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Professional Center
275/75, Prof. Stanley Jayasundara Mawatha,
Off Baudhahoka Mawatha,
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Editorial Office

Office of the SLDA,
Professional Centre,
275/75, Bauddhaloka Mawatha,
Colombo - 7.

Correspondence regarding editorial matters, articles, reviews and news items should be addressed to the Editor, SLDJ, Prof. Sunethra Rajapakse, Dept. of Oral Medicine & Periodontology, Faculty of Dental Sciences, University of Peradeniya, Sri Lanka.
Tele: 0812 397 552 / 0714 488 800

Correspondence regarding advertisements and financial matters should be addressed to Dr. Malcolm Stanislaus, 50, Hekitta Cross Road, Hendala, Wattala.
Tele: 011 - 2930368

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SRI LANKA DENTAL ASSOCIATION
Professional Center
275/75, Prof. Stanley Wijesundara Mawatha,
Off Bauddhaloka Mawatha,
Colombo 07, Sri Lanka.
Tel/Fax 0094 11 2595147

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EDITORIAL

NEED OF THE HOUR - PREVENTIVE ORAL HEALTH

Sri Lanka in recent times has produced specialists and super specialists in the field of dentistry. Their services however, are limited to a small sector of the population for numerous reasons which, I shall not discuss. In the backdrop of the above mentioned specializations, it is worthwhile to appraise the situation with regard to the prevalence of common preventable oral diseases in Sri Lanka.

According to the available published data on oral diseases in Sri Lanka, the occurrence of common oral diseases such as dental caries and periodontal diseases are widespread. Sri Lankan National Oral Health Survey 2002/2003, reports the prevalence of early childhood caries among 5-year olds as 65% and caries among 12 year olds as 40%¹. According to the same report, the periodontal disease status for ages 35-44 years and 65-74 years with periodontal disease of all severity were 89.9% and 98.1% respectively and the prevalence of caries in 35-44 year olds has been reported to be 91.5%.¹In addition, epidemiological studies carried out in different populations in the country show the prevalence of oral diseases among pregnant women, preschool children and elderly is high^{2,3,4,5}. In this context of high disease burden, it is more appropriate to focus on planning programmes geared for prevention of oral diseases. These programmes could be planned with specific population groups such as pregnant women, preschool children and elderly in mind.

It is imperative to ask ourselves a few important

questions with regard to prevention of oral diseases in Sri Lanka. Can we do it alone as an oral health care workforce? Do we have sufficient resources? Can we integrate our preventive messages into an already established primary health care system? What is the most economical approach in terms of manpower as well as cost effectiveness? Last but not least, do we have an adequate and reliable database of our own, needed to plan effective oral health promotion and disease preventive programmes suitable for Sri Lanka?

Due to high dentist to population ratio in the country (0.8:10000) there is a dire need to explore the possibility of utilizing alternative manpower resources that could be trained to deliver oral disease preventive programmes. Is it possible to incorporate oral health care in the already established primary health care service which is being provided in the medical sector? If it is feasible primary health care workers who operate island wide at grass root levels need to be trained to deliver messages on oral health promotion and disease prevention. The use of primary healthcare workers in oral health promotion appears to be a viable alternative as it has already been shown to be effective in oral cancer and precancer screening in the past⁷. Furthermore, utilizing primary healthcare workers as the first line of prevention in the delivery of oral health messages should be cost effective too.

In addition, utilizing preschool setting to deliver oral health messages through trained preschool teachers and promoting oral health in schools by integrating it into general health promotion, school curricula and activities would also strengthen the effectiveness of the preventive programmes as healthy behaviours and lifestyles developed at an early age are more sustainable. In conclusion, it is apparent that Sri Lanka needs a viable oral health care programme with a major preventive bias. It is the responsibility of every dental practitioner to contribute in some

form, towards preventive oral health and not confine themselves to curative, symptomatic or aesthetic care.

Further, there is a scarcity of research studies of public and professional knowledge, attitudes, and practices towards prevention of oral diseases. Hence, there is a need for research into these aspects and the generated data would be useful in developing tailored oral health promotion and disease prevention programmes. Similarly, the need for data on the impact of existing oral health services is a necessity to evaluate and to improve the existing preventive programmes.

Sunethra Rajapakse

BDS(SL), MPhil,(SL) PhD (Melbourne)
Senior Professor in Periodontology, Faculty of
Dental Science University of Peradeniya
At present (Visiting Scholar, Department of
Periodontics, University of Washington, Seattle,
WA, USA)

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Impact of prenatal oral health care provision for mothers on the caries experience of their children

**J.M.W. Jayasundara Bandara, W.P.M.M. Abeysekara, N.C. Wellappluli,
R.M.D.W. Ratnayake, K.G.A. Niroshini, D.P.M. Suraweera**

Abstract

Objective: Improving the oral health of pregnant mothers prevents complications of dental diseases during pregnancy, may reduce adverse pregnancy outcomes and has the potential to decrease early childhood caries in their babies. The aim of this study was to determine the difference in caries experience of the children of mothers who received oral health care and those who did not receive oral health care during pregnancy and the factors associated with the caries experience in the two groups of children.

Material and methods: A retrospective cohort study was carried out among 111 mothers exposed to oral health care during 2007-2008 at De Soysa Maternity Hospital (DMH) selected using simple random sampling technique from the pregnant mothers register maintained at the antenatal clinic of obstetric ward which sent these mothers to the oral health unit and their children were considered as the exposed population. A sample of 102 mothers was selected adopting the same sampling technique

used to select the exposed group from the pregnant mothers register maintained at the clinics where the mothers did not participate in the oral health care programme at DMH during 2007 and 2008. An interviewer administered validated questionnaire and a check list for intra oral examination were used as the study instruments. Data collection was carried out by visiting the residences of the study participants who agreed to participate in the study. Analysis was carried out using version 18 of the PASW statistical software package.

Results: Mean dmft of all the children (Mean age was 3 years) was 3.12 (SD± 3.8) and the median was 2.0. Mean DMFT of mothers at the time of data collection was 6.02 (SD ±4.38) and the median was 5.0. Mann Whitney U test ($Z=2.015$) indicated a statistically significant difference 1 between distribution of dmft of the children whose mothers had exposed to oral health care during pregnancy and whose mothers did not ($p < 0.05$).

Dr. N.C.Wellappuli (Correspondence)	MD, (Community Dentistry), MSc (Community Dentistry), BDS Ministry of Health, Nutrition & Indigenous Medicine, Sri Lanka. E-mail: nimalicw@yahoo.com
Dr. J.M.W. Jayasundara Bandara	MSc, (Community Dentistry), BDS Ministry of Health, Nutrition & Indigenous Medicine, Sri Lanka.
Dr. W.P.M.M. Abeysekara	MSc, (Community Dentistry), BDS Dental Institute, Colombo, Sri Lanka.
Dr. R.M.D.W. Ratnayake	MSc (Medical Administration), BDS Ministry of Health, Nutrition & Indigenous Medicine, Sri Lanka.
Dr. K.G.A. Niroshini	BDS, Institute of Oral Health, Maharagama, Sri Lanka.
Dr. D.P.M. Suraweera	BDS, Dental Institute, Colombo, Sri Lanka.

Exposure of the mothers to oral health care during pregnancy significantly associated with ECC of their children when adjusted for other factors (OR= 0.722, 95% CI= 0.619-0.942, $p < 0.05$). Mother's employment showed a significant adjusted odds ratio of 1.155 for the risk of dental caries of the children (95% CI= 1.002-2.004, $p < 0.05$).

Conclusion: Provision of oral health care during pregnancy was a significant factor influencing the caries experience of the children.

Key Words: Oral health care, dental caries, mothers, pregnant women, transmission.

Introduction

For more than three decades, it has been recognized that dental caries is an infectious and transmissible disease that is strongly modified by diet¹. Early childhood may be the most important time for future dental health. During this period, the primary teeth erupt, bacteria colonize on the teeth and dental health behavior begins to form².

A group of bacteria collectively known as the mutans streptococci (MS) has been implicated as the principal bacterial component responsible for dental caries in humans. The major source from which infants acquire MS is their mothers³. Children whose teeth are colonized earlier by MS show higher caries experience than those colonized later or not at all.^{1,4-5}. However, prenatal dental programs have been reported to be effective in improving oral health outcomes during pregnancy, most likely because women are particularly receptive to health education interventions at that time⁶⁻⁸.

Untreated dental problems are evident in pregnant women with low educational level and low income¹⁰⁻¹¹. Evidence supports that given the basic measures including an educative approach, removal of dental biofilm and elimination of infection foci may contribute to improve the

quality of life of the pregnant woman and the baby^{12-13,24}.

Early childhood caries (ECC) is a serious public health problem in both, developed and developing countries. It affects infants and toddlers worldwide. The prevalence of ECC in developing countries was reported to be as high as 70%¹⁶. Third national oral health survey 15 conducted by Ministry of Health, Sri Lanka revealed that in Sri Lanka, 65.5% of the 5 year-old children suffer from dental decay. On an average each child in this age group experience decay in more than 3 teeth¹⁵. Pregnancy is an opportune time to motivate women about preventing dental caries in young children, the most common childhood problem in most if not all countries. Hence Ministry of Health, Sri Lanka decided to provide special oral health care for antenatal mothers and a policy on provision of oral health care for pregnant mothers was formulated.

As a first step of this programme, provision of regular oral health care for pregnant mothers was started about three years ago at the De Soyza Maternity Hospital (DMH), Sri Lanka. It was considered as a pilot project prior to island wide programme for pregnant mothers to provide oral health care services as a special group. *These mothers were provided a package of oral health education and promotion*, prior to screening of the oral cavity. After the screening, necessary basic treatments were carried out to *minimize the density of harmful oral flora*. Those antenatal mothers who attended to the clinics conducted at DMH by two obstetric units had been directed to the dental clinic to participate in the oral health care programme. However those pregnant mothers in other obstetric units had not participated in the programme as they were not referred to oral health unit.

Recently, the oral healthcare services are gearing up to meet the demand of the oral health need of the pre natal mothers in Sri Lanka. Several significant steps have been taken in

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this regard in the recent past. One of the most important developments is the establishment of a special dental care clinics in DMH and Castle Street Hospital for women. They provide comprehensive oral care to pre natal mothers including oral health education and oral health promotion. It has been accepted as a policy to provide regular oral care service to pregnant mothers in view to promote good oral practices in their future children and healthy teeth in the future generations.

Since this has to be developed into an island wide activity involving all the spheres of health service providers, it is essential to evaluate the effectiveness of providing such care. Hence this study was aimed at assessing the difference in caries experience of the children of mothers who received oral health care and those who did not receive oral health care during pregnancy and the factors associated with the caries experience in the two groups of children. This study contrive towards providing evidence to a nationwide programme.

