 and 2020. G* power software, version 3.1.9 (available at http://www.gpower.hhu.de/en.html) was used to calculate sample size based on the results of previous studies. Individual group sample size was n=30, with the level of significance and power of test set at 5% and 80%, respectively (total 90). Sample selection is described in Fig 1. The inclusion criteria were thalassemic or hemophilic patients above the age of 14 years (individuals visiting “Institute of Dental Sciences” and “Institute of Medical Science and SUM Hospital”), healthy individuals without any bleeding disorder or systemic condition, visiting the Institute of Dental Sciences for a dental check-up. The exclusion criteria were: other bleeding or clotting disorders and chronic systemic conditions, hormonal disorders, fluoride therapy patients, medications affecting the salivary flow rate (β-blockers, antihistamines, antipsychotics, anti-inflammatory drugs, etc.), patients below the age of 14 years, and dental fluorosis (according to modified Dean’s fluorosis index i.e.: questionable/0.5 to severe/4).

All patients provided written informed consent (in the case of patients below the age of 18, written informed consent was taken from their parents/guardian). A sample size of 90 patients (n=30 in each group) was allocated using randomization software (www.randomization.com). Group A [Control group]: Healthy individuals (who did not have any bleeding or clotting disorder and satisfied the exclusion criteria) Group B: Thalassemic patients, Group C: Hemophilic patients. Each patient had a detailed case record and oral hygiene status, which included the DMFT (Decayed- Missed- Filled- Teeth) and (debris & calculus) OHI-S (Oral Hygiene Index- Simplified) indexes. A single dental practitioner performed the patients’ dental examinations (Fig 2). Five milliliters of unstimulated saliva was collected from each individual minimum 30 minutes after eating and stored separately in sterile sample collection bottles. Each patient’s saliva was collected between 10 a.m. to 3 p.m. (No specific instructions regarding oral hygiene maintenance was advised). Saliva (5 mL) were divided into Part I (4 mL) to determine salivary fluoride concentration and Part II (1 mL) to determine predominant microbialia and colony-forming units (Fig 1).

Measurement of salivary fluoride concentration

The 4 mL of saliva samples were centrifuged at 1500 rpm for 3 min, and the supernatant was stored in close lid containers at 4°C for a maximum of 48 hours. After the readings were standardized using fluoride standard solution, the saliva samples were diluted with TISAB III (Total Ionic Strength Adjustment Buffer- III) reagent (Merck India) and used to quantify salivary fluoride concentration using an Orion Star A 214ASIC pH meter with a fluoride ion-selective electrode. For each sample, the results were acquired three times, and the mean values for each group were recorded individually.
Fig 1. Flow chart of sampling of groups and methodology.

**Determination of the salivary microbial level**

One milliliter of saliva sample was stored in close lid containers at 37°C for a maximum of 48 hours and used for microbiological culture on nutrient agar and blood agar plates. The plates were incubated in a bacterial incubator for 24 hours at 37°C. The predominant colony was identified and subjected to Gram’s staining. The stained slides were observed under a microscope (Olympus India) at 40x and 100x magnifications in oil immersion. The predominant microflora for each sample was determined under the microscope. The number of colony-forming units (CFU) was counted for the predominant microflora of each sample. The results were recorded and subjected to statistical analysis.

**Statistical analysis**

Statistical analysis was performed using IBM SPSS statistics 24.0, South Asia Private Ltd. www.spss.co.in. The
test of association of patient group was done following cross tabulation procedure followed by Chi-square test of independence. The chi-square test of independence was used to determine if there was a significant relationship between two categorical variables. The null hypothesis for this test was that there is no relationship between the categorical variables.

Comparison of age, decayed teeth, filled teeth, calculus, and fluoride levels among the three groups of patients was performed following nonparametric Kruskal-Wallis test, as these variables failed to pass the Shapiro Wilki normality test. The Kruskal-Wallis H test, a is the nonparametric analog of one-way analysis of variance and detects differences in distribution location. The mean, SD and quartiles were calculated following a descriptive statistics procedure. Colony-forming units (CFUs) is the estimate of the number of viable bacteria or fungal cells in a sample and have been classified into three groups: 10+ to 20+, 30+ to 50+, and 80+ to 150+. The level of significance was kept at p<0.05.

