Comparative Assessment of Salivary Flow Rate, Buffering Capacity, Resting PH and Dental Caries in Children with Beta Thalassemia

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Abstract: The aim of the present study was to assess and compare the salivary flow rate, buffering capacity and resting pH of β thalassemic children with healthy children and to compare the proportion of dental caries in β thalassemic children with healthy children. A total of 60 children were included in the study, study group consisted of 30 diagnosed cases of Thalassemia attending the Indira Gandhi Institute of Child Health, Bangalore, and control group consisted of 30 healthy children who visited the Department of Pediatric and Preventive dentistry, V S dental college, Bangalore for a routine dental check-up. Salivary parameters like the flow rate, buffering capacity and resting pH were measured using saliva check buffer kit (GC Europe) and dental caries was recorded according to WHO criteria 1997. Salivary flow rate, buffering capacity and resting pH was lower in thalassemic children when compared to that of healthy children and the results were statistically significant. There was a positive correlation between salivary parameters and dental caries. Increased incidence of dental caries in children with thalassemia could be due to poor oral hygiene, poor motivation, endocrine problems, malocclusion, frequent blood transfusion and immune deficiency. In the present study reduced salivary flow rate, low buffering capacity and reduced resting pH has been observed in thalassemic children, which could be the causative factors for the increased incidence of dental caries in these children.

To cite this article
[Babu, N. S. V. & Shah, S. (2018). Comparative Assessment of Salivary Flow Rate, Buffering Capacity, Resting PH and Dental Caries in Children with Beta Thalassemia. *The Journal of Middle East and North Africa Sciences*, 4(3), 18-22]. (P-ISSN 2412- 9763) - (e-ISSN 2412-8937). www.jomenas.org**. 4**

Keywords: Beta Thalassemia, salivary flow rate, buffering capacity; resting pH; dental caries.

1. Introduction:

Thalassemia is a type of severe hemolytic anemia and is the most common single gene abnormality. It has over 200 mutations; most of them are very rare. Approximately 20 common alleles constitute 80% of the known thalassemia worldwide. About 3% of the world population carries genes of beta-thalassemia (Arora et al., 2014a) Most patients are transfusion- dependent from early infancy when they demonstrate severe anemia and hepatosplenomegaly. Children receiving regular transfusions grow normally but few complications are related to iron overload which is not only because of blood transfusion and increased gastrointestinal absorption but also from ineffective erythropoiesis and constant hemolysis which starts to appear such as liver cirrhosis, endocrine dysfunction, and pulmonary defect. Bone marrow hyperplasia is reflected at the skeletal level with the formation of a bone expanding erythroid mass. Malocclusions are observed as a consequence of severe maxillary protrusion, with the development of overbite and open bite (Al-Hadithi, 2011).

Saliva is a clear, slightly acidic mucoserous exocrine secretion. Whole saliva is a complex mix of fluids from both major and minor salivary glands and from the gingival crevicular fluid, which contains oral bacteria and food debris (Humphrey & Williamson, 2001). It is armed with immunological and enzymatic defense systems and has the ability to protect the mucosa against mechanical insults and promote its healing (Al-Jobouri & Al-Casey, 2011).

The most important caries protective function of saliva is the flushing and neutralizing effects which is dependent on the flow rate (FR) and buffering capacity (BC) of saliva (Lagerlöf, 1994). Reduction in the salivary FR can lead to rapid formation of caries lesions. There is an inverse relationship between salivary BC and caries susceptibility (Edgar et al., 1994). BC is related to both FR and resting pH of the saliva. BC is one of the best indicators of caries susceptibility as it reveals the host response. Saliva acts with its BC as a regulator of plaque pH, neutralizing acids produced by cariogenic bacteria (Arora et al., 2014b).

Dental caries is a widespread disease affecting all ages. The prevalence and severity of dental caries are affected by several factors, which includes diet, age, gender and socioeconomic factors. Dental caries involves internal defense factors such as saliva, tooth morphology,

general health, nutritional and hormonal status, and external factors like diet, microbial flora, oral hygiene and fluoride availability. Also, some illnesses predispose to high risk of dental caries, in addition to some medications (Kuriakose et al., 2013).

It has been observed that parents of children suffering from a life-threatening systemic disease like thalassemia major focus mainly on the medical procedures required to overcome the disease and in turn giving low priority to oral health status during early childhood. So, this poor oral health leads to further deterioration of systemic health in these children Although several studies have been done to determine the association, if any, in patients suffering from beta thalassemia with dental caries and their comparison with normal counterparts, till date only two studies have been reported to assess the salivary flow rate and resting pH in children with beta-thalassemia whereas there are no reported studies assessing the buffering capacity in children with beta-thalassemia and its association with dental caries.