Materials and methods

A retrospective cohort study was carried out to assess the impact of pre natal dental care provision for mothers on the caries experience of their children. The study population was the children of the mothers who attended De Soysa Maternity Hospital (DMH) for delivery during the years 2007-2008. Those mothers who received oral health care at dental clinic other than the clinic at DMH were excluded from the study. Sample size was calculated using WinPepi software²⁸. As according to the systematic review by the oral health research group at Murdoch children's research institute New Zealand (2008)²⁵ expected difference between mean dmft values of the children whose mothers exposed to pre natal dental care and non-exposed to prenatal dental care groups was considered as 1 and a standard deviation of 2.5 was expected for the calculation of sample size. Power was set as 80% for the calculation of sample size. With

allowance of 10% non-response rate, calculated sample size was 111 for each group. A sample of 111 mothers exposed to oral health care during the years 2007-2008 at DMH was selected using simple random sampling technique from the pregnant mothers register maintained at the unit and their children were considered as the exposed population. A sample of 102 mothers was selected adopting the same sampling technique used to select the exposed group from the pregnant mothers register maintained at the clinics where the mothers did not participate in the oral health care programme at DMH during 2007 and 2008.

Respective Public Health Midwife (PHMM) areas where the mothers belonged to were determined using their addresses recorded at the registers. According to the addresses the mothers were principally from Colombo, Gampaha, Kalutara and Ratnapura Districts. Permission was obtained from the four Regional Directors of Health Services and the in-charge Medical Officers of Health (MOHH) of the selected PHMM areas were contacted through them. With the help of MOHH the respective PHMM were contacted and they were educated about the study. Informed consent was obtained from the mothers via the PHMM prior to the collection of data. It was possible to achieve a response rate of 90.9% by contacting the mothers through PHMM. Obtaining of prior consent enabled to identify the non-respondents without physically approaching them which in turn improved the feasibility of the study. Contact details of those mothers those who have left the area were found out with the help of PHMM and they were approached via the PHMM of the areas where they were residing at the time of data collection.

An interviewer administered validated questionnaire and a check list for intra oral examination were used as the study instruments. The questionnaire consisted of 5 components to obtain information about mother's pre natal dental history, socio demographic data, oral

health practice of the child and the dietary habits of the child. It was pre tested at two clinics at the Institute of Oral Health, Maharagama with 20 mothers visited there with the children. The technique of intra oral examination of the dental surgeon which used to record the caries status of the children and their mothers was standardized and calibrated by a consultant in Community Dentistry at the Institute of Oral Health, Maharagama with 20, three year old children and 20 mothers who visited the institution. A qualified Dental surgeon carried out the oral health examination to ensure the quality of data.

Data collection was carried out by visiting the residences of the study participants who agreed to participate in the study. Dental surgeon was accompanied by the area Public Health Midwife. This improved the corporation of the mothers during data collection. Dental Surgeon carried out the oral examination under artificial light while the subjects were seated on an ordinary chair. Instruments needed for the oral examination were sterilized and stored in sterilized trays with lids each morning prior to the field visits. Dental therapist was utilized as a data recorder for the study. The recorder sat in close proximity to the examiner, which enabled the recorder to hear the examiner clearly. Also this allowed the examiner to check whether the data were accurately recorded. DMFT of mothers as well as dmft of their children were recorded and same criterion (caries in the dentine) that was used to detect caries in children used for the mothers as well.

Procedure was carried out for 92 days from 25th May 2012. Autoclaved instruments were provided by the Institute of Oral Health, Maharagama for intra oral examination. Once the instruments were used they were not used again without autoclaving.

Analysis was carried out using version 18 of the PASW statistical software package²⁶. For the purpose of this study “pre natal dental care” was defined as obtaining of any kind of dental

treatment during pregnancy. “Decayed” was defined as active dental caries (caries in the dentine) at any stage on any surface of the tooth. “Filled” was defined as presence of any kind of restoration done due to dental caries on any surface of the tooth. “Missing” was defined as tooth loss due to dental caries. The associations between categorical variables were determined by Chisquare test. Having checked for multicollinearity and interactions, those variables that were significantly associated ($P < 0.05$ level) with caries prevalence of child in the bivariate analysis were included in a multiple logistic regression model to determine the independent associations of those variables with the dependent variable (caries prevalence of child). Model was modified by adding biologically important variables in to that.

Ethical Approval for the present study was obtained from the Ethical Review Committee of the Faculty of Dental sciences, University of Peradeniya.

Results

Mother was the care giver of the 76.7% of the children. When mother was not the care giver, 50% of the children were looked after by their grandparents. Approximately 67% of the mothers were unemployed. Majority of the employed mothers were in the private sector. About 77% of the mothers at least had secondary education. Out of all the families in the sample 71.3% had a monthly income between 10,000 to 30,000 rupees.

Good intra-examiner Kappa values at tooth levels were obtained from mothers as well as from children (0.93 and 0.78, respectively). Total number of children in each group was 101 with a response rate of 91% for each group. All the children were at three years of age and the mean age of the mothers was 32 years ($SD \pm 4.62$). Intra oral examination of all the children and their mothers was carried out. Mean dmft of all the children was 3.12 ($SD \pm 3.8$) and the median was

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2.0. Mean DMFT of mothers at the time of data collection was 6.02 (SD \pm 4.38) and the median was 5.0.

Mean DMFT of the mothers who were exposed to oral health care during pregnancy was 6.4 (SD \pm 4.1) and median was 6.0. Mean DMFT of the mothers who were not exposed to oral health care during pregnancy was 7.4 (SD \pm 5.3) and median was 7.0. Mean dmft of those children of the mothers who were exposed to oral health care during pregnancy was 2.5 (SD \pm 3.4) and the median was 2.0. Mean dmft of those children of the mothers who were not exposed to oral health care during pregnancy was 3.7 (SD \pm 4.07) and the median was 3.0.

There was statistically significant difference in the dmft between children of exposed and unexposed mothers ($p < 0.05$).

Majority (60%) of the children had brushed their teeth twice a day and care taker had been the person who brushed the teeth of 49% of the children. About 64% of the children were consuming sweets and 50.5% of them consumed sweets more than thrice a week.

Bivariate analysis of data of the present study revealed exposure of mother to oral health care service at DMH, employment status of the mother, DMFT of the mother of tooth, frequency of tooth brushing by child, current breast feeding practices of the mother and sweet consumption by child were factors significantly associated with the high caries risk of the child (Table 1).

When adjusted for employment status of the mother, frequency of tooth brushing, current breast feeding practice and sweet consumption by the child the adjusted odds ratio for the

Table 1. Factors associated with dental caries of children. Bivariate Analysis

Variable	Experienced Caries (n=94)		No experienced caries (n=108)		Significance
	No.	%	No.	%	
Exposure of mother to oral health care service at DMH					
Yes	24	25.5	77	71.2	$\chi^2 = 19.43$ $df = 1, p < 0.001$ $OR = 0.65(0.52-0.87)$
No	70	74.5	31	28.8	
Mother is care taker of the child					
Yes	37	39.3	61	56.4	$\chi^2 = 2.19$ $df = 1, p = 0.13$ $OR = 0.78 (0.95-1.45)$
No	57	60.7	47	43.6	
Employment status of the mother					
Yes	56	59.6	41	38.0	$\chi^2 = 12.76$ $df = 1, p < 0.001$ $OR = 2.42 (1.95 - 3.00)$
No	38	40.4	67	62.0	
DMFT of mother					
< 6	34	36.1	97	89.8	$\chi^2 = 5.96$ $df = 1, p = 0.42$ $OR = 0.92 (0.66- 0.99)$
≥ 6	60	63.9	11	10.2	

Cont..... Table 1

Characteristics	Experienced Caries (n=94)		No experinced caries (n=108)		Significance
	No.	%	No.	%	
Frequency of tooth brushing					
Once a day	53	56.3	35	32.4	$\chi^2 = 20.25$ $df = 1, p < 0.001$ $OR = 2.03 (1.64-2.52)$
Twice a day	41	43.7	73	67.6	
Current breast feeding					
Yes	68	72.3	59	54.6	$\chi^2 = 13.36$ $df = 1, p < 0.001$ $OR = 1.49 (1.20-1.84)$
No	26	27.7	49	45.4	
Sweet consumption					
Yes	76	62.4	52	40.7	$\chi^2 = 14.18$ $df = 1, p < 0.001$ $OR = 2.42 (1.95-3.00)$
No	18	37.6	56	59.3	
Monthly income of the family (Rs)					
≤ 20,000	69	73.4	76	70.3	$\chi^2 = 7.67$ $df = 1, p = 0.08$ $OR = 0.53(0.27-1.02)$
>20,000	25	26.6	32	29.7	

exposure to oral health care at DMH by their mothers was 0.72 (95% CI: 0.619- 0.942). Mother's employment showed a significant

adjusted odds ratio of 1.155 for the risk of dental caries of the children (Table 2).

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Table 2. Factors associated with dental caries of children. Multiple Logistic Regression Model (n= 202)

Independent variable	Adjusted Odds Ratio		95% CI		Significance (p-value)
			Lower	Upper	
Exposure to oral health care service at DMH	Yes	0.722	0.619	0.942	0.041
	No	1 ^b			
Employment status Of the mother	Yes	1.155	1.002	2.004	0.046
	No	1 ^b			
Frequency of tooth brushing	Once a day	0.595	0.139	2.550	0.484
	Twice a day	1.336			
	Irregular	1 ^b			
Current breast Feeding	Yes	1.813	0.266	1.846	0.111
	No	1 ^b			
Sweet consumption	Yes	1.015	0.101	1.245	0.106
	No	1 ^b			

a. The reference category is: No experienced caries

b. This parameter is set to 1 because it is redundant

Discussion

Many investigators have suggested that provision of oral health care to the mothers during pregnancy improves the oral health of the children. Since it is unethical to conduct a clinical trial to test this hypothesis a retrospective cohort study was conducted. Those mothers who had an experience of oral health care during pregnancy from institutions other than DMH were excluded to control for that confounding effect since those mother who have had oral care from outside are more likely to reject oral health care at DMH but the oral health status of their children might have the influence of the oral health care they have had. Mean dmft of this sample of children was 2.5 and median dmft of them was 2. Early childhood caries has

been affecting children below three years, the disease is currently considered a public health problem¹⁶⁻¹⁷. Its etiology is complex and results from the interaction of several factors, such as high consumption of fermentable carbohydrates, lack of oral hygiene, low family socioeconomic status, and mother's limited educational level as well as poor oral hygiene of the mother¹⁶⁻¹⁷.

Pregnant women are at higher risk of tooth decay for several reasons, including increased acidity due to decrease in PH and buffer effect in the oral cavity, sugary dietary cravings, and limited attention to oral health²³. Therefore improving the oral health status of mothers may benefit their children in two general ways. Firstly, interventions that increase maternal

knowledge of their own oral health issues, such as limiting carbohydrate intake and improving oral hygiene habits are likely to impact on how they care for their infant's oral health. Secondly an improvement in maternal oral health may reduce the number of mutans streptococci present in their oral cavity thereby reducing the potential risk of transmission of these cariogenic bacteria to their children. It is probable that the majority of children acquire mutans streptococci eventually but the critical period for early childhood caries (ECC) development is the first few years of life. Interventions that aim to prevent transmission of mutans streptococci by either reducing levels of the bacteria in the mother's oral cavity or by reducing behaviours that promote salivary transfer may be important in reducing the risk of ECC in early childhood. Interventions that focus on improving oral awareness more generally may have long lasting, ongoing positive influences on the whole family unit.

