Evaluation of Salivary Sialic Acid Levels in Acute Lymphoblastic Leukemic Children and Its Correlation with Dental Caries Experience

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Aim- *The aim of present study was to evaluate the salivary sialic acid levels in Acute Lymphoblastic Leukemic (ALL) children and to correlate it with dental caries experience.* ***Method-*** *A total of 120 children aged 4-10yrs were divided into 4 groups of 30 each. Dental caries experience was recorded followed by un-stimulated saliva collection.* ***Results-*** *The mean salivary sialic acid levels of the study group were much higher when compared with the control group, which was statistically significant. A linear relationship between salivary sialic acid levels and dental caries in leukemic children was observed.* ***Conclusion -*** *The findings of our study conclude that salivary sialic acid levels may have a potent activity in dental caries process and can be used as a useful marker in cancer.*
Keywords: *Sialic Acid, Saliva, Dental caries, Acute Lymphoblastic Leukemia(ALL)*

INTRODUCTION

Of all the diseases affecting mankind till date, the most dreaded illness has been cancer. Although, its manifestations are innumerable, the exact underlying oncogenic etiology is yet to be deciphered. Management of cases of malignancies, especially in the late stages remains a challenge to the pathologist and the clinician. Treatment modalities for malignancies i.e., chemotherapy and radiotherapy envisage debatable aftermath effects on the patients. Leukemia, a malignancy of connective tissue is associated with disseminated proliferation of immature or blast cells of the bone marrow, which replaces the normal marrow elements and tends to accumulate in various tissues of the body.[1]

Leukemia constitutes approximately 30% of all childhood cancers and Acute Lymphoblastic Leukemia (ALL) is the most common type. Nearly 75% of the childhood leukemia's are of ALL type-3. Treatment modalities widely accepted in ALL children is chemotherapy alone and chemotherapy with irradiation.[2] Chemotherapy associated toxicity compromises the oral mucosa and impedes repair of mucosal injury. Emesis can increase further mucosal damage. Oral dryness, ulceration and increased dental decay seen are due to reduced salivary flow rate.[3]

Sialic acid found on carbohydrate branches of glycoprotein, exists in conjugate form on external surface of cell membrane as a membrane receptor of insulin, interferon, and serotonin. It regulates external and internal surface of cell membrane. Sialic acid affects amino acid transport on cell membrane and also retards synaptic transmission. Plasma sialic acid levels is a marker of acute phase response.[4] Serum sialic acid levels are found to be raised in storage diseases, cardiovascular diseases and cancers including Leukemia.[5]

Salivary sialic acid levels are found to be raised significantly in subjects suffering from lung cancer than normal healthy subjects.[6] The salivary free sialic acid levels are found to be significantly higher in well-differentiated squamous cell carcinoma than in moderately differentiated carcinoma.[7] Salivary sialic acid levels are found to be affected by oral diseases or oral manifestations of systemic diseases.[6,7] Free sialic acid levels are raised in serum of leukemic patients.[5] Since saliva acts as a "mirror of body's health",[8] changes can be also seen in the salivary sialic acid levels of leukemic children.

Salivary sialic acid, an implicated chelating agent was found to be positively correlated with active dental caries.[9] The species of *Viridans streptococci*, most commonly associated with dental caries are found to utilize salivary sialic acid for the adherence to the dental tissues & extra oral sites.[10]

Hence, the present study evaluates the sialic acid levels in saliva and correlates it with dental caries experience in Acute Lymphoblastic Leukemia children.

MATERIALS AND METHOD

In the present cross-sectional study, 120 children (90 leukemic children, study group and 30 normal healthy siblings, control group) between the age 4-10 yrs reporting to the Institute of Child Health, Chennai, were selected by simple random sampling method. Ethical committee of the Institute approved the study. Parental written and oral consent was obtained for each participant. The total time duration for the study was 6 months period. The grouping was done as follows-

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Table 1. Mean values of salivary sialic acid levels in study group & control group. *

	Group	N	Mean (mg/dl)	Std. Deviation	Sig. *P value*
Salivary Sialic acid levels (mg/dl)	**Group 1**	30	2.64	± 0.24	<0.001 (hs)
	Group 2	30	4.06	± 0.27	
	Group 3	30	7.62	± 0.40	
	Group 4 (control)	30	0.46	± 0.29	

hs= highly significant

*The statistical comparison of salivary sialic acid levels between study and control group was evaluated by One way ANOVA and Kruskal-Wallis H test (Non-parametric analysis of variance (ANOVA) test). P-value <0.05 is considered to be statistically significant.

Table 2. Mean values of dental caries experience in study group & control group.*

	Group	N	Mean	Std. Deviation	Sig. *P value*
Dental caries Experience	**Group 1**	30	0.87	± 0.67	<0.001 (hs)
	Group 2	30	1.66	± 0.60	
	Group 3	30	3.40	± 0.88	
	Group 4 (control)	30	0.70	± 0.87	

hs= highly significant

*The statistical comparison of Dental caries experience between study and control group was evaluated by One way ANOVA and Kruskal-Wallis H test (Non-parametric analysis of variance (ANOVA) test). P-value <0.05 is considered to be statistically significant.

Group 1 - 30 ALL patients without chemotherapy, **Group 2** - 30 ALL patients up to 1-2 weeks of chemotherapy, **Group 3** - 30 ALL patients with more than 2 weeks of chemotherapy, **Group 4** - (Control group) 30 normal healthy siblings of the study population.

The patients included in the study group were diagnosed cases of Acute Lymphoblastic Leukemia (ALL) who reported during the study period and only chemotherapy was the treatment protocol. A Standard drug regimen protocol designed in collaboration with National Cancer Institute (USA) of MCP 841 for ALL children11 being used at various centers in India including Institute of Child Health (ICH) was followed. Acute Lymphoblastic Leukemi (ALL) children receiving the chemotherapeutic regimen for 1 to 2 weeks and more than 2weeks were part of the study.

Acute Lymphoblastic Leukemic children suffering from any other disease, children who were on chemo-radiation therapy and on the maintenance phase were excluded from the study.

Clinical procedure

Before the collection of saliva samples, caries assessment was done using 'DMFT' index for permanent dentition and 'dft' index for primary dentition.[12] A pilot study was carried out by three examiners to minimize the inter examiner variability. Among the three examiners, one trained examiner was chosen for further oral examination and saliva collection along with one recorder. The chosen examiner examined the children in the study group and the control group. A standardized protocol was used for the collection of saliva of the patients in study group and control group. Saliva collection was performed at the pediatric oncology unit of Institute of Child Health (ICH). The patients were made to sit on the bed, and examination was done in the day light using latex gloves, plain mouth mirror, probes, tweezers, container, cotton/gauze to remove any debris and dry the oral mucosa.

All collections were performed between the hours of 8:00 a.m. and 10:00 a.m. to avoid circardian variations in salivary flow.[13] The subjects were instructed to refrain from eating or drinking for a minimum of 2 hours before the saliva sample were collected. Before treatment un-stimulated saliva (1.0 - 1.5ml) was collected by allowing the patient sit in the coachman position, the patient was asked to passively drool into a funnel inserted into a graduated cylinder for 5 min.14 The un-stimulated salivary flow (USF) rate was calculated using the following formula:[14]

$$USF = \frac{\text{Total volume of collected saliva}}{\text{Time period for collection of saliva}}$$

The collected saliva was stored in glass or plastic vials, then in the chiller at *4°C*. The sialo-chemical analysis was done to evaluate sialic acid levels in saliva with in 24 hours.[15]

Sialochemical analysis

Saliva samples were centrifuged (centrifugal force: 1,000 g) to remove bacteria and other extraneous material. The resulting clarified supernatant fluid was used for the biochemical assay to measure salivary sialic acid levels. Sialic acid was estimated by the method of Winzler *et al*. A protein precipitate of saliva containing sialic acid reacted with diphenylamine producing purple color which was quantitatively measured on a spectrophotometer at 530 nm.[15]

The statistical comparison of mean values of salivary sialic acid levels, dental caries experience and salivary flow rate with respect to the three study groups and one control group was evaluated using one way ANOVA, Kruskal-Wallis H test (Nonparametric analysis of ANOVA test), Tukey's Post Hoc Test, Kappa analysis to check the inter-observer variability and Pearson's coefficient of correlation with SPSS data processing software version 17.0.