So the present study was conducted to compare salivary flow rate, buffering capacity and resting pH and its association with dental caries in children with beta thalassemia.

2. Materials and Methods:

A total of 60 children aged 5-18 yrs were included in the study, study group consisted of 30 diagnosed cases of thalassemia attending the Indira Gandhi Institute of Child Health, Bangalore and control group consisted of 30 healthy children who visited the Department of Pedodontics and Preventive dentistry, V S dental college and hospital Bangalore for a routine dental check. Institutional ethical clearance was obtained. Signed written informed consent was obtained from the parents/guardians of the children participating in the study.

Examination of the children of both Thalassemic group and the healthy group was done on a dental chair under natural light.

The child was draped and made to sit on the dental chair for intraoral examination such that the floor of the mouth is maintained parallel to the ground. Salivary parameters were measured using Saliva- check buffer kit (GC Europe) for both the groups.

The salivary flow rate of unstimulated saliva was noted by visual inspection of the level of hydration, by everting the lower lip gently, blotting the labial mucosa with a small piece of gauze and observing the mucosa under good light for droplets of saliva at the orifice of the minor salivary glands. Time was assessed for visible production of saliva. If the time noted was greater than 60 seconds unstimulated salivary flow rate was considered to be low.

Patients were instructed to chew the piece of wax provided in the kit for 5 minutes to measure the stimulated salivary flow rate. Saliva was collected into the collection cup at regular intervals. The quantity of saliva was measured by checking the ml marking on the side of the cup. If the quantity of saliva at 5 minutes was <3.5ml then it was considered as very low, between 3.5-5.0ml then it was considered low and >5.0ml was considered as normal salivary flow rate.

pH strips were placed in the collected salivary sample for 10sec and then the colour change was compared with the testing chart provided in the Saliva-check buffer kit, and the values were assigned accordingly. pH of 5.0-5.8 was considered highly acidic, 6.0-6.6 as moderately acidic and 6.8-7.8 was considered as healthy saliva.

Buffering capacity of saliva was measured using buffer test strips, sufficient saliva was drawn from the collection cup and then dispensed one drop onto each of the three test pads on the buffer strip. The test pads began to change colour immediately and after 2 minutes the final results were calculated by adding the points according to the final colour of each pad. A total of 0-5 was considered as very low buffering ability of saliva, 6-9 was considered as the low buffering ability of saliva and 10-12 was considered as the normal buffering ability of saliva.

Caries evaluation was performed under the standardized condition, using optimal artificial light, mouth mirror and probe. Dental caries was recorded according to World Health Organization(1997) criteria.

2.1.Statistical Analysis:

Descriptive and analytical statistics were done. The chi-square test was used to check differences in proportions. The normality of data was analyzed by the Shapiro-Wilk test. The non-parametric Mann-Whitney U test was used to check differences in mean scores between groups. SPSS (Statistical Package for Social Sciences) Version 20.1 (Chicago, USA Inc.) was used.

3.Results:

The present study was conducted to compare the salivary flow rate, buffering capacity, resting pH and dental caries in healthy children and children with β thalassemia.

The unstimulated salivary flow rate was found to be normal in 24 (66.7%) healthy children and 12(33.3%) thalassemic children, whereas unstimulated salivary flow was found to be low in 6 (25.0%) healthy children, and 18 (75.0%) thalassemic children. The normal unstimulated salivary flow ranges from 0.25 to 0.35ml/min, low ranges from 0.1 to 0.25ml/min. Comparison of unstimulated salivary flow rate between both the groups showed statistically significant results (p-0.003). The unstimulated salivary flow rate was observed to be low in thalassemic children when compared to that in healthy children (Table A).

The stimulated salivary flow was found to be normal in 21(80.8%) healthy children and 5(19.2%) thalassemic children, whereas stimulated salivary flow was found to be

low in 9(47.4%) healthy children and 10(52.6%) thalassemic children. The very low stimulated salivary flow was observed in 15(100%) thalassemic children, none of the children in healthy group had very low stimulated salivary flow. Normal stimulated salivary flow ranges from 1 to 3ml/min, low ranges from 0.7 to 1.0ml/min and if less than 0.7ml/min then it is considered as very low stimulated salivary flow. Comparison of stimulated salivary flow between healthy and thalassemia group was statistically significant (p-< 0.001) (Table B).