Most dental care activities during pregnancy are safe, and may aid in preventing early childhood caries because maternal transmission is the primary way in which children are inoculated with the bacteria, mutans streptococci, associated with early childhood caries¹⁸.

A prospective study carried out by Gomaz (2001)¹⁹ consisted of a preventive dental program (PDP) instituted in pregnant women comprising an educational and a preventive part was evaluated when their children were five and six years of age. It was identified the mean dft of the PDP children five and six years of age was 0.4 and 0.2, respectively, versus 1.3 and 1.4 for the control children and the differences were statistically significant. The results of Gomaz's study (2001) is in accordance with the current study and confirms the preventive dental program which started in pregnant women and continued in the mothers and their children were highly effective for a long-term reduction of dental caries.

A prospective clinical long-term study investigated the effects of a long-term preventive program on dental and oral health of teenagers at the age of 13 to 14 years²⁷. The entire study was subdivided into four phases. Phase I comprised an individual preventive care during pregnancy ("primary-primary prevention"), phase II assessed the effect of preventive oral health care for mothers and their young children until the age of 3 years ("primary prevention") and in phase III assessed the effect of preventive oral health care for mothers and children at the age of 6 years were investigated. In phase IV of the study, the oral health of 13- to 14-year-old teenagers was examined (13.4 +/- 0.5 years; n = 29). All phases consisted of an examination, education about oral health care, and treatment based on the concept of an early oral health care promotion. The control group consisted of randomly selected adolescents at the same age (n = 30). Decayed/ missing/filled teeth (DMF-T) and decayed, missing, and filled surface teeth index were assessed. The teenagers of the "prevention" group of phase IV of this prospective study revealed 89.7% caries-free dentitions (65.5% sound; 24.2% caries-free with fillings). Mean DMF-T was 0.55 +/- 1.0. The control group showed a significantly higher mean DMF-T of 1.5 +/- 1.5 ($p < 0.05$) and revealed 56.7% of caries-free dentitions (30% sound, 26.7% caries-free with restorations). The data clearly document that an early oral health care promotion starting during pregnancy may cause a sustained and long-term improvement of the oral health of children.

Also in multivariate analysis of another study was found exposure of mothers for the oral health care during pregnancy act as protective factor for occurrence of early childhood caries (OR=0.61, 95% CI: 0.61-0.94)²⁰. The unique approach of provision of oral health care for pregnant mothers preventing or reducing early childhood caries will potentially enable greater benefits for the individuals as well as country level.

Impact of prenatal oral health care provision for mothers on the caries experience of their children

A study consisted of 117 severe early childhood caries children and 129 caries-free children less than 4 years old in Beijing, China²¹ identified lower maternal education level, poor knowledge of mother on oral hygiene, night-feeding, and excessive sugar intake by children were important contributors to the development of early childhood caries. Even current study supports the findings of the China study as it was also identified sweet consumption, frequency of sweet consumption increased the dmft of the children in the study sample.

However another cross sectional study²² was carried out among 6-19 months children in Thailand shows children belonged to low-income families, mothers/care givers with low education, and mothers/caregivers with decayed teeth had higher early childhood caries (ECC) scores ($P < 0.05$). Except mothers with decayed teeth other factors were not identified as risk factors of ECC in current study. The reason behind is ECC is not only a public health problem but also a social problem in Thailand, because it relates to family income and education level unlike in Sri Lanka.

Information bias may have affected the results. There is a possibility for the subjects to under report pre metal dental history, oral health practice of the child and the dietary habits of the child. Although steps were taken to minimize this bias to the best possible extent, it may not have been eliminated completely.

Conclusion

Provision of oral health care during pregnancy was a significant factor influencing the caries experience of the children.

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Role of Antioxidants in Disease Progression and Malignant Transformation of Oral Submucous Fibrosis (A Preliminary Study)

J.D.R. Perera, R. Sivakanesan, R.D. Jayasinghe, B.M.R. Bandara and W.M. Tilakaratne

Abstract

Oral submucous fibrosis (OSF) is a chronic, progressive, scarring and potentially malignant disease of the oral mucosa. It has a malignant transformation rate of 7-12%. To date there are no established predictive markers for malignant transformation of OSF, although some genes related to malignant transformation have been identified in the recent past. There is evidence that reactive oxygen species (ROS) promote DNA damage through oxidative stress, protein oxidation and lipid peroxidation.

The objective of the study was to compare antioxidants and lipid and protein oxidation markers in different patient groups including betel quid chewers without OSF or oral squamous cell carcinoma (OSCC), OSF, OSF with dysplasia, OSCC and a control group. Eighty two new patients were recruited and blood samples were collected to assess serum antioxidant levels and protein and lipid oxidation. The total antioxidant levels in blood serum were determined by DPPH radical scavenging assay and ferric reducing ability of plasma (FRAP) assay. Thiobarbituric acid reactive substances (TBARS) for lipid peroxidation and 5, 5'-

dithiobisnitrobenzoic acid (DTNB) assay for total protein thiol content were carried out to assess the extent of lipid and protein oxidation respectively. Data were analyzed using one way ANOVA.

Low mean % reduction of DPPH activity of (34.4 %) was seen in groups of OSF patients with dysplasia and OSCC whereas it was high (37.2%) in the control. Protein thiol was lowest in patients with OSF (583.0 $\mu\text{mol/L}$) and it was highest in patients with OSCC (665.1 $\mu\text{mol/L}$). FRAP and DTNB results indicate higher antioxidant levels in patients with OSCC and betel chewers compared to the control group. Antioxidant estimated according to DPPH assay showed higher levels in normal controls than the other 4 groups. Results of the DTNB assay for protein thiol was similar to those of FRAP assay. Different groups revealed a difference in antioxidant levels. Significant difference was observed among the groups in FRAP assay. However the clinical significance of the above finding need to be further investigated.

Introduction

Oral submucous fibrosis (OSF) is a chronic,

Ms. J.D.R. Perera	Department of Chemistry, Faculty of Science, University of Peradeniya
Prof. R. Sivakanesan	Department of Biochemistry, Faculty of Medicine, University of Peradeniya
Dr. R.D. Jayasinghe	Department of Oral Medicine and Periodontology, Faculty of Dental Sciences, University of Peradeniya
Prof. B.M.R. Bandara	Department of Chemistry, Faculty of Science, University of Peradeniya
Prof. W.M. Tilakaratne (Correspondence)	Department of Oral Pathology, Faculty of Dental Sciences, University of Peradeniya E-mail: wmtlak@pdn.ac.lk

progressive, scarring and high-risk potentially malignant disease of the oral mucosa seen primarily in the Indian subcontinent and in South East Asia. OSF is characterized by fibrosis of the sub mucosa, atrophy of the epithelium and reduction of the vasculature. Schwartz first described this condition in 1952 and named it as 'atrophiaidiopathicatropicamucosaoris'. Joshi in 1953 renamed it as oral submucous fibrosis. Although various factors have been proposed as etiological factors in the past, arecanut use is the only proven factor in the aetiology of OSF^{1,2}. Arecoline, an alkaloid found in arecanut stimulates fibroblastic proliferation and collagen synthesis leading to accumulation of collagen causing the disease. In addition to the local factors, various systemic factors such as anaemia, chronic iron and vitamin B complex deficiency and genetic predisposition have been implicated in the causation of the disease^{1,3,4}.

OSF is diagnosed by its characteristic clinical features. Clinical criteria include mucosal blanching, hardening and the presence of characteristic vertical fibrous bands. OSF starts at the back of the mouth and progresses to the anterior parts of the mouth as the disease advances. Fibrosis and sub epithelial hyalinization account for most of the clinical features. Fibrous bands can be observed at the faucial, buccal and labial mucosa. The presence of fibrous bands is minimal in mild disease. The degree of mouth opening can be used as an objectively verifiable criterion to assess the severity of the disease (functional stage)⁵. Site of the fibrous bands can be used to classify the disease clinically (clinical stage)⁵. Mouth opening has been used as a predictor of the severity and extent of OSF⁵. An incisional biopsy may be needed in order to confirm the diagnosis in a minority of patients.

OSF is a premalignant condition with a malignant transformation rate of 7-12%¹. The evidence to suggest the premalignant potential of the disease includes histopathological diagnosis of

squamous cell carcinoma (SCC) in patients with OSF without any clinical features suggestive of SCC, high prevalence of leukoplakia and high prevalence of epithelial dysplasia in patients with OSF.

To date there are no established predictive markers of malignant transformation of OSF, although some genes related to the same have been identified in the recent past^{1,3}. There is evidence that reactive oxygen species (ROS) promote oxidative stress causing DNA damage, protein oxidation and lipid peroxidation in OSF. The potentially damaging ROS are removed by a variety of endogenous low-molecular-weight antioxidants such as glutathione, uric acid, thiol groups, ascorbic acid, vitamin E, bilirubin and ubiquinol^{6,7}. The concentration of antioxidants present in physiological samples represents the capacity to remove ROS of a given tissue, organ or body fluid, indicating the extent and efficiency of inherent defensive antioxidant mechanisms. The total antioxidant levels of appropriate body fluids can thus be used to predict the malignant transformation potential of OSF patients. Therefore, it may be of relevance to determine the levels of endogenous antioxidant levels in blood serum of OSF patients. We hypothesised that a strong association exists between clinical and histopathological stage, malignant transformation potential and blood serum antioxidant level in patients with oral sub mucous fibrosis.

Aims and objectives

Objective of the present study was to assess the serum antioxidant level and evidence for protein and lipid oxidation in different patients with OSF but without dysplasia, patients with OSF with dysplasia, patients with OSCC without evidence of OSF, betel quid chewers without OSF or OSCC and normal controls.

Materials and Methods

The subjects of the study were recruited from the newly diagnosed patients attending

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the University Dental Hospital, Peradeniya. Incisional biopsy was performed in order to arrive at a diagnosis and for management purposes and the histopathological information was used to categorise the patients into groups. Age and sex matched control group was selected from the patients with temporomandibular disorders (TMD).

The patients were divided into five groups.

- Group I - Controls (patients with TMD)
- Group II- Betel chewers without OSF/ OSCC
- Group III- Patients with OSF but without epithelial dysplasia
- Group IV- Patients with OSF with epithelial dysplasia
- Group V- Patients with OSCC

All patients were examined by experienced clinicians, and general data including the history of complaint, medical history and oral health related habits (especially in relation to tobacco usage and alcohol consumption) and any previous treatments were recorded.

After obtaining the informed consent, blood samples were collected from all the subjects. The serum was separated from blood to assess the antioxidant levels and protein and lipid oxidation.

