A DESCRIPTIVE ASSESSMENT OF ORAL HYGIENE AND SALIVARY PARAMETERS AMONG IDENTICAL AND NON-IDENTICAL TWINS

ABSTRACT

AIM: This study was to evaluate saliva parameters of identical and non identical twins with their dental caries and oral hygiene status. **MATERIAL AND METHODS:** A cross sectional study was conducted to assess the oral health status and investigate the salivary parameters of identical and non–identical twins in Kodhini Village, Kerala. The WHO Oral Health Assessment Form was used to record the oral health status. The Simplified Oral Hygiene Index and Gingival Bleeding Index were also included. Unstimulated saliva was collected. Immunoglobulin A levels in saliva was estimated by Turbidimetric Immunoassay. Salivary Cortisol was analysed using ELISA method. For TAC thiobarbituric acid reactive from Fenton's reaction. **RESULTS:** Since the absolute difference of the salivary parameters between the pairs of twins was not symmetrically distributed, we report median and interquartile range to describe these differences. There were no observed significant variations in the above salivary parameters. **CONCLUSION:** The similarity in their environmental factors as well as perhaps the similar genetic predisposition on salivary factors has led to the similarity in the dental caries experience in these twin children.

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KEYWORDS

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INTRODUCTION

Saliva is a complex fluid in the oral cavity, composed of a mixture of secretary products from the major and minor salivary glands.[1] Saliva has multifunctional roles in the oral cavity, and is very important for the maintaining of oral health.[2,3] Thus the saliva research field is rapidly advancing. Saliva has various defense mechanisms such as immunological and enzymatic defense systems against bacteria, viruses, fungi, protection of mucosa and promotes its healing.[4] One of the important defense mechanism is antioxidants system.[5] Antioxidants have many health benefits that made their evaluation in disease process very popular.[6]

Caries is one of the most common oral health problems and its prevention is one of the most important strategies in many countries. The levels of antioxidants could be changed in response to an infection, inflammation or disease.[7] Also Tulunglu showed that total protein and total antioxidant level of saliva were increased with caries activity.[8]

A village situated 35 km south of Calicut in Mallapramditrict, kerala entered international spotlight when a survey found an unusually large number of twin births in the region of which the phenomenon yet to be understood.Family and twin studies have found a strong genetic component in caries, tooth.[9-11] Genetic factors have also been shown to affect inter-individual variation in children's susceptibility to caries.[12]

However, very little studies have been discussed about the saliva parameters of identical and non identical twins with their dental caries. Therefore, the aim of this study was to evaluate saliva parameters of identical and non identical twins with their dental caries and oral hygiene status.

MATERIAL AND METHODS

Kodini village in the southern state of Kerala, India came to the limelight with the exceptionally large number of twins unheard of in any other part of the globe. No prior oral health study was conducted on these twins as to ascertain their oral health parameters in between and among the twins. The Twin and Kin organisation was involved with the treatment camp organised by the department of Public Health dentistry, Yenepoya Dental Colleges.

A cross sectional study was conducted to assess the oral health status and investigate the salivary parameters of identical and non – identical twins in Kodhini Village, Kerala.The study samples were selected using the convenience sampling method based on the availability of twins born in Kodhini Village at time of a dental treatment camp. The study samples consisted of 40 pairs of twins and one set of triplets. Of the total 83 samples, 45 were non-identical and 38 were identical twins.

Ethical clearance for the study was obtained from the Ethical clearance committee of Yenepoya University, Mangalore.

Prior to commencement of oral examination relevant medical and dental history was elicited from the parents. The subjects were selected based on the following inclusion and exclusion criteria. Twins born in Kodhini Village and who provided consent/ assent for the study were included in the study. Subjects undergoing orthodontic treatment, with severe systemic disease and taking antihistamines, antiepileptic drugs, anticholinergics, antibiotics or any other drugs influencing salivary constituents were excluded.

The WHO Oral Health Assessment Form (1997) was used to record the oral health status. A single examiner and recorder carried out intra-oral type-III examinations as recommended by American Dental Association and adopted by the World Health Organization (WHO, 1999). The Simplified Oral Hygiene Index (OHI-S by John C Greene & Jack R Vermillion in 1964) and Gingival Bleeding Index (GBI by Carter H.G & Barnes G.P in 1974) were also included to assess the oral hygiene and gingival status of the subjects.

Unstimulated saliva was collected from every subject. Subjects were instructed not to eat, drink, perform any oral hygiene practices or put anything into their mouth before the collection time. Saliva was collected by making the patient sit in an upright position. Every subject was asked to allow saliva to drool from the oral cavity into sterile, labelled disposable containers. 5 ml saliva was collected from each subject and transported immediately in a thermostat container maintained at -4 degree Celsius for 10 hours and then stored at -40 degree Celsius at Yenepoya Research Centre until the samples were analysed.

The approximate pH of each salivary sample was determined at the site using pH strips while a pH meter was used at the research center.

Immunoglobulin A levels in saliva was estimated by Turbidimetric Immunoassay using QUANTIA –IgA reagents. Two ml of each salivary sample was centrifuged at 4000 rpm for 20 min, to remove the particulates. 5µl of the supernatant saliva was used for the analysis.[13]

Salivary Cortisol was analysed using ELISA method by VITROS ECiQ Immunodiagnostic System (Ortho Clinical Diagnostics kit by Johnson & Johnson).[14]

The assay measured the capacity of the biological fluids to inhibit the production of thiobarbituric acid reactive (TBARS) substances from sodium benzoate under the influence of free oxygen radicals derived from Fenton's reaction. A solution of 1mmol/litre uric acid was used as standard.[15]

The buffering capacity was analysed by "titration method". 10µl of Hydrochloric acid

was titrated into the saliva sample in the measurement chamber of the pH meter, and allowed to stabilize for a few seconds; then pH measured again. HCL titration was repeated up to a total of 30 µl acid was titrated. At the point of 30µl titrated HCL, salivary buffering capacities were ranked into one of the following three categories according to the measured pH; High buffering capacity (pH above 5.5), medium buffering capacity (pH between 4.5 & 5.5) and low buffering capacity (pH below 4.5).

Data were entered in Microsoft Excel (2010) and statistically analysed using SPSS 15.

RESULTS

The descriptives of the salivary parameters and the dentition status has been described based on the identical and non-identical twins and based on the gender of the twins (all female, all male and male/female). Since the absolute difference of the salivary parameters between the pairs of twins was not symmetrically distributed, we report median and interquartile range to describe these differences.

Among the data available for 31 pair of twins of them 15 were identical and 16 were non-identical. In table 1, shows the percentage of all female identical twins was 20% and non-identical were 17.5%. In the all-male category27.5% were identical and 12.5% were non-identical. Out of the total study sample 47.5% were identical twins and 52.5% were non-identical.

Table 2 shows the differences of the dentition status among the twins. According to this in primary dentition, around 60% and 81.2% had no differences in the dentition status among the identical and non-identical twins. The permanent dentition when observed showed 41.7% and 56.2% of the identical and non-identical twins had difference of up to 3 teeth in their dentition status.

Table 3 shows the descriptives of the salivary parameters i.e. pH, buffering capacity at 30µL and 60µL, and the total antioxidant levels in saliva of the twins. There were no observed significant variations in the above salivary parameters.

Table 4 shows the descriptives of salivary Ig A and salivary cortisol levels which were found to have no significant variations among the identical and non-identical twins.

Salivary pH measured using pH strips in the cross tabulation between the twins in table 5 shows out of the 40 pairs of twins, 34 pairs (85%) have identical pH values.

Table 6 shows the community periodontal index scores available for 29 pairs out of the 40 pairs , 23 (79.31%) pairs have identical CPI highest scores.

Intra group comparison of the salivary parameters using Mann Whitney U test as

shown in table 7, observed no significant differences among the identical and non-identical pairs of twins.

Table 1. Number and percentage of twins examined.

Twins		n	Percentage
All female	IDENTICAL	8	20.0%
	NON IDENTICAL	7	17.5%
Male/female	NON IDENTICAL	9	22.5%
All male	IDENTICAL	11	27.5%
	NON IDENTICAL	5	12.5%
Total	IDENTICAL	19	47.5%
	NON IDENTICAL	21	52.5%

Table 2. Differences among the dentition status among the twins.

Dentition		IDENTICAL		NON IDENTICAL	
		n	Percentage	n	Percentage
PRIMARY	No difference	9	60.0%	13	81.2%
	Difference of up to 3 teeth	3	20.0%	2	12.4%
	Difference of more than 3 teeth	3	20.0%	1	6.2%
	Total	15	100.0%	16	100.0%
PERMANENT	No difference	4	33.3%	6	37.6%
	Difference of up to 3 teeth	5	41.7%	9	56.2%
	Difference of more than 3 teeth	3	25.0%	1	6.2%
	Total	12	100.0%	16	100.0%

Table 3. The descriptives of the salivary parameters (pH, buffering capacity at 30µL, 60 µLand TAC).

Twins		INITIAL pH			30 µL			60 µL			TAC		
		Median	Q1	Q3	Median	Q1	Q3	Median	Q1	Q3	Median	Q1	Q3
All female	IDENTICAL	.98	.21	1.38	1.08	.34	1.77	.93	.38	.98	32.55	8.80	184.58
	NON IDENTICAL	.78	.69	.82	.43	.18	.99	.64	.20	.98	313.30	115.90	540.80
Male/Female	NON IDENTICAL	.41	.21	.65	.48	.38	1.05	.84	.66	1.21	120.20	94.40	201.60
All male	IDENTICAL	.46	.35	.64	.65	.53	7.51	.55	.24	.78	115.90	68.70	300.90
	NON IDENTICAL	.98	.15	1.21	.61	.56	7.23	1.15	.16	.35	219.00	29.60	351.90
Total	IDENTICAL	.49	.31	1.03	.70	.35	2.20	.55	.32	.82	98.70	17.10	206.10
	NON IDENTICAL	.69	.30	.88	.56	.38	1.05	.84	.27	1.03	201.60	94.40	321.90

DISCUSSION

Dental caries was examined in twin populations early in the 20th century. One of the first studies evaluated 301 pairs of twins, of which 130 were monozygotic and 171 were dizygotic.[16] The evaluation compared the caries incidence of monozygotic twins with same-sex dizygotic (93 pairs) and different-sex dizygotic (78 pairs) twins. The results demonstrated that monozygotic twins had a more similar caries incidence than dizygotic twins and that different-sex dizygotic twins had the greatest variance.

Bachrach and Young found caries prevalence to be greater in monozygous than in dizygous pairs, the difference between them

regarded as statistically insignificant. These findings were interpreted to suggest that heredity played a minor role in the etiology of dental caries.The statistical analysis suggested that there was no statistical difference in salivary parameters and caries experience in the twin children. The similarity in their environmental factors as well as perhaps the similar genetic predisposition on salivary factors has led to the similarity in the dental caries experience in these twin children.[17]

Table 4. The descriptives of the salivary parameters (Ig A, Cortisol).

		IGA			CORTISOL			OHIS			GBI		
		Median	Q1	Q3	Median	Q1	Q3	Median	Q1	Q3	Median	Q1	Q3
All female	IDENTICAL	7.05	1.80	8.00	.06	.00	.18	.27	.10	.40	.02	.00	.15
	NON IDENTICAL	3.50	3.00	6.20	.21	.12	.43	.20	.00	.40	.00	.00	.00
Male/Female	NON IDENTICAL	7.50	3.00	11.80	.25	.11	.36	.50	.00	.50	.00	.00	.00
All Male	IDENTICAL	3.30	1.70	5.10	.11	.10	.50	.20	.00	.30	.00	.00	.04
	NON IDENTICAL	8.40	8.20	9.00	.15	.15	.23	.30	.20	.70	.08	.00	.21
Total	IDENTICAL	3.60	1.70	7.40	.11	.02	.21	.20	.00	.30	.00	.00	.05
	NON IDENTICAL	6.20	3.40	9.00	.21	.11	.36	.30	.00	.50	.00	.00	.00

Table 5. pH levels among the twins.

		TWIN B pH (STRIPS)								
		IDENTICAL			NON IDENTICAL			Total		
		6	7	8	6	7	8	6	7	8
TWIN A pH (STRIPS)	6	3	1	0	11	0	0	14	1	0
	7	0	4	1	0	5	0	0	9	1
	8	0	2	8	0	2	3	0	4	11

Table 6. Community periodontal index scores among the twins.

		TWIN B – CPI HIGEST SCORE					
		IDENTICAL		NON IDENTICAL		Total	
		1	2	1	2	1	2
TWIN B – CPI HIGEST SCORE	1	6	0	7	2	13	2
	2	2	3	2	7	4	10

The descriptives of the salivary parameters in our study i.e. pH, buffering capacity at 30µL and 60µL, and the total antioxidant levels in saliva of the twins showed no significant variations.Rudney*et al* deduced that genetic regulation of hormones or neurotransmitters could be responsible for heritable patterns of saliva secretion rates or composition.[18]

Intra group comparison of the salivary parameters we computed the absolute difference between twins for the variables pH, 30 µL, 60 µL, TAC, IGA, Cortisol, OHIS and GBI. These differences are compared between identical and non-identical twins.We did not

find difference in the differences between twins, among identical and non-identical pairs, for any of the variables at 5% level of significance. Mann Whitney U test was performed to compare the differences. This is in agreement with the study conducted by Taranath A et al showed no significant difference in the caries experience in twins' children and no statistical difference in the salivary parameters amongst the twins.[19, 20]

Table 7. Intra group comparison with salivary parameters.