Table A: *Comparison of unstimulated salivary flow rate between the groups.*

Groups	Unstimulated Salivary Flow rate		X^2 Value	P-Value*
	Normal	Low		
Healthy N (%)	24 (66.7)	6 (25.0)	10.000	0.003†
Thalassemia N (%)	12 (33.3)	18 (75.0)		

Table B: *Comparison of stimulated salivary flow rate between the groups.*

Groups	Stimulated Salivary Flow rate			X^2 Value	P-Value*
	Normal	Low	Very Low		
Healthy N (%)	21 (80.8)	9 (47.4)	0 (0.0)	24.899	<0.001†
Thalassemia N (%)	5 (19.2)	10 (52.6)	15 (100.0)		

Buffering capacity of saliva was found to be normal in 28(66.7%) healthy children and 14(33.3%) thalassemic children, whereas low in 2(11.1%) healthy children and 16(88.9%) thalassemic children. On comparison of buffering capacity in both the group's results were found to be statistically significant(P<0.001). Buffering capacity was low in the thalassemic group when compared to that of the control group (Table C).

Table C: *Comparison of salivary buffering capacity between the groups.*

Groups	Buffering Capacity		X^2 Value	P-Value*
	Normal	Low		
Healthy N (%)	28 (66.7)	2 (11.1)	15.556	<0.001†
Thalassemia N (%)	14 (33.3)	16 (88.9		

On comparison of salivary resting Ph between the groups, the results were found to be statistically significant (p-0.003). Normal salivary Ph ranges from 7.1 to 7.5. Mean salivary Ph in healthy group was found to be 7.58 whereas in a thalassemic group it was 7.29. Salivary resting ph was observed to be higher in the thalassemic group when compared to that in healthy group (Table D).

Table D: *Comparison of salivary resting pH between the groups.*

	Ph	
Groups	Healthy	Thalassemia
N	30	30
Mean	7.5800	7.29
S.D.	.21238	0.38
Min.	7.00	6.40
Max.	7.80	7.80
Z-Value	-2.920	
P-Value*	0.003†	

Mean DMFT in the healthy group was found to be 0.73 and 2.16 in the thalassemic group, which was statistically significant (p-0.015). The filling component (F) of the DMFT was found to be higher in the healthy group as compared to that of thalassemia group and was statistically significant(p- 0.036) (Table E).

Mean dmft in the healthy group was found to be 1.16 and 2.46 in the thalassemic group. The results were found to be statistically significant (p- 0.046) (Table F).

4. Discussion:

In the present study salivary flow rate, buffering capacity and pH were found to be significantly lower among the thalassemic children when compared to that of the healthy children. This is comparable to the findings of the study done by Al-Jobouri and Al-Casey (2011), who also found that the salivary flow rate and pH were significantly lower in the thalassemic group. As there are no reported studies assessing buffering capacity in thalassemic children, the present study was the first to assess the same. Buffering capacity was observed to be low in patients with thalassemia when compared to that in healthy children. This reduction in salivary flow rate, buffering capacity and resting pH might be due to the fact that iron deposits in thalassemic patients can directly affect the salivary glands, causing painful inflammation, with either normal or diminished salivary flow, low pH and reduced buffering capacity.

However, in contrary Siamopoulou et al. (1992) concluded that Parotid saliva flow rates in thalassemia major (TM) patients were not significantly different from those in the healthy controls.

In the present study mean DMFT/ dmft was found to be significantly higher in patients with thalassemia. Al – Raheem et al. (2009), Al-Wahadni et al. (2002) and Arora et al. (2014a) also reported that dental caries was significantly higher in patients with thalassemia. Predisposing factors like the difference in morphological properties of teeth including; pits, fissures, tubercles, prominence and protuberances lead to increase in the severity of dental caries in thalassemic patients.