2, 2 -Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

DPPH reacts with antioxidants and is converted into its reduced form which is a colourless compound. Twenty μL of blood serum was transferred into an Eppendorf tube and 500 μL of 10 mM (pH = 7.4) sodium phosphate buffer and 500 μL of 0.1 mM methanolic DPPH solution were added and mixed. After keeping the mixture at room temperature for 30 min, the solution was centrifuged for 15 min. Finally, the absorbance of the sample was measured at 520 nm using a spectrophotometer. The absorbance of a DPPH solution without serum (blank) was

measured and used as the control. Activity (% DPPH reduction) was calculated using the following formula⁸.

$(A-B/A) \times 100$ where A= absorbance of blank and B= absorbance of sample

Ferric reducing ability of plasma (FRAP) assay

FRAP reagent consists of ferric ions (Fe^{3+}), 10 mmol/L tripyridyltriazine (TPTZ) in hydrochloric acid (HCl) and 300 mmol/L (pH=3.6) acetate buffer. Ferric ions in the FRAP reagent react with the antioxidants in the sample and get reduced to Fe^{2+} and the colour of the solution changes to blue purple from pale yellow. FRAP reagent was prepared by mixing 300 mmol/L (pH=3.6) acetate buffer, TPTZ and 20 mM FeCl_3 in 10:1:1 ratio. Twenty μL of blood serum was transferred to a test tube. One mL of FRAP reagent was added to 20 μL of blood serum (for the standard, 20 μL of 1000 μM FeCl_2 was used) and vortexed and left to stand exactly for 4 min. Finally the absorbance was measured at 593 nm in a spectrophotometer⁹.

5,5'-Dithiobisnitrobenzoic acid (DTNB) assay

DTNB reacts with thiol groups in the sample to form the dianion 2-nitro-5-thiobenzoate (TNB^{2-}) which is yellow in colour and absorbs strongly at 412 nm. First, 50 μL of blood plasma was transferred to a test tube and 1 mL of phosphate buffer (0.1 M, pH 7.4) and 200 μL of DTNB (2 mM) was added. The mixture was incubated at 37°C for 5 min. Finally, the absorbance was measured at 412 nm in a spectrophotometer¹¹.

Thiobarbituric acid reactive substances (TBARS) assay

Lipid peroxidation by reactive oxygen species in serum samples results in an end product malondialdehyde (MDA) which reacts with TBA to produce a MDA-TBA adduct which is pink in colour and absorbs strongly at 535 nm.

Mixed reagent for the assay was prepared by mixing 15% trichloroacetic acid (TCA) (w/v),

HCl (0.25 N) and 0.375% TBA (w/v) in 1:1:1 ratio. Blood serum, 0.25 mL, was transferred to an Eppendorf tube and 1 mL of mixed reagent was added, and the mixture was centrifuged for 15 min. One mL of supernatant was transferred into a test tube and mixed with 0.25 mL of TBA. The mixture was left in a boiling water bath for 60 min. Finally, the absorbance was measured at 535 nm using a spectrophotometer¹⁰.

Ethical clearance for the study was obtained from the Ethical Review Committee of the Faculty of Dental Sciences, University of Peradeniya. SPSS version 17.0 and one way ANOVA were used for the statistical analysis.

Results

Serum samples from 82 patients were used in the study. The 82 cases were categorised into five groups (Table 1). DPPH reduction was highest in group I whereas the lowest DPPH reduction was observed in group IV. Higher FRAP activity was observed in the group V (patients with OSCC) whereas high TBARS levels were observed in group II betel chewers without OSF or OSCC. Low DTNB levels were seen in patients with OSF (group III). Mean values for each test and group are given in Table 1.

Table 1. Analysis of serum in patient groups by antioxidant assays.

Groups of patients	N	Mean \pm SD			
		% DPPH reduction	FRAP activity / μ M	Protein thiol/ μ mol/L	TBARS activity/ μ mol/L
Controls (Group I)	22	37.1 \pm 6.4	864.7 \pm 139.4	600.9 \pm 109.9	1.48 \pm 0.60
Betel chewers without OSF/ OSCC (Group II)	18	35.6 \pm 6.7	885.4 \pm 174.7	639.8 \pm 100.8	1.94 \pm 1.05
Patients with OSF but without epithelial dysplasia (Group III)	17	36.0 \pm 4.3	840.4 \pm 209.8	609.3 \pm 62.5	1.49 \pm 0.91
Patients with OSF with epithelial dysplasia (Group IV)	16	34.4 \pm 6.5	856.1 \pm 177.5	664.1 \pm 146.6	1.54 \pm 0.96
Patients with OSCC (Group V)	9	34.6 \pm 2.3	1034.1 \pm 120.8	672.1 \pm 121.1	1.64 \pm 0.89

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Table 2. One-way ANOVA

Assay		Sum of Squares	Df	Mean Square	F	Sig. P<0.05
% DPPH reduction	Between Groups	80.26	4	20.06	0.59	0.669
	Within Groups	2606.70	77	33.85		
	Total	2686.96	81			
FRAP activity/ μM	Between Groups	255049.46	4	63762.36	2.21	0.075
	Within Groups	2220038.32	77	28831.66		
	Total	2475087.78	81			
Protein thiol/ μmol/L	Between Groups	62004.02	4	15501.00	1.28	0.283
	Within Groups	928755.25	77	12061.75		
	Total	990759.28	81			
TBARS activity/ μmol/L	Between Groups	2.74	4	0.68	0.87	0.484
	Within Groups	59.68	76	0.78		
	Total	62.43	80			

Discussion

In this study, the hypothesis that there is a correlation between the antioxidant level and the malignant transformation potential of OSF into OSCC has been tested. A difference in antioxidant levels is evident from the results obtained for the different groups using the four spectrophotometric assays, indicating that there is an imbalance in antioxidant levels in the five groups. Therefore, different stages of malignant transformation seem to reflect different antioxidant levels.

DPPH radical scavenging assay and FRAP assay give a direct measurement of the antioxidant levels in the system¹³. The results of DPPH assay indicate that betel chewers have a lower antioxidant level when compared to normal controls. OSCC patients and OSF patients with dysplasia seem to have the lowest antioxidant levels, whereas OSF patients without dysplasia have the second highest antioxidant levels. The results of FRAP assay indicate that OSCC patients have the highest levels of antioxidants, while both OSF without dysplasia and OSF with dysplasia show lower levels of antioxidants than in normal controls. TBARS assay does not give a direct measurement of the antioxidant levels. Instead it gives a measurement of the oxidative

stress that system is under. Therefore, this assay gives a measure of the antioxidant capacity of the system that provides protection against lipid peroxidation by ROS¹⁴. The results of TBARS assay reveal that the antioxidant protection is lowest in the betel chewers. Both OSF without dysplasia and OSF with dysplasia show lower antioxidant protection than normal controls. Normal controls showed the least TBARS activity indicating the least levels of oxidative stress. OSCC patients seem to have the second highest TBARS activity indicating higher oxidative stress levels than the OSF groups.

The results of DPPH and TBARS assays show a general trend in which the betel chewers have lower antioxidant levels than normal controls, indicating high ROS levels that are consistent with the association of betel chewing in the etiology of cancer and OSF. The low antioxidant levels in betel chewers may be attributed to arecoline, an active alkaloid present in betel nuts, which stimulates the fibroblasts to increase the production of collagen causing a juxtaepithelial inflammatory reaction in the oral mucosa leading to OSF¹⁵. Since ROS is produced in the system as a result of an inflammatory response during the above pathogenesis stimulated by arecoline¹⁶, high levels of ROS can be expected leading to

lower levels of antioxidants. Furthermore, the DPPH and TBARS assays show a trend revealing a decrease in antioxidant levels when going from normal controls to OSF patients without dysplasia, OSF patients with dysplasia and OSCC patients. Hence, the observed decrease in antioxidant levels is consistent with the increase in malignancy. This is in agreement with the proposed hypothesis that high ROS levels and thus low antioxidant defense mechanisms are expected with the increase in malignant transformation potential. However the statistical results from the One way ANOVA table (Table 2) indicate that all four assays have a P value much greater than 0.05. FRAP assay shows a P value of 0.075 which indicates a marginal statistical significant difference of the results between different groups. According to the results of the FRAP assay, an increase in the antioxidant levels is implicated with the malignant transformation of OSF into OSCC. Both OSF with and without dysplasia are precancerous states which are at the early stages of malignant transformation and they show lower levels of antioxidants than normal controls and when it reaches the final stages of malignant transformation which is OSCC, higher levels of antioxidants than normal controls can be seen. A probable explanation for this behaviour in antioxidant levels is that the body and the cancerous tissues must be maintaining higher levels of endogenous antioxidants (e.g. glutathione, alpha-lipoic acid, coenzyme Q, ferritin, uric acid, bilirubin, metallothioneine, L-carnitine and melatonin) when compared to normal cells to counteract the effects of ROS produced by cancer cells¹⁷. Under oxidative stress conditions such as in cancer, the body and the cancer tissues adapt to different mechanism to survive under these harsh conditions¹⁷. As one such mechanism, high levels of endogenous antioxidants seem to be maintained in tissues under oxidative stress.

The results of DTNB assay cannot be used directly to predict the antioxidant levels in a blood sample. DTNB assay measures the

total content of protein thiols in blood which act as antioxidants¹⁸. However, the total blood protein levels vary in different individuals¹⁹. Albumin contributes to a large portion of the serum protein content and it is responsible for about 70% of the serum antioxidant capacity as protein thiols²⁰. Therefore, a person with higher serum protein content will naturally have a higher protein thiol content which contributes to the total antioxidant level. Therefore the serum protein thiol content should be given as a percentage of the total serum protein content.

Several attempts had been used in the recent past to identify the relationship between antioxidant levels and malignant transformation in OSF. Aggarwal et al in 2011¹² have identified beta carotene as an important factor in the pathogenesis of OSF. The results obtained and the trends they interpret also suggest that the antioxidant level in the system depends not only on the amount of ROS (oxidative stress) present in the system, but also on many other biochemical factors that need to be addressed in order to determine the relationship of antioxidants in the malignant transformation of OSF into OSCC. Although the endogenous antioxidant levels are controlled by internal biochemistry of the body, the exogenous antioxidant levels that contribute to the total antioxidant count largely depend on factors like dietary habits of the patients, and their medication including vitamin supplements. Fresh fruits and vegetables contain high levels of vitamin C, while vegetable oils contain high levels of vitamin E. Polyphenolic antioxidants are present in high quantities in tea, coffee etc²¹. Therefore, it is expected that a person who consumes green vegetables, fruits, tea, coffee and vitamin supplements on a daily basis would have higher antioxidant levels. Antioxidant levels are also affected by smoking and medical conditions such as diabetes that are seen very commonly in people²². Also, most often patients with cancer and precancerous conditions in Sri Lanka seek traditional medicinal treatment and vitamin supplementation before they attend the

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oral pathology clinic. The nature of traditional medication is such that it contains large amounts of herbs that contain considerable amounts of antioxidants²³. Therefore if patients have undergone such treatment, their antioxidant levels would be much higher than in normal controls. These factors could be probable explanations for the high antioxidant levels observed in the OSCC patients group in the FRAP assay. Therefore, these factors need to be considered in each patient when interpreting their antioxidant levels in relation to their stage of the disease. Furthermore, the high levels of antioxidants observed in the OSCC patients group in the FRAP assay could be due to the presence of outliers. These outliers may be due to the above mentioned factors. However, the sample sizes of the groups are not large enough for effective removal of these outliers, especially the OSCC group (n = 9). The presence of these outliers can lead to misinterpretation of the data. Before drawing final conclusions the above factors the dietary issues, vitamin supplementations and herbal medications must be addressed employing a large sample size.