RESULTS

Children with ALL from the study group had higher levels of sialic acid in saliva than the controls. Mean sialic acid levels in control group were as low as 0.46 mg/dl, whereas these levels increased from 2.64 mg/dl in group 1, 4.06 mg/dl in group 2 up to 7.62 mg/dl in group 3 of the study group. This increase in salivary sialic acid levels occurred with the duration of chemotherapy and the results were found to be statistically highly significant (ANOVA: 2608.467, Kruskal Wallis H test, $p<0.001$). (Table 1).

DMFT/dft values in group 1(0.87) leukemic children were not statistically significant when compared with control group (0.7) ($p=0.818$). The DMFT/dft values in group 2 was 1.66 and in group 3 was 3.40. The increased mean value of DMFT/dft in group 3

Table 3. Mean values of Salivary flow rate in study group & control group.*

	Group	N	Mean	Std. Deviation	Sig. *P value*
Salivary Flow Rate (ml/min)	**Group 1**	30	0.46	± 0.20	<0.001 (hs)
	Group 2	30	0.35	± 0.16	
	Group 3	30	0.12	± 0.09	
	Group 4 (control)	30	0.85	± 0.31	

* The statistical comparison of Salivary Flow Rate between study and control group was evaluated by One way ANOVA and Kruskal-Wallis H test (Non-parametric analysis of variance (ANOVA) test). P-value <0.05 is considered to be statistically significant.

leukemic children who were for more than 2weeks of chemotherapy were statistically highly significant when compared with other study groups and the control group.(ANOVA:73.914, Kruskal Wallis H test p<0.001).(Table 2). Amongst the DMFT/dft values, the values for decayed teeth (D/d) were higher than missing (M) or filled teeth (F) in the three study groups.

The salivary flow was reduced in all the three groups with least mean value of 0.12ml/min seen in group 3, who were on long term chemotherapy which was statistically highly significant (ANOVA, Kruskal Wallis H test, p<0.001). (Table 3)

A positive correlation was observed between the total salivary sialic acid levels, and dental caries experience among the study groups, suggesting that as the duration of chemotherapy increases, the sialic acid levels in saliva increase, with increase in the dental caries experience, which was statistically highly significant (r = 0.775, p<0.001).(Table 4)

An inverse correlation was observed between the salivary flow rate and dental caries experience among the study groups, suggesting that as the duration of chemotherapy increases, salivary flow rate decreases, the dental caries experience increases, which was statistically highly significant (r = 0.534, p<0.001). (Table 5).

Post hoc Analysis showed statistically significant difference for the salivary sialic acid levels and dental caries experience between the 1-2,1-3,1-4, 2-3,2-4, and 3-4 groups. (Tukey's Test, p<0.001). (Table 6 & 7).

Table 5. Comparison of mean values of dental caries experience among the study groups and its correlation with salivary flow rate.*

Group	N	Mean Salivary flow rate (ml/min)	Mean DMFT/dft (Dental Caries experience)	Correlation between salivary flow rate and DMFT/dft
1	30	0.46	0.87	r = 0.534 P = 0.001(hs) N=120
2	30	0.35	1.66	
3	30	0.12	3.40	
4 (control)	30	0.85	0.70	

hs= highly significant.

* Correlation between salivary flow rate and DMFT/dft was evaluated by Pearson's correlation coefficient.

Table 4. Comparison of mean values of dental caries experience among the study groups and its correlation with salivary sialic acid levels.*

Group	N	Mean Sialic acid levels (mg/dl)	Mean DMFT/dft (Dental Caries experience)	Correlation between total Sialic acid and DMFT/dft
1	30	2.64	0.87	r = 0.775 *P* = 0.001(hs) N=120
2	30	4.06	1.66	
3	30	7.62	3.40	
4 (control)	30	0.46	0.70	

hs= highly significant.

* Correlation between total sialic acid and DMFT/dft was evaluated by Pearson's correlation coefficient.

Percentage of Agreement between Examiner 1vs 2 and Examiner 2 vs 3 was 97.5% with the Kappa value of 0.96, which shows that it was in excellent agreement between the examiners. Also, the percentage of agreement between the Examiner 1and 2 was 100% with the Kappa value of 1.00 which shows that it was in absolute agreement between the Examiners.(p<0.001). (Table 8).

DISCUSSION

Leukemia is a common type of malignancy.[2] Nearly 75% of childhood leukemias are of Acute Lymphoblastic type (ALL) which is a debilitating disease suppressing children's general and dental health, rendering them immunocompromised.[3] Children treated with chemotherapy forming a part of treatment schedule for the same, exhibit acute complications in the oral cavity and long term complications in dental and craniofacial development. Higher prevalence of dental diseases like dental caries, oral mucositis, gingival inflammation are reported for children with leukemia when compared to systemically healthy children.[4,22,26,27] Similar findings were observed in the present study with increased severity of disease process.

Table 6. Post Hoc analysis of salivary sialic acid levels.* Tukey's HSD.

	I group	J group	Mean Difference (I-J)	P value
Salivary Sialic acid levels.	1	2	-1.42	0.001
		3	-4.98	0.001
		4	2.18	0.001
	2	1	1.42	0.001
		3	-3.55	0.001
		4	3.60	0.001
	3	1	4.98	0.001
		2	3.55	0.001
		4	7.16	0.001
	4	1	-2.18	0.001
		2	-3.60	0.001
		3	-7.16	0.001

*Post hoc Analysis of salivary sialic acid levels was evaluated by Tukey's test. p-value <0.05 considered as statistically significant.

Table 7. Post hoc analysis of dental caries experience.* Tukey's HSD

	I group	J group	Mean Difference (I-J)	*p- value*
Dental Caries Experience	1	2	-0.78	0.001
		3	-2.53	0.001
		4	0.17	0.001
	2	1	0.78	0.001
		3	-1.75	0.001
		4	0.95	0.001
	3	1	2.53	0.001
		2	1.75	0.001
		4	2.70	0.001
	4	1	-0.17	0.001
		2	-0.95	0.001
		3	-2.70	0.001

*Post hoc Analysis of Dental Caries Experience was evaluated by Tukey's test. p-value <0.05 considered as statistically significant.

Table 8. Inter observer variability in relation to Dental Caries Experience.*

	Kappa value	% of Agreement between the examiners	*p-value*
Measure of Agreement between Examiner 1 vs 2	0.96	97.5%	0.001
Measure of Agreement between Examiner 1 vs 3	1.00	100%	0.001
Measure of Agreement between Examiner 2 vs 3	0.96	97.5%	0.001

*Inter observer variability analyzed by Kappa statistics. p-value <0.05 considered as statistically significant.

Leukemic patients were found to be at various levels of risks for developing oral problems, the reason for which could be due to the time lapse between the onset of the pathology and the clinical diagnosis of the condition among them. Other factors influencing include the drug, type of the malignancy, patient's age and the level of oral health before and during the therapy.[4,16]

Chemotherapy causes decreased salivary flow rate which can be attributed to the fact that salivary gland hypoplasia occurs in patients receiving chemotherapeutic agents,[4,17] the results of which are consistent with the above findings, where the most affected group was the one who received chemotherapy for more than 2 weeks.

Sialic acids are terminal sugar components of the oligosaccharide chains of glycoproteins and glycolipids. In human beings it is present in body fluids (blood plasma, breast milk, sweat, gastric juices and urine) and salivary glands. It is also present as constituent of membrane glycoprotein's of erythrocytes, leucocytes and platelets.[18]

The structure, occurrence and general functions of sialic acids have been extensively reviewed.[5,6,7,18] Sialic acids contribute significantly to the overall negative charge of cell surface and glycoprotein. The negative charge contributes to cell to cell repulsion (ant adhesion effect), functioning stability and survival of glycoprotein in blood circulation and cell-to-matrix interactions. Due to the shielding effect, sialylated glycans protect parts of a glycoprotein from proteolytic attacks.[18]

Sialic acid is an important structural component of salivary glycoproteins, enhancing bacterial aggregation as well as participating in the formation of the acquired pellicle and dental plaque. In any diseased state, free radicals produced are higher. These free radicals may play an important role in tissue damage and are increased in cancer. It is an important cancer marker. Salivary sialic acid (SA) levels are affected by oral diseases.[19] Avsara *et al* (2009) showed that salivary sialic acid levels were raised in children who were passive smokers.[20] This shows that salivary sialic acid increased with salivary oxidative stress and is linked with cancer.[19,20]

The degenerative diseases that afflict humanity have their origin in deleterious free radical reactions. Reactive oxygen species are related to both the arrest of growth and start of cell differentiation. Human cancer is accompanied by a strong oxidative predominance in blood. In cancer, ingestion of drugs has been reported to cause increased production of oxygen free radicals through auto-oxidation. Generation of oxygen free radicals by auto- oxidation is widely believed to be one of the main causes of oxidative stress in cancer.[16,17] In the present study, salivary sialic acid levels were raised in leukemic children when compared with the healthy siblings. The increase in salivary sialic acid levels was found to be proportional to the duration of chemotherapy in leukemic children. This rise in salivary sialic acid levels may be attributed to the increased salivary oxidative stress.

The dental caries experience in leukemic children was found to increase when compared with the control group. Sialic acid, implicated as an important chelating agent has been found to be positively associated with active dental caries.[9] *Viridans streptococci,* an important species of microorganism associated with dental caries, utilizes salivary sialic acid for adherence to dental tissues.[10] Sarkarya (2003) showed that adherence of *S.mutans, S.sobrinus* and *S.sanguinus* is mediated by sialic acid and sialic acid-containing receptors.[23] Also, Yarat (1999) showed raised sialic acid levels with increased oxidative stress and increased dental caries experience in Down syndrome subjects.[21]

In the present study, the sialic acid levels in saliva were found to be raised along with dental caries experience in leukemic children. The levels of salivary sialic acid and dental caries experience increased with the duration of chemotherapy and thus showing the positive correlation. This could be due to fact that as salivary flow rate decreases with the duration of chemotherapy as in our study, protein concentration increases especially mucins in saliva.[24]

Sialic acid being a constituent of human salivary mucin is rapidly released, broken down by the bacterial enzyme systems and utilized for bacterial metabolism compared to other hexosamines. The reaction involving the release of sialic acid from salivary mucins leads to its precipitation under mildly acidic or neutral conditions play an important part in formation of dental plaque, thereby resulting in dental caries.[24]

The increase in dental caries experience in the present study could also be due to the fact that maintenance of oral hygiene was difficult for the patients who were on chemotherapy that lead to increased oral mucosal inflammation resulting from the decreased salivary flow rate.[22,25,26,27] Conflicting results, however, were reported by Yang *et al*.[28]

Dental caries is a multi-factorial disease, associated with numerous factors. Increased levels of sialic acid in saliva of leukemic children may be used to assess the activity of dental caries. However, the use of sialic acid as a risk indicator for dental caries activity in children irrespective of any underlying systemic disease invites further research.

The findings of our study finally conclude that salivary sialic acid levels may have a potent activity in dental caries process and can be considered as a useful marker in cancer.

Due to the paucity of literature of salivary sialic acid levels in leukemic children, more research may be done before a concrete conclusion be derived.

CONCLUSION

The salivary sialic acid levels were higher in leukemic children when compared to the healthy siblings. A linear relationship was found to be present between salivary sialic acid levels and dental caries in leukemic children. An inverse relationship was seen between salivary flow rate and dental caries in leukemic children.

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