Table E: *Comparison of mean DMFT between the groups.*

Groups	D		M		F		DMFT	
	Healthy	Thalassemia	Healthy	Thalassemia	Healthy	Thalassemia	Healthy	Thalassemia
N	30	30	30	30	30	30	30	30
Mean	0.50	1.96	0.13	0.20	0.83	0.13	0.73	2.16
S.D.	0.86	1.77	0.57	0.48	1.46	0.43	1.28	2.56
Min.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Max.	3.00	7.00	3.00	2.00	5.00	2.00	5.00	9.00
Z-Value	-3.647		-1.141		-2.096		-2.421	
P-Value*	<0.001†		0.254		0.036†		0.015†	

Table F: *Comparison of mean Dmft between the groups.*

Groups	D		M		F		Dmft	
	Healthy	Thalassemia	Healthy	Thalassemia	Healthy	Thalassemia	Healthy	Thalassemia
N	30	30	30	30	30	30	30	30
Mean	0.70	1.80	0.30	0.43	0.23	0.16	1.16	2.46
S.D.	1.57	2.12	0.74	0.72	0.62	0.53	2.29	2.82
Min.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Max.	6.00	6.00	3.00	2.00	2.00	2.00	9.00	8.00
Z-Value	-2.262		-1.090		-.411		-1.992	
P-Value*	0.024†		0.276		0.681		0.046†	

There is a significant correlation between the effect of blood transfusion and dental caries, which is related to the variation in concentration of iron in serum which leads to changes in the concentration of iron in the teeth, which also might lead to the severity of dental caries.

Al-Jobouri & Al-Casey (2011) and Kataria et al., (2012) stated that Salivary flow rate plays an important role in relation to dental caries in which the cleansing activity of saliva is very important in the clearance of food as well as bacteria. This might be the reason that the risk for caries is significantly increased when salivary flow rate is pathologically low (hyposalivation and xerostomia) and this evidence indicates that clinically relevant chronic reduction in salivary flow rate is a strong risk factor for caries prevalence and incidence, which is in accordance to the result of the present study

Kuriakose et al., (2013) conducted a study to compare the salivary buffering capacity(BC), flow-rate(FR), resting pH and salivary immunoglobulin-A(s-IgA) levels in children who are caries resistant and who have rampant dental caries, it observed that a reduction in salivary FR, BC, resting pH and s-IgA in children with rampant caries which might have led to dental caries formation. In the present study the salivary flow rate, buffering capacity and resting pH was observed to be high in healthy children because of which there might be decrease in the caries incidence

A study conducted by Gomber and Dewan (2006) also showed a higher prevalence of dental caries in children with thalassemia. They concluded that certain oral structural changes that take place in thalassemic patients due to maxillary enlargement result in protrusion of anterior teeth, increased space between teeth, over-bite or open-bite and varying degrees of malocclusion, which further predispose to caries. These clinical findings were in accordance to the study done by Babu and Amitha (2014) in which they observed similar features like maxillary prognathism, short roots, enlarged marrow spaces, salt and pepper appearance of the skull, hair on end appearance of a cranial vault, and alteration of the trabecular pattern on radiographic examination.

Reduced salivary flow rate in thalassemic patients causes colonization of Streptococcus mutans, which may also have a role in the higher caries incidence. These patients are so preoccupied with their main life-threatening illness that they neglect basic and preventive dental care. A higher caries incidence in thalassemias can also be attributed to poor oral hygiene, improper dietary habits and lack of motivation of these patients.

This result was in contrast to the study done by Arora et al., (2014b) where there was no statistically significant difference in the dental caries status of children with beta-thalassemia major and their normal counterparts.

In the present study, the main difference between thalassemic and healthy children was found in the filling component (F) of mean DMFT, which was lower in thalassemic children when compared to that of healthy children. This indicates lack of conservative treatment provided to thalassemic children. This lack of care could be due to neglect on the part of caregivers and also the financial status of these families who are already burdened with other life-threatening diseases. These findings were

comparable to the study done by Qureshi et al., (2010) where the F component was found to be significantly higher in healthy children when compared to that of thalassemic children.

Thus the results of the present study conclude that the salivary flow rate, buffering capacity and resting pH were low in thalassemic children as compared to that of healthy children, and there is a positive correlation of these salivary parameters and incidence of dental caries.

5.Conclusion:

In the current study, Thalassemic children had reduced salivary flow rate, low buffering capacity, and reduced resting pH when compared to that of healthy children. In addition, high incidence of caries was observed in thalassemic children as compared to that of healthy children. Furthermore, a positive correlation was observed between the salivary parameters and incidence of dental caries in both the groups.

The reason for increased dental caries in children with thalassemia could be due to poor oral hygiene, poor motivation, endocrine problems, malocclusion, frequent blood transfusion and immune deficiency. Reduced salivary flow rate, buffering capacity and resting pH has been observed in thalassemic children, which could be the causative factors for the increased incidence of dental caries in these children.

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Received January 02, 2018; revised February 16, 2018; accepted February 18, 2018; published online March 01, 2018