RESULTS

The demographic details are the mean age of patients in healthy individuals, thalassemic and hemophilic were 18.73 ± 2.59, 20.20 ± 8.91, 18.27 ± 4.23 years respectively. The difference in the distribution of age among the three groups was not significant (p=0.374). In hemophilic group all the patients were males. The male-female proportions in the control group were 46.7% and 53.3% and in the thalassemic group were 56.7% and 43.3%, respectively.

The mean debris index among groups A, B, and C was 0.55 ± 0.43, 0.61 ± 0.46, and 0.46 ± 0.47, respectively. The median debris among the three groups was in the range of 0.915 with IQR (interquartile range): 0.000 to 1.000 to 0.330 with an IQR: 0.00 to 1.00. The mean calculus index among groups A, B, and C was 0.33 ± 0.48, 0.18 ± 0.34, and 0.15 ± 0.34, respectively. The median calculus among the three groups was in the range of 0.000 with IQR: 0.000 with IQR: 0.00 to 0.000 to 0.000 with IQR 0.000 to 1.000. The OHI-S (debris and calculus) among the groups was statistically insignificant (Tables 1&2).

The DMFT (decayed and filled) score for group A (control) was high (1.93 ± 1.86, 1.67 ± 1.92) compared to groups B (0.40 ± 0.77, 0.67 ± 1.37) and C (0.47 ± 0.68, 0.30 ± 0.54). The DMFT scores among Groups B and C were statistically insignificant compared to Group A (p=0.000). However, clinically Group C showed lower DMFT scores (Figs 3&4). The fluoride concentrations among the three groups were 0.06 ± 0.07, 0.12 ± 0.13, and 0.12 ± 0.13 ppm. The mean difference among the three groups was statistically insignificant (p=0.566) (Table 3).
TABLE 1. Comparison of Debris among groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mean ± SD</th>
<th>Q1</th>
<th>Q2 (Median)</th>
<th>Q3</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>30</td>
<td>0.55 ± 0.43</td>
<td>0.000</td>
<td>0.580</td>
<td>1.000</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Thalassemic</td>
<td>30</td>
<td>0.61 ± 0.46</td>
<td>0.000</td>
<td>0.915</td>
<td>1.000</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Hemophilic</td>
<td>30</td>
<td>0.46 ± 0.47</td>
<td>0.000</td>
<td>0.330</td>
<td>1.000</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

Kruskal Wallis Test 'p' value 0.465

TABLE 2. Comparison of Calculus among groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mean ± SD</th>
<th>Q1</th>
<th>Q2 (Median)</th>
<th>Q3</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>30</td>
<td>0.33 ± 0.48</td>
<td>0.000</td>
<td>0.000</td>
<td>1.000</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Thalassemic</td>
<td>30</td>
<td>0.18 ± 0.34</td>
<td>0.000</td>
<td>0.000</td>
<td>0.330</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Hemophilic</td>
<td>30</td>
<td>0.15 ± 0.32</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

Kruskal Wallis Test 'p' value 0.288

Fig 3. Comparison of mean Decayed tooth among groups.

Fig 4. Comparison of mean Filled tooth among groups.
TABLE 3. Comparison of Fluoride concentration (in ppm) among groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mean ± SD</th>
<th>Q1</th>
<th>Q2 (Median)</th>
<th>Q3</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>30</td>
<td>0.06 ± 0.07</td>
<td>0.019</td>
<td>0.027</td>
<td>0.054</td>
<td>0.002</td>
<td>0.254</td>
</tr>
<tr>
<td>Thalassemic</td>
<td>30</td>
<td>0.12 ± 0.13</td>
<td>0.018</td>
<td>0.036</td>
<td>0.230</td>
<td>0.001</td>
<td>0.453</td>
</tr>
<tr>
<td>Hemophilic</td>
<td>30</td>
<td>0.10 ± 0.11</td>
<td>0.014</td>
<td>0.025</td>
<td>0.201</td>
<td>0.001</td>
<td>0.321</td>
</tr>
</tbody>
</table>