Even though standard assays were used for the analysis of antioxidants, there are some limitations in evaluating antioxidant parameters in blood samples. The DPPH method is only able to measure direct reactions with DPPH radical, which depend on the structure of the antioxidant compound, and can only give a general indication of the radical scavenging abilities of antioxidants²⁴. Since the human blood serum constituents are more or less constant in individuals, these effects can be assumed to cancel out during comparisons²⁴. Recently, carotenoids have been reported to interfere with the DPPH assay because they absorb light at 520 nm²⁵. In the FRAP assay, the reduction of ferric ions to ferrous ions involves only one electron. Therefore, the antioxidants that react with ferric ions should be able to undergo one electron oxidation reactions. If not, the amount of ferrous ions produced will not be proportional

to the amount of antioxidants present in the sample. Studies done on FRAP assay reveal that it is responsive to uric acid which constitutes the largest portion of plasma antioxidants, but does not adequately measure the antioxidant activity of many important antioxidants such as albumin²⁶. However, the FRAP activity values obtained from FRAP assay still effectively reflect the antioxidant capacity of the system⁹. TBARS assay is relatively more specific towards its target molecule malondialdehyde. However, interferences of bilirubin, heme pigments and sodium caseinate have been reported²⁷. The DTNB assay shows minimum interferences due to its high specificity towards thiol groups²⁸.

The subjects for this study were patients visiting the Oral Pathology clinic, Faculty of Dental Sciences, Peradeniya. As further improvements, larger sample sizes will be employed for each category and the patient's dietary habits, medication and vitamin supplements can be controlled with the patient's consent.

Conclusion

A difference in antioxidant levels at different stages of malignant transformation is evident. A dependence of the antioxidant levels on the malignant transformation potential is indicated. The antioxidant level obtained from two of the assays appears to show the expected trend, but not statistically significant enough. To determine the exact relationship between the groups, further studies should be conducted with larger sample sizes.

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Associations between dental fluorosis dental caries and periodontal status in 15 year old school children residing in a high fluoride area in Sri Lanka

R.M. Jayasinghe, N. Abeygunawardhana, R.D. Jayasinghe, L. Ekanayake

Abstract

Objectives: The objectives of the present study were to determine the prevalence of dental fluorosis, dental caries and periodontal diseases in 15-year-old school children living in a high fluoride area in Sri Lanka and to assess the associations between dental fluorosis, dental caries and periodontal status.

Materials and methods: The sample consisted of 415 15-year-olds attending 8 randomly selected schools in Maho area in the Kurunegala district. They were life-long residents of the area and obtained their drinking water from a ground water source. Data was collected using a self-administered questionnaire and an oral examination. The Dean's index was used to record dental fluorosis and periodontal status was assessed in terms of gingival bleeding on probing (BOP) which was recorded on 6 sites of all teeth present in the mouth. Dental caries was recorded according to the WHO Basic Methods.

Results: Information was gathered from 415 students and the response rate was 409 (99%). According to the Dean's index, 79.5% had mild to severe dental fluorosis.

Prevalence of dental caries in the sample was

46.4%. In 25% of the children, Over 20% of gingival sites bled when probed. A binary logistic regression analysis revealed that dental fluorosis (OR=5.749, 95% CI=3.069-10.772) and use of fluoride tooth paste (OR=0.346, 95% CI=0.224-0.535) were independently associated with dental caries. Chi square analysis revealed that dental fluorosis was not associated with bleeding on probing. (p=0.162)

Conclusions

There was a significant positive association was observed between dental fluorosis and dental caries. However, no significant association was identified between dental fluorosis and BOP. (p>0.001)

Introduction

Caries preventive role of fluoride is now well established. Though fluoride has a beneficial effect on dental caries, excessive fluoride ingestion during the period of tooth formation could cause dental fluorosis.

Many researchers have studied the association between dental caries and enamel fluorosis^{1,2,3}. Lida and Kumar⁴ in their study on US school children have reported that permanent maxillary first molars with fluorosis are more

Dr. R.M. Jayasinghe
(Correspondence)

Consultant in Restorative Dentistry, Teaching Hospital, Kurunegala. Tel: 0777 806 314
E-mail: manoripathiraja@yahoo.com

Dr. N. Abeygunawardhana

Restorative Dental Unit, Teaching Hospital, Karapitiya, Galle.

Dr. R.D. Jayasinghe

Dept of Oral Medicine and Periodontology, Faculty of Dental Sciences, University of Peradeniya.

Prof. L. Ekanayake

Dept of Community Dental Health, Faculty of Dental Sciences, University of Peradeniya.

resistant to caries than permanent maxillary first molars without fluorosis. Moreover a significant negative association between fluorosis status and caries experience in the permanent dentition has been observed in South Australian children⁵. Similar findings have been reported from Saudi Arabia as well (Al Dosari et al 2010)³. A Sri Lankan study conducted among 14 year olds residing in a high fluoride area revealed that those children with milder forms of enamel opacities had a lower caries experience compared to those with severe forms of enamel opacities⁶.

Also, a higher level of gingival inflammation has been observed in high fluoride areas compared to non-fluoride areas^{7,8,9}. A recent study conducted in India has reported that as the severity of fluorosis increased, the occurrence of gingivitis and periodontitis also increased¹⁰.

Dental fluorosis - caries relationship has been assessed in the Sri Lankan children in the past⁶. However; to the best of knowledge no study has been conducted to assess the association between dental fluorosis and periodontal status in the Sri Lankan population.

Therefore, the objectives of the present study were to determine the prevalence of dental fluorosis, dental caries and periodontal diseases and the association between dental fluorosis, dental caries and periodontal status in 15 year old school children residing in a high fluoride area in Sri Lanka.

Materials and Methods

Ethical approval for the present study was obtained from the Ethics committee of the Faculty of Dental Sciences/University of Peradeniya. Written informed consent was obtained from all participants and their parents. Also permission to conduct the study was obtained from the zonal education director and the principals of the selected schools.

The study was carried out in the Divisional

Secretary's division of Maho in the Kurunegala district, where fluoride levels in ground water is high (0.5-1.0ppm)¹¹. The sample consisted of school children who had completed their 15th, but not the 16th birthday and who have been life-long residents of the area. Moreover they have obtained their drinking water from a ground water source. Children with the mixed dentition were excluded from the study sample. The sample size was determined using the formula for estimating a population proportion with absolute precision. According to the National Oral Health survey, the prevalence of caries and periodontal disease among 15 years old in the Kurunegala district are 45% and 56% respectively. For the purpose of calculating the sample size the prevalence of dental caries (45%) in 15- year olds in Kurunegala district was used. Accordingly the minimum sample size required for the present study using that prevalence estimate at 95% confidence level and accepting a sampling error of 5% was 380. This was increased by 10 % to compensate for non-responses.

According to information obtained from the education authorities, 15 year olds attend 15 schools in the area. Eight schools were selected randomly from these 15 schools and all children who satisfied the inclusion criteria were included in the sample.

Data were collected by means of a self administered questionnaire and an oral examination. Caries status was recorded according to WHO Basic methods¹² while Dean's index was used to record dental fluorosis. Periodontal status was assessed in terms of bleeding on probing as it is an accepted method of identifying active periodontal inflammation. Bleeding on probing was assessed on 6 sites of each tooth.

Oral examinations were carried out by two calibrated dentists; both examiners examined all children from the area. Intra- and inter examiner

variability was ascertained by re-examining every tenth student examined on a particular day. Following the oral examination, children who needed dental treatment were provided with necessary care which included restorative and periodontal care as well as microabrasion of fluorosed teeth.

SPSS 17.0 version software was used for data analysis. The Chi square test was used to determine the associations between categorical variables. Explanatory variables that were associated with the dependent variable (dental caries) at $p < 0.05$ in the bivariate analysis were then included in a binary logistic regression analysis to determine the independent effects of the explanatory variables on the dependent variable. As none of the explanatory variables considered was associated with BOP in the bivariate analysis logistic regression analysis was not carried out for BOP.

Results

Information was gathered from 415 students and the response rate was 99%. Of the sample, 50.1% were males and 71.7% were using water from deep dug wells for drinking. According to the Dean's index, 79.5% (325) had mild to severe dental fluorosis.

Prevalence of dental caries in the sample was 46.4% (190). In 25% of the children, Over 20% of gingival sites bled when probed. Bivariate analysis revealed that gender; father's education, mother's education and frequency of tooth brushing were not associated with dental caries. However, there was a significant inverse association between use of fluoride tooth paste and dental caries. Also presence of dental fluorosis was significantly associated with the presence of dental caries. (Table 1)

Use of fluoride tooth paste (OR=0.346, 95% CI=0.224-0.535) and presence of dental fluorosis (OR=5.749, 95% CI=3.069-10.772) were independently associated with dental caries (Table 2).

Table 3 shows the associations between selected variables and bleeding on probing (BOP). Gender, frequency of brushing, and presence of fluorosis were not associated with BOP (Table 3).

Discussion

Dental fluorosis may result in varying degrees of structural damage, superficial porosity, and loss of continuity of dental enamel layer¹³. Dental fluorosis is a global public health issue that is endemic in areas with above-optimal fluoride levels in the drinking water^{14,15}.

The prevalence of dental fluorosis was very high (99%) in the sample. Also nearly 72% of the sample had stated that they obtain their drinking water from dug wells. Maho is in the dry zone of Sri Lanka and fluoride level in ground water in the dry zone is higher than in the wet zone of Sri Lanka¹¹. Also it has been reported that water in dug wells contains very high levels of fluoride¹⁶. This may be a reason for the high prevalence of dental fluorosis observed in the sample. Literature indicates that nearly 80% of the 15 year old children residing in the dry zone of Sri Lanka^{17,18} have fluorosed teeth; mostly in the milder forms.

The prevalence of dental caries in the present study was 46% and almost similar that the prevalence of caries in 12-15 year olds living in endemic fluorosis areas in Mexico (49 %)¹⁹. It can be described with relevance to the use of fluoridated tooth paste as observed in the study and role of dietary factors among school children.

The results revealed a significant positive association between dental fluorosis and dental caries and however, according to the literature this association is not consistent.