Difference measured between the twins	Non Identical			Identical			pvalue
	Median	95% Confidence Interval		Median	95% Confidence Interval		
		Lower bound	Upper bound		Lower bound	Upper bound	
pH	.65	.210	.995	.53	.374	.818	0.549
30 µL	.54	.354	.870	.72	.495	1.394	0.613
60 µL	.56	.285	.990	.88	.486	2.060	0.310
TAC	98.80	27.518	236.573	167.40	97.385	315.931	0.194
IGA	3.90	2.452	8.310	5.10	3.278	7.592	0.748
Cortisol	.17	.094	.405	.17	.094	.364	0.705
OHIS	.70	.500	1.055	.76	.339	.961	0.943
GBI	.15	.000	.679	.21	.000	.857	0.771

Table 8. Descriptives of Oral hygiene and bleeding index among the twins.

		OHI-S			GBI		
		Median	Q1	Q3	Median	Q1	Q3
All female	IDENTICAL	.27	.10	.40	.02	.00	.15
	NON IDENTICAL	.20	.00	.40	.00	.00	.00
Male/Female	NON IDENTICAL	.50	.00	.50	.00	.00	.00
All Male	IDENTICAL	.20	.00	.30	.00	.00	.04
	NON IDENTICAL	.30	.20	.70	.08	.00	.21
Total	IDENTICAL	.20	.00	.30	.00	.00	.05
	NON IDENTICAL	.30	.00	.50	.00	.00	.00

Studies using different twin designs have indicated that the risk of dental caries has a genetic basis, but these examinations have not been able to specify which of the different aetiological factors– diet, saliva, tooth morphology or microbial flora – are most important.[21] Analyses of twins reared apart have provided the strongest evidence that genetic factors contribute to caries incidence, as the confounding issues of common environmental effects within twin pairs are overcome. Although twin studies have provided strong evidence of a genetic contribution to caries risk, they have not provided any evidence of linkage to, or association with, specific genes. Genetic

linkage studies based on well-defined populations have been identified by Schuler as the necessary next step in analysing the relationship between inheritance and dental caries.[22]

Salivary pH measured using pH strips in the cross tabulation between the twins in table-5 shows out of the 40 pairs of twins, 34 pairs (85%) have identical pH values. This suggests strong genetic influence on salivary Ph.Few studies have contributed to the knowledge so far.

Table 6 shows the community periodontal index scores available for 29 pairs out of the 40 pairs , 23(79.31%) pairs have identical CPI highest scores. This may not be entirely due to genetic effect. They are other contributing factors like oral hygiene practices. Since the study group shares almost similar environment and same knowledge and attitude towards oral hygiene practices, this could possibly confound the result. While the hereditary basis for susceptibility to dental caries is rather well-founded, the heritability regarding periodontitis is not so. This is due to rather to the relative complexity of the disease, continually emerging new knowledge about its pathogenesis, vagaries of clinical diagnosis and statistical quantification and the profession's own nomenclature for classifying these diseases, which keeps evolving even today.[23] A recent investigation of periodontitis and gingivitis in 64 MZ twin pairs and 53 DZ twin pairs reared together confirmed that approximately 50% of the variance in adult periodontitis could be attribute to genetic factors (Bonney, 1984).[24] Using a gingival index (GI), Michalowicz*et al.* (2000) estimated gingival bleeding to have a genetic component of about 50%.[25] They found that the frequency of dental cleanings altered the most correlations of the gingival index among monozygotic and dizygotic twins. Since all this studies does not support high heritability of periodontitis, our result may be confounded by the shared environmental factors.

CONCLUSION

Our study was designed to establish genetic influence on the descriptive of salivary parameters with dental caries in twin children .The statistical analysis suggested that there was no statistical difference in salivary parameters and caries experience in the twin children. The similarity in their environmental factors as well as perhaps the similar genetic predisposition on salivary factors has led to the similarity in the dental caries experience in these twin children. The reason for the similarity in caries experience among twin children or even in siblings is mainly due to the role played by the similar dietary habits, oral hygiene practices etc., unless they are reared apart. It is possible to conclude that the caries experience is well correlated to salivary factors which are definitely under the genetic control

though confounding factors exist. Hence we suggest further genome wide association scan studies on salivary parameters to establish this. This study had the patronage of the Yenepoya University in carrying out the study.

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