The proportions of S. mutans and Lactobacillus in group A were 86.7% and 10.0%, group B 93.3% and 6.7%, and group C were 80.0% and 20.0%, respectively. The predominant microorganisms did not have a significant association among the groups (p=0.323). The association between decayed teeth and different colony forming groups was 0.71±0.95 in the 10+ - 20+ CFU group, which increased to 3.36±2.20 in the 80+ - 150+ CFU group compared to the control group. In group B, 0.13 ± 0.35 in 10+ - 20+ CFU group which increased to 2.00 ± 1.00 in 80+ - 150+ CFU group and for group C was 0.00 ± 0.00 in 10+ -20+ CFU group which increased to 1.17 ± 0.75 in 80+ - 150+ CFU group. The number of colony-forming units was highest in healthy individuals and lowest in thalassemic patients. The increased CFU level has a significant association with a higher number of decayed teeth (Tables 4&5).

The correlation of age, number of decayed teeth and fluoride showed that age did not have a significant correlation with the number of decayed teeth or fluoride in the healthy and hemophillic groups. However, age had a significant positive correlation of 0.40 with fluoride in the thalassemic group (p<0.05). The number of decayed teeth showed no statistically significant correlation with fluoride in any of the three groups (p>0.05).

DISCUSSION
Saliva is an important predictor of oral health status. The preference for unstimulated saliva in this study is attributable to the fact that “stimulated saliva has increased flow rate, dilution, and change in pH”. During the collection of a saliva sample, it was observed that the salivary flow rate was higher in young individuals than in adults, which is in accordance with a meta-analysis that revealed that the maturing process is directly associated with a decreased salivary flow rate and is unaffected by medications.

The hemophilic group had only male patients considering “hemophilia inherits in an x-linked recessive pattern that occurs primarily in males”. Females become

TABLE 4. Association of Predominant Microorganisms in groups.

<table>
<thead>
<tr>
<th>PM</th>
<th>Control No.</th>
<th>Control %</th>
<th>Thalassaemic No.</th>
<th>Thalassaemic %</th>
<th>Hemophilic No.</th>
<th>Hemophilic %</th>
<th>Total No.</th>
<th>Total %</th>
<th>χ², p</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Mutans</td>
<td>26</td>
<td>86.7</td>
<td>28</td>
<td>93.3</td>
<td>24</td>
<td>80</td>
<td>78</td>
<td>86.7</td>
<td>χ² = 4.671</td>
</tr>
<tr>
<td>Candida</td>
<td>1</td>
<td>3.3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1.1</td>
<td>p=0.323</td>
</tr>
<tr>
<td>Lactobacillus</td>
<td>3</td>
<td>10</td>
<td>2</td>
<td>6.7</td>
<td>6</td>
<td>20</td>
<td>11</td>
<td>12.2</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>100</td>
<td>30</td>
<td>100</td>
<td>30</td>
<td>100</td>
<td>90</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>
carriers and are usually asymptomatic, although in rare situations, they may develop hemophilia symptoms.\textsuperscript{18,19} A case-control study found that children and adolescents with hemophilia had similar caries experiences and had no significant differences in oral hygiene or dietary habits.\textsuperscript{20,21} Group B’s age ranged from 15 to 54 years old, whereas group C’s age ranged from 14 to 32 years old. Thalassemic patients have low IgA levels in their saliva and endocrine dysfunction, which increases their risk of decay.\textsuperscript{22,23} The results of the present study are in accordance with the aforementioned studies.

When compared to healthy persons, patients with blood disorders had compromised oral health, particularly poor periodontal conditions (gingival and plaque index) in - thalassemia and sickle cell anemia patients.\textsuperscript{24,25} Individuals affected and their families’ physical and psychological well-being was impacted (involves regular visit, chelation therapy, and uncertainties about the future), and studies have shown that thalassemia patients have a higher rate of caries, which could be attributed to aberrant tooth morphology, abnormal pits and fissures, and changes in salivary components and volume.\textsuperscript{13,23} Despite the foregoing reasoning, the current investigation found a minor rise in mean clinical debris (0.61 ± 0.46), an improved calculus index (0.15 ± 0.32) and DMFT score (0.47 ± 0.68) when compared to the control group. Among the groups (A: 0.06 ± 0.07, C: 0.10 ± 0.11), group B (0.12 ± 0.13) had a higher clinical fluoride level. The proportion of the predominant microorganisms \textit{S. mutans} and \textit{Lactobacillus} was slightly greater than that in the control group, but the difference was statistically insignificant (p=0.323). Only one patient in the control group exhibited Candida, whereas groups B and C had none.

In a study of hemophilic’s oral and general health-related quality of life, researchers noted that psychological behavior and mental health were lower, but that self-assessing oral health state and regularly perceiving dental treatment needs were higher than in healthy people.\textsuperscript{26} This is consistent with the findings of the current investigation, in which these patients were well-versed in the consequences of poor dental hygiene and the risk of caries. When compared to the thalassemia and control groups, the debris index (0.46 ± 0.47), calculus index (0.15 ± 0.32) and DMFT score (0.47 ± 0.68) were lower. The control group’s DMFT scores were statistically significant (p=0.000).

Salivary fluoride concentration was clinically evident in groups C and D when compared to the control group. This could be because these patients are more aware of the importance of maintaining good dental hygiene. There was no test of gender association in the hemophilic group because all of the cases were males. In comparison to the thalassemic and control groups, the proportions of \textit{S. mutans}, \textit{Candida}, and \textit{Lactobacillus} were lower (80.0%, 0%, and 20.0%, respectively) in the hemophilic group. Previous research has found that patients with blood problems had a greater \textit{S. mutans} and \textit{Lactobacillus} count in their saliva than healthy people.\textsuperscript{27,28} The presence of a certain microorganism was not revealed by an increase

### TABLE 5. Comparison of mean number of decayed teeth by CFUs within groups.

<table>
<thead>
<tr>
<th>CFU</th>
<th>Group</th>
<th>Decayed tooth</th>
<th>Control</th>
<th>Thalassaemic</th>
<th>Hemophilic</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>Mean ± SD (IQR)</td>
<td>N</td>
<td>Mean ± SD (IQR)</td>
<td>Mean ± SD (IQR)</td>
<td>Mean ± SD (IQR)</td>
</tr>
<tr>
<td>10+ - 20+</td>
<td>7</td>
<td>0.71±0.95 (0,2)</td>
<td>15</td>
<td>0.13±0.35 (0,0)</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30+ - 50+</td>
<td>12</td>
<td>0.33±0.65 (0,75)</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>80+ - 150+</td>
<td>11</td>
<td>2.00±1.00 (0,1)</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total</td>
<td>30</td>
<td>1.93±1.86 (0,75)</td>
<td>30</td>
</tr>
</tbody>
</table>

Kruskal Wallis Test ‘p’ value: 0.004, 0.003, 0.003
in the number of colony-forming units in the groups (p ≥ 0.05).

The current study revealed that thalassemic and hemophilic patients were well aware of the conditions and potential problems associated with poor oral health. The majority of patients visited the dentist every six months for a routine dental examination, and the parents /guardian of patients under the age of 18 yrs. were well aware of the issue. Any dental invasive surgery should be performed after factor replacement/ transfusion. Hematologists should inform such patients about dental problems and treatments, and encourage them to visit the dentist on a frequent basis to avoid complications. Further studies into the impacts of various fluoride therapy methods, specific microbiological load, and pain perception during dental treatment can be considered for a large thalassemic and hemophilic population (different location, depending on socioeconomic status of the individual and their families).

CONCLUSION

The current study concludes that the OHI-S (debris & calculus) index and mean salivary fluoride concentration was statistically insignificant among the groups (p>0.05). DMFT scores were less in thalassemic and hemophilic patients compared to the healthy individuals. The number of colony-forming units was higher in healthy individuals and lowest in thalassemic patients. Dentists should be aware of and knowledgeable about the risks involved when treating thalassemic and hemophilic patients. Early detection and recognition of oral health concerns will help patients financially and in terms of reducing the risk. According to the current findings, the incidence of caries, gingivitis, and microorganism in thalassemic and hemophilic patients is clinically significant. To receive safe, comprehensive oral care, these patients' families, guardians, physicians, and dental clinicians must work collaboratively.

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REFERENCES