A study carried out on 12-16 year olds in a high fluoride area in Iran has revealed that severe

Table 1. Bivariate associations between selected variables and dental caries

Variable	Caries Present		Caries Present		P value
	Number	Percentage	Number	Percentage	
Sex	90	44.1	114	55.9	0.344
Female (204)	100	48.8	105	51.2	
Male (205)					
Father's education	34	49.3	35	50.7	0.637
Less than O/L (69)	150	46.2	175	53.8	
O/L and above (325)					
Mother's education	42	44.2	53	55.8	0.792
Less than O/L (95)	135	45.8	160	54.2	
O/L and above (295)					
Frequency of brushing-					0.708
once/day (119)	57	47.9	62	52.1	
- twice or more times/day (290)	133	45.9	157	54.1	
Use of fluoride tooth paste					<0.001
No (154)	97	63	57	37	
Yes (255)	93	36.5	162	63.5	
Dental Fluorosis					<0.001
Absent (84)	14	16.7	70	83.3	
Present (325)	176	54.2	149	45.8	

Table 2. Bivariate associations between selected variables and dental caries

Variable	Odd's ratio	95% confidence interval	P value
Use of fluoride toothpaste			
No (154)	0		<0.001
Yes (255)	0.35	0.224-0.535	
Dental fluorosis			
Absent (84)	0		0.001
Present (325)	5.75	3.07-10.77	

Table 3. Bivariate associations between selected variables and bleeding on probing

Variable	BOP* ≤20%		BOP>20%		P value
	Number	Percentage	Number	Percentage	
sex	151	73.7	54	26.3	0.511
Male (205)	156	76.5	48	23.5	
Female (204)					
Frequency of brushing-	92	77.3	27	22.7	0.501
Once/day (119)	215	74.1	75	25.9	
Twice or more times/day (290)					
Dental fluorosis					0.162
Absent (84)	68	81	16	19	
Present (325)	239	73.5	86	26.5	

*Percentage of sites which bled when probed

dental fluorosis is associated with higher caries occurrence²⁰ and is consistent with the findings of the present study. Moreover, a positive relationship between dental fluorosis and dental caries was observed across various tooth types in moderate and high fluoride areas in Ethiopia²¹. In contrast Do *et al*⁵ in their study in South Australian children found a significant negative association between fluorosis status and caries experience in the permanent dentition. Also a cross-sectional study among 13 and 15 year old school children residing in an endemic fluorosis area in India found that the DMFT reduced with the increase in the severity of fluorosis²². Findings from a study conducted in the US indicate that molars with fluorosis are more resistant to caries than molars without fluorosis⁴.

Al Dosari *et al*³ found that there was no significant changes in caries experience or in the prevalence of dental fluorosis when the fluoride level in water increased from 0.3 ppm to 0.6 ppm. However when the fluoride level in water was above 0.6ppm, it was observed that with the increase in the severity of fluorosis, the caries experience reduced.

Authors of a study in India in 2011 had highlighted that as many local and systemic factors influence the etiology and pathogenesis of periodontal disease and one such factor could be fluoride²³. Some work on the effect of fluoride on specific bacteria suggests that fluoride has a bactericidal effect on microorganisms and is important in the treatment of periodontal disease^{24,25}. However, there was no significant association between dental fluorosis and BOP (p=0.162) in the present study. In contrast, findings from a study carried out in India have shown that there was a strong association between dental fluorosis and gingivitis; as the severity of fluorosis increased the severity of gingivitis also increased¹⁰. The authors of that study had concluded that it would take a considerable time to elucidate the role of fluoride in the etiology of periodontal disease.

Also Murray²⁶ found that the prevalence of gingivitis and gingival recession (loss of attachment) in 15-65 year old residents of Hartlepool where the fluoride concentration in water ranged from 1.2-2.0ppm was higher than in York, England where fluoride levels in drinking water was very low (0.15-0.2 ppm).

In conclusion, it was evident from the findings that there was a significant positive association between dental fluorosis and dental caries while use of fluoride tooth paste was inversely associated with dental caries. However, BOP was not associated with dental fluorosis.

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Glandular Odontogenic Cyst - Dilemma in Management

Viraj Indika Jayasinghe, S.P.I. Silva, A.H.T.S. Karunatileke

Abstract

The glandular odontogenic cyst (GOC) is a rare developmental odontogenic cyst found in the teeth bearing area of the jaws, more often in the anterior mandible. Its' behaviour is locally aggressive and tends to recur after enucleation. This case report is based on a GOC of a 53 year old male, which responded very well to decompression. However, this method of surgical treatment has not been emphasized in the literature in the treatment of GOC. The prognosis after treatment of this GOC had been good with no clinical and radiological evidence of recurrence for six years.

The finding of this case report highlights the benefits and the importance of this minimally invasive surgical treatment in the management of GOC. In conclusion we propose that this method should be evaluated as a cost effective alternative treatment option in the management of GOC.

Key words: Glandular odontogenic cyst, locally aggressive, anterior mandible, decompression, enucleation.

Introduction

The GOC was first introduced by Padayachee and Van Wyk in 1987 and proposed as "sialo odontogenic cyst"¹. One year later Gardner

introduced the term "glandular odontogenic cyst". In 1992 World Health Organization accepted glandular odontogenic cyst as a distinct pathological entity and the preferred term GOC since the possible salivary gland origin of the cyst has not been established².

GOC has been reported in 14 - 85 year old individuals and majority of them being above 40 years of age². The occurrence of GOC has also been reported to be common in the 6th decade of life with the mean age of 49 years³. There is a slight male preponderance and the reported prevalence of this cyst is 0.017 % to 0.2 % of all odontogenic cysts⁴. The usual presentation is disfigurement, swelling, resorptive change in alignment of teeth, difficulty in wearing the denture and rarely pain or tenderness^{5,6,7}.

The histological and immunohistochemical investigations of this cyst have been carried out extensively using paraffin embedded sections stained with hematoxylin and eosin, periodic acid Schiff- Alcian Blue, reacted with a range of monoclonal cytokeratin antibodies according to the alkaline phosphatase-anti-alkaline phosphatase method⁵. The cyst consists of well-defined stratified squamous non keratinized epithelium of varying thickness and a cyst wall. Superficial layers of the epithelium show either cuboidal or columnar cells with cilia. Epithelial

Dr. Viraj Indika Jayasinghe
(Correspondence)

Registrar, Oral and Maxillofacial Surgery, PGIM Colombo. Tel: 077 392 53 09 E-mail: Virajjayasinghe76@gmail.com

Dr. S.P.I. Silva

Con OMF Surgeon DI Colombo. E-mail: spadmasilva@gmail.com

Dr. A.H.T.S. Karunatileke

Con OMF Surgeon CSTDH Kalubovila. E-mail: ahtska@gmail.com

plaque formation is a common feature and there are pseudo-glandular spaces containing periodic acid Schiff positive material and mucicamine positive material. The other possible findings are micro-cysts within the epithelium and goblet cells in the superficial layers of the epithelium. Only a few cases reported with hyaline bodies. The cyst wall contains a few inflammatory cells confirming its developmental origin⁴.

The histopathological features of GOC are described as a combination of findings from a botryoid odontogenic cyst and a mucoepidermoid carcinoma, often causing a diagnostic dilemma for pathologists⁸. The epithelial plaque formation is seen in lateral periodontal cyst and the botryoid cyst. The pseudo-glandular spaces and micro-cysts formation are seen in low grade intra-osseous mucoepidermoid carcinoma. Since these three pathologies share common histological features accurate diagnosis is mandatory in the management of GOC⁴.

The radiological findings are not pathognomonic as the radiolucency can be either unilocular or multilocular. When it is unilocular, it simulates jaw cysts such as dentigerous cyst, radicular cyst and odontogenic keratocyst or tumors with unilocular radiolucency such as some odontogenic tumors, central giant cell lesions, vascular lesions or intra-osseous malignancy. When it appears as a multilocular radiolucency, picture may resemble bone lesions such as ameloblastomas or bone cysts or tumors which give rise to multilocular radiolucency⁹. Almost always the lesions tend to have well-demarcated and sclerotic borders which are scalloped or smooth. When the lesion exceeds the size of 6cm, it often tends to perforate the cortical plate. Tooth displacement and dental root resorption may be a feature. The multilocularity might be a size related phenomenon, and the cortical perforation shows its aggressive behavior¹⁰.

In the management of GOC the following treatment modalities are being practiced;

1. Enucleation and surface curettage for small unilocular lesions.(+/_ thorough extirpation)
2. Marginal, en-bloc or segmental resection for large multilocular lesions.
3. Marsupialization with a second surgery of enucleation and curettage where vital structures to be preserved^{11,12,13,14}.

Irrespective of the method used in the treatment of these cysts, it is mandatory to follow up the patients for several years due to reported recurrences of the lesion as long as 7 years after initial treatment¹⁵.

Due to paucity of published data, there are many questions remaining to be resolved concerning histogenesis, biologic behavior and appropriate treatment of these lesions.

The purpose of this case report is to highlight another treatment option available which was experienced as an alternative approach to management of GOC with minimal morbidity and maximum outcome.

Case report

A 53 year old man reported to oral and maxillofacial surgery unit with the complaint of swelling of the lower jaw as a referral from the OPD dental clinic, Colombo South Teaching Hospital Kalubowila Dehiwala.

He had observed the gradual disfiguring of his chin region for about 1 ½ years' and mild changes in the alignment of the lower teeth. There was no pain or tenderness over the affected area or the teeth in the affected region and no numbness or abnormal sensation felt over the affected gingiva or the lower lip.

Significant facial asymmetry was observed due to the swelling of the mental region mainly towards the left side. Distortion of the anterior mandible could be felt and it extended bilaterally from the mental region to parasymphiseal regions.

Both jaws were partially edentulous, teeth of the clinically affected area of the mandible did not show discoloration or pathological level of mobility. Swelling was apparent labially and lingually. Labial swelling was extending from right lower canine region to the left lower premolar region. Lingual swelling was extending from the right lower lateral incisor region to the left lower lateral incisor region and appeared in a bluish gray color through the labial alveolar mucosa. It was fluctuant but did not blanch on pressure. Very mild mal-alignment was observed in relation to two lower central incisor teeth and the lower left lateral incisor tooth.

With this clinical picture, radiological investigations were carried out to assess the intra-bony lesion.

The following differential diagnoses were considered;

- I. Odontogenic cyst - orthokeratinized odontogenic cyst
- II. Odontogenic tumor - unicystic ameloblastoma
- III. Non odontogenic cyst- unicameral bone cyst, aneurysmal bone cyst, traumatic bone cyst
- IV. Non odontogenic tumor- enchondroma
- V. Central giant cell lesion- osteoclastoma, histiocytosis X
- VI. Central vascular lesion- hemangioma

The following radiographs were obtained and evaluated for extensions of the lesion, the behavior and features aid in the diagnosis:

- A. Intra oral periapical radiographs of lower anterior teeth and right and left premolar regions
- B. Lower standard occlusal radiograph
- C. Dental panoramic tomography

The radiolucent lesion with ill-demarcated margin was extending from right lower first molar region to the left lower first molar region. (Figure 1) The radiolucency extended in between roots of teeth to varying heights destroying the lamina

dura of the alveolar bone with no obvious root resorption. The inferior dental nerve had been displaced inferiorly. As the lesion was extensive and the margins were not demarcated, it was re-evaluated with computed tomography (CT).

An incisional biopsy was performed for histopathological diagnosis. A biopsy specimen of 1.0 x 0.5 x 0.2 cm sent to the Department of Oral Pathology, Faculty of Dental Sciences, Peradeniya. The microscopic findings showed a fibrous cyst wall lined by thin epithelium with two to three cell layers in most areas and focal areas of elongated basal cells with a mild degree of reverse polarity. The cyst wall was not inflamed. Even though those histopathological features were not pathognomonic, the most likely diagnosis was unicystic ameloblastoma.

A second set of periodical radiographs were taken during a waiting time for surgery to assess the root resorption of the affected teeth, revealed the lamina dura formation of pathologically affected alveolar process. It was decided to perform a second incisional biopsy for re-evaluation of the lesion in the absence of pathognomonic features in the histopathological evaluation and radiological finding of lamina dura formation of alveolar process. The second biopsy revealed non-keratinized stratified squamous epithelium with focal epithelial plaque formation, focal pseudo glandular spaces, surface papillary projections and glandular metaplasia. These histopathological features were consistent with those of GOC.

The new pathology report led to evaluate the lesion with a second CT. The comparison of the two CTs indicated a significant bone regeneration of the anterior cortex over the previous bony defect; measurements being 3.2x1.4 cm in the first CT vs. 3.2x0.6cm in the second CT, which has taken place during the course of two months. The IOPA finding of lamina dura formation and CT finding of bone apposition, suggested that surgical sites of two incisional biopsies have served as means of surgical decompression.

With the initial diagnosis of unicystic ameloblastoma, the treatment plan was a marginal resection of the mandible with pathological lesion, reconstruction of the surgical bone defect with a bone graft and mini-plate osteosynthesis under general anesthesia.

However the observed change of behavior of the lesion, led the clinician to follow “wait and see” policy. With the consent of the patient, it was decided to follow up the patient regularly with dental panoramic tomography.

Patient did not have any complaint one year of follow up. A sinus opening in the labial alveolar mucosa was observed in relation to the right lower central incisor tooth. The third CT reporting confirmed the findings of the radiographs. Currently the patient is on regular follow up for the sixth year and there is no asymmetry of the face, no visible or palpable bone distortion over the previously diseased area of the mandible, but only a sinus tract of roughly 0.5 cm in diameter in the labial gingiva in-relation to the midline. Last radiograph (March 2014- Figure 3) showed remnants of a radiolucent lesion in few focal areas, with signs of bone apposition.

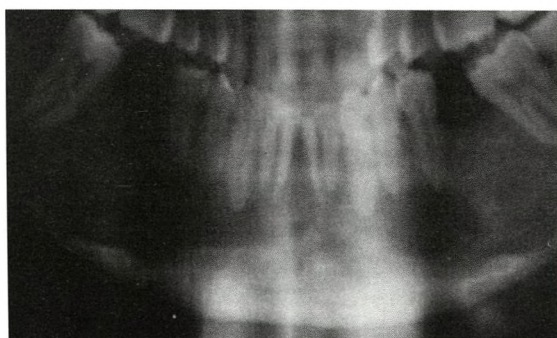


Figure 1. First panoramic tomograph showing the lateral extensions of the radiolucent lesion from right lower first molar region to left lower first molar region and vertical extension in between dental roots and inferior displacement of the inferior dental nerve in left side.

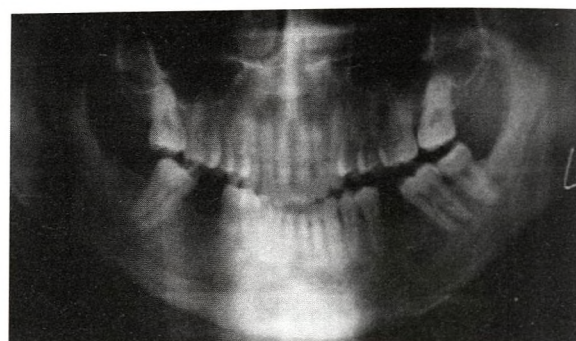


Figure 2. Intermediate - dental panoramic radiograph showing reduction of the right lateral and vertical extensions of the radiolucent lesion.

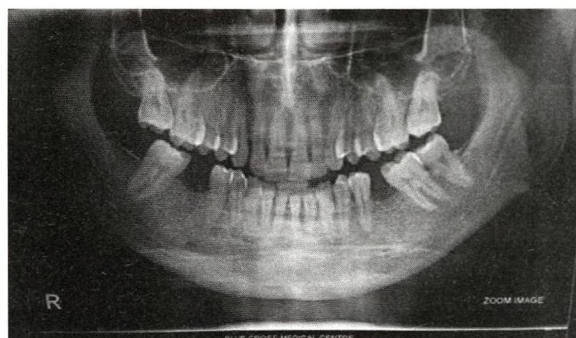


Figure 3. Last dental panoramic tomograph showing few irregularly ovoid radiolucent areas in relation to apices of right second premolars to left lower premolars.

Discussion

Until 1987, no one knew this cyst as glandular odontogenic cyst and, that does not necessarily mean such cyst did not exist, but could have been misdiagnosed as some other cystic lesion and treated differently.

In 1988 this cyst was introduced as a rare cyst of the jaw¹⁶. Almost after 20 years it has remained a relatively rare cystic lesion of the jaw¹⁷. As the current microscopic criteria have not been universally accepted; many GOCs have been identified as central mucoepidermoid carcinomas and GOC may not be as rare as it was thought¹¹. Several treatment modalities have been used in

the treatment of GOC. These include curettage, enucleation with careful dissection of the margins of bone (Peripheral ostectomy), marginal resection, en-bloc resection and segmental resection of jaw bones. The prognosis of this cyst remains unclear. However, the aggressive nature of GOC has been reported, and at least 25% to 55% recur following curettage making it imperative to follow up the patient carefully for several years after curettage or enucleation. Due to high recurrence rate and aggressive growth potential, some authors believe that radical resection may be more reliable treatment for GOC¹⁸. Therefore both conservative and radical methods of treatment for GOC have been suggested in the literature^{19,20}.

The treatment plan for each lesion should be customized, by utilizing the reliable evidence in the literature and patients' preferences. Those that are treated with conservative approaches must be followed up with regular radiological follow ups.

It may be difficult to define the characteristics and behavior of GOC, leading to dilemma in selecting a proper treatment protocol due to less number of reported cases. The above mentioned case would help to enrich the literature in future treatment planning. This unintentional finding prevented a planned resective and reconstructive surgery and avoided possible surgical morbidity, mortality and the hazards of general anesthesia. The logical and timely taken clinical decision led to a favourable outcome of this clinical scenario. Although the marsupialization is a form of decompression and used in the treatment of GOC, it is for the preservation of vital structures as a preparation for definitive surgical treatment. The other important aspects of this case scenario are the importance of re-evaluation and observing the lesion after interfering with its natural history and re-evaluation of the initial diagnosis in the absence of pathognomonic features.

However the assumption of decompression as a promising treatment for GOC may be wrong in the following circumstances;

1. If the histopathological diagnosis was questionable
2. If the pathology was an unknown sub-type of the diagnosed lesion
3. Individual variation of the pathology
4. Individual variation of the host factors such as subjective immunity
5. Microbiological factors of the individual and
6. Any combination of the above mentioned reasons

The unexpected outcome of this clinical scenario reveals a possible alternative treatment approach which is beneficial to the patient in several ways and rewarding the surgeon and also possibility of expanding it into a treatment protocol which would be useful in reducing the burden on health economics. However, such behavior of GOC has not been reported in literature, this treatment procedure should be further evaluated by; carrying out case control studies to assess the behavior of GOCs following decompression. The findings of this case report contributes to the current understanding of GOC and may pave way forward for the practice of evidence based dentistry and also may help to prevent unnecessary resection of mandibles and maxillae of patients with GOCs.

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Non - Syndromic Multiple Unerupted Supernumerary Teeth A Case Report

A.A.A.K. Wimalarathna

Abstract

The exact aetiology of supernumerary teeth is still unknown even though many theories have been proposed to explain their presence. Abnormal reactions to a local traumatic episode, environmental factors or a developmental anomaly, and certain syndromes have been implicated as causative factors. Although a single supernumerary tooth or few teeth have been widely reported in the literature, multiple supernumerary teeth are not a common occurrence. Multiple supernumerary teeth affecting all four quadrants of the jaw is a rare dental anomaly which generally is an accidental finding on routine dental radiography. This article reports the case of non-syndromic multiple supernumerary teeth, affecting all four quadrants in a 23-year-old male, who presented to the Faculty of Dental Sciences, University of Peradeniya for routine dental treatment without any significant medical or dental abnormality. A brief review of the literature on multiple supernumerary teeth is also presented.

Key Words: Radiography; Unerupted teeth; Non - Syndrome; Supernumerary teeth.

Introduction

Supernumerary teeth or hyperdontia are defined as the existence of an excessive number of teeth in relation to the normal dental formula and may or may not mimic the normal shape^{1,2}. They may occur singly, multiply, unilaterally or bilaterally and in one or both jaws. Rarely these may occur in all four quadrants of the jaw bone as in the index cases. Supernumerary teeth can be classified according to their form and position as shown in Table 1^{2,4}.

While impaction of single tooth is not uncommon, development of multiple impacted teeth is a rare condition and is often found in association with syndromes or developmental anomalies such as Gardner's syndrome, cleidocranial dysplasia, trichorhino phalangeal syndrome and cleft lip and palate^{3,4}. However, supernumerary teeth can occur in patients without any associated syndromes or developmental anomalies.

Classification based on form		Classification based on position	
Conical	(supernumerary peg shaped teeth)	Mesiodens	(present in the incisor region)
Tuberculate	(more than one cusp or tubercle)	Paramolar	(present beside a molar)
Supplemental	(resemble to normal teeth)	Distomolar	(present distal to the last molar)
Odontome	(a mass of dental tissue)	Parapremolar	(present beside a premolar)

Table 1. Classification of supernumerary teeth

The exact aetiology of supernumerary teeth is still unknown. Several reasons such as abnormal reactions to a local traumatic episode, environmental factors, development anomaly, and supernumerary teeth associated syndrome have been implicated as causative factors. However, many theories have been proposed to explain their presence; i.e.: - 'The dichotomy theory' of tooth germs states that the tooth bud splits into two equal or different sized parts, resulting in two teeth of equal size or one normal and one dimorphic tooth respectively. Further, the theory of hyperactivity of the dental lamina, the autosomal recessive inheritance or linked to the X chromosome, are accepted widely^{5,6,7}.

The presence of supernumerary teeth can cause problems for development of a normal dentition such as, failure of eruption of adjacent permanent teeth, crowding and rotation, occlusal problems, dental malformations, displacement and ectopic eruption, formation of diastema, root resorption of adjacent dentition, dilaceration of adjacent dentition and loss of vitality, formation of dentigerous cysts or they can even be just asymptomatic^{4,8}.

The unerupted asymptomatic supernumeraries are better kept as they are, without surgical intervention, to prevent the damage to the vital anatomical structures such as inferior dental nerve or mental nerve. It was suggested that a periodical radiographic observation is needed to detect early formation of any cystic degeneration

associated with the unerupted teeth. Then, it is advisable to remove these supernumerary teeth immediately, if it starts to show any pathologic change. Further, it is necessary to carry out further clinical examination to rule out possible syndromes.

Case Report

A 23-year-old male patient visited the department of Restorative dentistry with a complaint of pain in relation to the decayed teeth in left upper and lower posterior region. The patient was generally healthy, and his family's medical and dental history was noncontributory, and the extra oral examination was normal. Intraoral examination confirmed that the upper left first molar (#26) and lower left second molar (#37) had deep dentinal caries. (Figure 1 a, b).

The Intra-Oral-Periapical (IOPA) radiographs relevant to both carious teeth were obtained as a baseline investigation prior to dental treatment. Routine intraoral radiographs revealed the presence of multiple supernumerary teeth located in both upper and lower arches (Figure 2 a, b). The dental panoramic tomography (DPT) was required as a compulsory investigation to identify all the unerupted supernumerary teeth. The DPT radiograph revealed a total of fourteen (14) supernumeraries in all four quadrants and all of them were unerupted. The DPT showed no other abnormality or evidence of cystic degeneration around any of the unerupted supernumeraries (S^{UE}) (Figure 3) and dental formula is charted in Table 2.

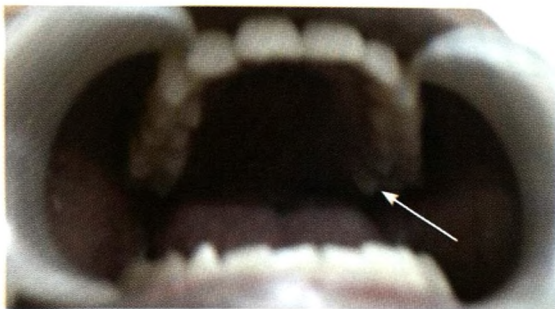


Figure 1. (a, b) Carious upper left first molar (#26) and lower left second molar (#37)

Non - Syndromic Multiple Unerupted Supernumerary Teeth
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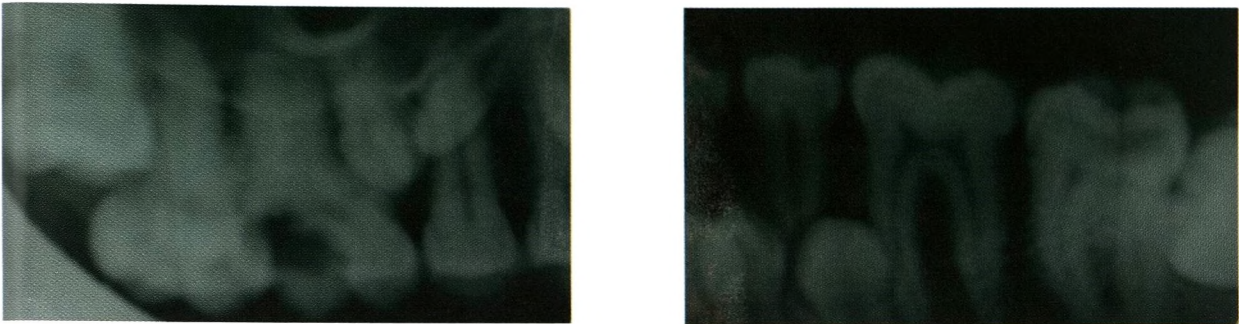


Figure 2. (a, b) The Intra-Oral-Periapical (IOPA) radiographs in relation to both carious teeth

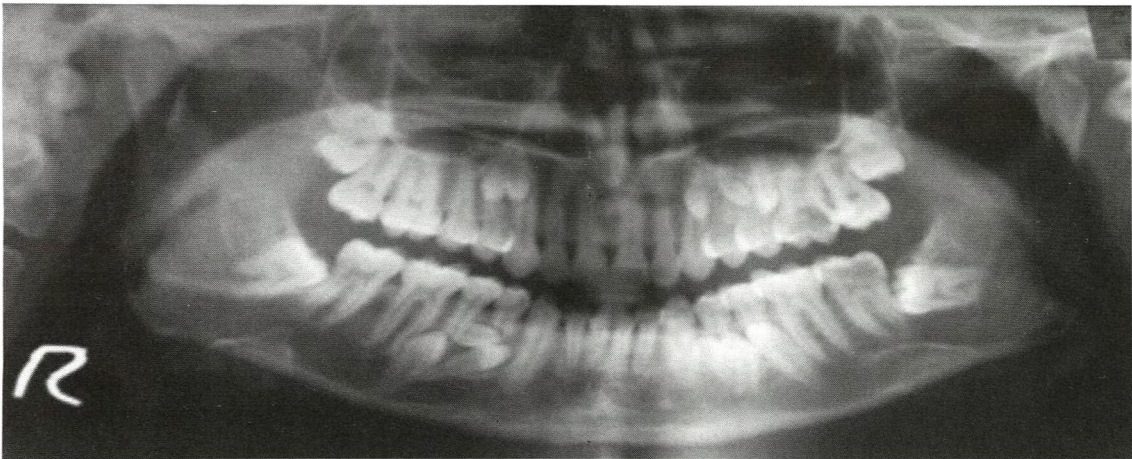


Figure 3. The DPT showing a total of fourteen (14) supernumeraries involving all four quadrants and all of them being unerupted.

S ^{UE}	S ^{UE}	18 ^{UE}	17	16	15	14	S ^{UE}	S ^{UE}	13	12	11	21	22	23	S ^{UE}	24	25	S ^{UE}	S ^{UE}	26	27	28 ^{UE}
48 ^{UE}	47	46	S ^{UE}	45	S ^{UE}	S ^{UE}	44	43	42	41	31	32	33	S ^{UE}	34	S ^{UE}	S ^{UE}	S ^{UE}	35	36	37	38 ^{UE}

Table 2. Chart showing location of unerupted supernumeraries (S^{UE})

Based on the history, clinical examination and radiographic investigations, it was confirmed as Non-Syndromic Multiple Supernumerary Teeth.

All the teeth were vital except the upper left first molar (#26). Indirect pulp capping and temporary filling was done for the lower left second molar (#37) and it was decided to do the endodontic treatment for the non-vital upper left first molar (#26). It was suggested to the patient that a follow-up DPT would have to be taken every two years to identify any cystic degeneration as-

sociated with these unerupted teeth. Further, the patient was clinically examined to confirm and rule out syndromes.

Discussion

Non-Syndromic multiple supernumerary teeth itself is a rare condition and involvement of the all four quadrants of the jaw is very rare. The lower arch premolar region is the most common site of occurrence of supernumerary teeth⁹. This case shows the presence of (fourteen (14) multiple supernumerary teeth in the premolar area of

all four quadrants. The occurrence of non-syndromic supernumerary teeth is more common in the maxilla than in the mandible¹⁰, and in males than in females¹, in permanent dentition than in primary dentition¹², and unilaterally than bilaterally¹². Non-syndromic supernumerary teeth might be related to heredity factor; therefore a thorough family' history should be carefully investigated.

Published research on prevalence of supernumerary teeth shows variations according to the location from where the data had been collected.. The prevalence of supernumerary teeth in permanent dentition ranges from 0.15% to 3.8%^{13,14}. It is less common in the deciduous dentition with a reported incidence of 0.3% to 1.7% of the population^{13,14}. However, where multiple supernumerary teeth are taken to mean five or more supernumerary teeth, the prevalence has been reported as less than 1%. The incidence of multiple supernumeraries is lower than that of single and double supernumeraries. According to the reported literature, 68.6% were single supernumeraries, 20.3% were double and 11.1 % multiple supernumeraries. Approximately 75% of supernumerary teeth are impacted and asymptomatic, and most of these teeth are diagnosed coincidentally during radiographic examination¹⁵.

Early diagnosis is important in order to minimize the risk of complications resulting from unerupted supernumerary teeth. Surgical intervention is recommended if they have caused delay or non-eruption of permanent teeth, displacement of permanent teeth, root resorption of adjacent teeth due to the pressure and if there are signs of cystic formations.

Symptomatic multiple unerupted supernumeraries may have to be surgically removed after appropriate education of the patient and parent about the risk of damage to adjacent teeth and vital anatomical structures. For asymptomatic supernumerary teeth, long term radiological

follow up is recommended for early diagnosis of any pathologic changes in relation to the unerupted teeth. If patients who visit for routine dental consultation with multiple supernumeraries are associated with other dental anomalies and syndromes those related clinical problems must be prioritized before treating the complications arising from presence of the supernumerary teeth.

Conclusion

It is rare that a dental surgeon may witness asymptomatic multiple supernumeraries involving all four quadrants. While it could be associated with syndromes, when it is not it is usually an incidental radiographic finding especially in the case of unerupted asymptomatic teeth. The treatments are controversial and are generally reserved for symptomatic teeth. Periodic radiological examination is essential to identify any pathologic change or other related problems.

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Management of giant salivary sialolith: A case report

Sudhir Ramiseti, Rajasekhar Gaddipati, K. Rajeev Reddy, Laxman Roy Chittaluri

Abstract

Sialolithiasis is the formation of calculi in the salivary gland or duct which results in the obstruction of salivary flow. Sialolith is a calcareous substance, which may form in the gland or the duct of the major or minor salivary glands. Sialolithiasis is known to be one of the most common diseases of the salivary glands in middle aged individuals. The majority of sialoliths (80%) occur in the submandibular gland or its duct and are a common cause of acute and chronic infections of the gland. This case report describes a patient presenting with an unusually large sialolith in submandibular duct, treated surgically through intra oral approach with no subsequent post-operative complications. Also included is a brief literature review discussing the aetiopathogenesis, diagnosis, various diagnostic aids, and treatment modalities for management of salivary gland calculi depending on their site and size.

Keywords: Salivary gland; Giant Sialolith; Submandibular duct; Infection; sialolithiasis

Introduction

Sialolithiasis is the formation of calculi in the

salivary gland or duct which results in the obstruction of salivary flow. Sialolith is said as a calcareous substance, which may form in the gland or the duct of the major or minor salivary glands. Although any of the salivary gland or duct may be obstructed by the formation of sialolith, nearly about 80% of the sialoliths form in the submandibular gland. This is because a long, curved wharton's duct has increased chance of entrapment of organic debris; the secretions of this gland is higher in calcium content and thick in consistency, and the position of the gland increases the chances for stagnation of the saliva. The factors like inflammation, local irritation or drugs can cause salivary stagnation leading to the buildup of an organic debris, which eventually will calcify. The size varies from less than 1 mm to a few cm in size, with most calculi being less than 10 mm in size⁽¹⁾.

Sialolithiasis affects approximately 12 in 1000 of the adult population with males being affected twice as much as females and children are rarely involved⁽²⁾. Giant salivary gland stones measuring greater than 15 mm have rarely been reported in the literature⁽³⁾. Sialoliths close to the hilum of the gland tend to become large

Dr. Sudhir Ramiseti
(Correspondence)

MDS, Associate professor, Department of Oral and Maxillofacial Surgery, Mamata Dental College and Hospital, