IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 14, Issue 12 Ver. V (Dec. 2015), PP 98-102
www.iosrjournals.org

Evaluation of oral hygiene status and salivary biochemistry of patients with Thalassemia major: A clinical study

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I. Introduction:

Thalassemia are a group of inherited haematological defects in the synthesis of either α or β polypeptide chains of the globin portion of the haemoglobin molecule, and therefore, referred to as α or β type and are characterized by hypochromic, haemolytic anaemia of varying degrees. Based on their clinical and genetic orders, thalassemia is classified mainly into major (homozygous) and minor (heterozygous) types. Thalassemia major or β -thalassemia, or Cooley's anaemia, exhibits the most severe clinical symptoms while thalassemia minor or α -thalassemia is mild and considered to be clinically asymptomatic. An intermediate form of thalassemia may also occur.**[1]** Thalassemia is a disease which not only affects the patient but also leaves a devastating psychosocial effect on the family of the patient. Furthermore, frequent blood transfusion is very expensive, and preventive and restorative dental care programmes also increase financial burden. The prevalence and severity of periodontal diseases have been documented to be associated with a number of diseases including β -thalassemia.

Beta Thalassemia Major (TM) is widely prevalent in South Asian countries including India and accounts for 10,000 of 1, 00,000 patients of Thalassemia major born worldwide every year. Not much information is available on the association of dental caries and gingival status with Beta thalassemia which affects both general and oral health related quality of life.[2] Studies showed a higher experience of periodontal problems and dental caries in thalassemia patients[3]. Delayed eruption of both deciduous and permanent teeth and high frequency of caries were observed; moreover, dental caries were related to the severity of systemic disease. A significant inverse correlation was observed between transfusion requirements and caries in mixed dentition by Luglie *et al* [4]. However very few studies have been done on the affected patients from the population of Central India. The purpose of this study was to assess the prevalence and distribution of dental caries and oral hygiene conditions in a 3-14 years old group of patients affected by TM in central India region. Furthermore, salivary biochemical composition of the total saliva was evaluated as related to dental caries.

II. Material and methods

2.1 Sample selection

The present work was carried out in the department of Pedodontics and Preventive Dentistry at Sharad Pawar Dental College and department of Biochemistry, Jawaharlal Nehru Medical College and Hospital, DMIMS (DU), Sawangi (Meghe), Wardha. The study was approved by Institutional Ethical Committee from Datta Meghe Institute of Medical Sciences (DU). Written informed consent was obtained from all the parents of children participating in the study before their examination. The sample consisted of 30 patients in study group and 30 patients in control group. Both experimental and control group had 10 girls and 20 boys (Table 1). Study group was taken from previously diagnosed patients suffering from beta thalassemia major TM in the age group of 3-14 yrs and control group consisted of normal unaffected children attending to Department of Pedodontics and Preventive Dentistry for routine dental check up. Matched controls (1:1) were randomly paired for age and sex and selected from a sample of the population living in the Central India region.

2.2 Clinical parameters

A clinical record was filled out for each patient with two parts. One part included gathering personal data and a second part included dental examination. The present number of teeth was recorded and the decayed, missing, and filled teeth (DMFT/dmft) index was quantified.[5] Oral hygienic conditions were recorded using the oral hygiene index (OHI)-S.[6] (Table 2)

2.3 Laboratory analysis

Saliva samples: Unstimulated saliva samples were collected according to Dawes` method.[7] To ensure standardisation of samples and minimise the effect of diurnal variation, the saliva collection was carried out at the same time of the day between 9 am to 11 am on a routine basis. Prio information was provided to the subjects to refrain from eating and drinking at least 60 min before the collection. During sample collection, the subject was seated in a normal chair instead of the dental chair to maintain a stress-free environment. The unstimulated saliva was collected by asking the subjects to pool the saliva on the floor of the mouth and then made to expectorate it into a collection cup. The collected saliva was placed in Eppendorf tubes (**Figure 1**) and preserved at –80°C before chemical analysis. The samples were brought to room temperature and centrifuged at 1,500 rpm for 10 minutes. Concentrations of calcium, phosphorous, urea, potassium and sodium, were assayed with Robonic EXE Biochemistry analyser, RX Daytona and ISE 5000 analyser respectively.

2.4 Inclusion criteria

- Age group between 3 and 14 years.
- Only those patients who were diagnosed previously for Beta Thalassemia major were considered as cases in the study.
- Matching of age, sex of cases and controls.
- The controls were free of thalassemia, both the major and minor forms.

2.5 Exclusion criteria

1. Those already undergoing dental treatment.
2. Those suffering from other diseases known to influence dental caries or severity of periodontal disease such as Diabetes or Congenital Heart Disease.

The study consisted of an interview, intraoral examination and collection and bio chemical analysis of saliva sample. Autoclaved plain mouth mirror and explorer were used to examine the oral cavity. Single examiner and single recorder were maintained throughout the study period. Statistical analysis was done using appropriate statistical software. Chi square test and student t-test was used for the comparison of study and control groups. The level of significance was set at $p<0.05$.

III. Results

Total 60 children were examined for DMFT/dmft score, OHIS and salivary biochemical levels of calcium, potassium phosphorus and urea that included 30 thalassemic children in experimental group and 30 normal children in control group. These patients were broadly divided in two groups one below 9 years and other above 9 years. It was found that DMFT/dmft score, OHIS and salivary urea levels for Thalassemia major group differed with age. The DMFT/dmft index showed a slightly higher mean value in the TM group, but no statistically significant difference was observed between the two populations. Thalassemia major patients <9 years of age had a mean DMFT score greater as compared to older patients.Their OHIS and salivary urea levels also had statistically significant differences. (**Table 3**)

IV. Discussion

The main purpose of our investigation was to determine relationships between the oral and chemical conditions of saliva in Thalassemia major patients. The DMFT index showed a slightly higher mean value in the TM group, but no statistically significant difference was observed between the TM and control group. The oral hygiene status observed was similar in the two groups. Previous studies [8-10] suggest a higher prevalence of gingivitis in TM patients and correlation to local factors or the maxillofacial characteristics of thalassemic disease. Orthodontic problems such as crowding, extreme maxillary overjet, crossbite, and oral breathing are mainly implicated in gingival disease as stated by Helm S et al.[11, 12] Our patients with TM underwent transfusion therapy at very early ages and were on a regular blood transfusion thus reducing the typical facial characteristics and related problems.

Saliva is a complex biological fluid containing several compounds which collaborate to prevent dental caries by mechanical washing, antimicrobial function, remineralization and regulation of oral pH by its buffering capacity. Saliva not only physically removes dietary substrates and acids produced by plaque from the mouth, but also has an important role in buffering the pH of saliva and plaque. [13] Thus it is critical for preserving and maintaining the health of oral tissues and has been used as a source of non-invasive investigation of metabolism and the elimination of many drugs. However, it receives little attention until its quantity diminishes or its quality becomes altered. At present, saliva represents an increasingly useful auxiliary means of diagnosis. Sialometry and sialochemistry can be used to diagnose systemic illnesses, monitoring general health,

and as an indicator of risk for diseases creating a close relation between oral and systemic health. [14] Concentrations of the biochemical components in saliva play an important role in oral diseases, but only few studies examined this in connection with Thallasemia Major. [10]

The salivary urea concentration was lower with a statistical significance in our study group of Thallasemia Major Patients. Urea is secreted continuously in the range of 3–10 m Mol in saliva and gingival crevicular fluids of healthy individuals [15] and is rapidly hydrolyzed by the urease enzymes of oral microflora. Existing data indirectly support a major role for ureolysis in plaque pH homeostasis. In addition, ammonia released by ureolysis can promote remineralization of the tooth enamel.[16] The lower salivary urea concentration detected in the Thalassemia major group is similar to results previously described.[17] Elevated salivary urea and ammonia concentrations correlate with marked reductions in the extent and duration of plaque acidification following a carbohydrate challenge.[18] Urea hydrolysis can neutralize plaque acid and may positively influence plaque ecology by preventing the pH from falling to levels that select for the outgrowth of aciduric, cariogenic micro-organisms. Salivary levels of urea, calcium and phosphorus were found to vary with age in both control and experimental group.

Results of examined thalassemia patients and healthy controls are suggestive of variations in their oral hygiene as measured by OHI-S which was found to be increased in Thalassemic patients. Dental caries experience was significantly higher in thalassemia patients of younger age groups. The higher caries prevalence can be attributed to the poor oral hygiene, improper dietary habits, lack of dental knowledge, poor motivation, reduced salivary urea concentration. This could also be attributed to parental overprotection or negligence in these systemically ill patients. So the clinical, salivary, and microbiological data allow us to affirm that Thalassemia major patients might be considered at risk for caries. However, it is very questionable to say whether this is related to the systemic disease. Similar study conducted by Luglie *et al* [4] found similar biochemical saliva composition in the two groups; with lower urea concentration in TM with statistically significant difference. He also studied the salivary levels of mutans streptococci and found their increase at detectable levels in TM patients. [4]

Navpreet Kaur *et al* [2] suggested that in patients with Beta thalassemia, dental caries was significantly higher than the normal children but no significant increased levels of gingivitis or plaque accumulation was seen in Beta thalassemia patients than in controls. But the present study showed increased gingivitis and plaque accumulation in TM than in controls.

However in our study, salivary concentrations of urea and calcium were significantly lower in the younger children of both groups. Younger thalassemic children in our study showed more dental caries as compared to older children. This could be attributed to parental overprotection, high consumption of cariogenic food like chocolates and candies provided on demand to these children of high socioeconomic group or dental care neglect of those affected children by parents in low socioeconomic groups due to devastating effects of systemic illness of these children (**Figure 2**). These parents are more concerned with the serious physical problems, paying lesser attention to the dental ailments, and only seek dental care when the child is in pain. Therefore, emphasis to educate such group in the prevention of dental caries should be considered. High caries prevalence in thalassemia patients can be attributed to poor oral hygiene, improper dietary habit and lack of motivation of these patients. Our findings confirm that, although no substantial differences were found between the two observed groups, further investigations are needed to determine the theoretical risk of oral diseases in thalassemic patients

V. Clinical significance

The findings and application of this study have a strong implication for dental caries prevention in thalassemia patients in Central India.

VI. Conclusions

1. The risk of oral disease in TM patients remains high and prevention against this oral disease is very important, for the higher life expectancy of these patients and the role of good oral status in better quality of life.
2. Emphasis to educate such group of individuals and their parents in the prevention of dental caries and periodontal disease should be considered and oral health education must be given to beta thalassemia patients right from the detection of disease to prevent dental diseases.
3. Such patients should receive regular follow up care and timely dental treatment.

Conflict of interest

There is no conflict of interest related to the submitted manuscrip

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Figures

Figure 1: Eppendorf tubes of unstimulated saliva

Figure 2: Cycle of overprotection

Tables:

Table 1: Gender wise Distribution of Subjects

Gender	Cases	Control
Male	20(66.7%)	20(66.7%)
Female	10(33.3%)	10(33.3%)
Total	**30(100.0)**	**30(100.0)**

Table 2: Comparison of Biochemical parameters between control and experimental groups

Parameters	**Control group**	**Experimental group (TM)**	**Z value**
OHI-S	0.95+0.29	1.05+0.47	1.07*
UREA	21.06+6.92	21.92+10.30	0.140*
PHOSPHORUS	12.87+7.15	11.10+8.71	0.165*
CALCIUM	5.36+2.10	5.48+3.53	0.860*
SODIUM	14.19+5.21	11.10+8.71	0.420*
POTASSIUM	15.51+3.72	14.23+2.97	1.69*

*Not significant, p>0.05

Table 3: Age wise Comparison of Biochemical parameters in experimental groups

Biochemical Parameters	**Age (yrs)**		**Z value**
	< 9 years Mean + SD (n = 19)	**> 9 years Mean + SD (n = 11)**	
OHIS	1.95+0.44	0.81+0.42	1.49*
DMFT	3.44+2.28	1.67+1.89	2.37**
Urea	17.60+6.86	29.39+11.23	2.93#
Calcium	4.56+3.02	7.08+3.91	3.159#
Phosphorous	9.90+5.08	13.16+12.89	1.83*
Sodium	14.21+3.14	14.27+2.80	0.8035*
Potassium	16.22+3.44	20.87+10.02	0.0559*

* Not Significant ($p > 0.05$) **Significant ($p < 0.05$) # Highly Significant ($p < 0.01$)

Legend

Figure 1: Eppendorf tubes of unstimulated saliva
Figure 2: Overprotection cycle
Table 1: Distribution of subjects
Table 2: Comparative analysis amongst control and experimental groups (T-test)
Table 3: Age-wise comparative analysis amongst thalassemic patients (T-test)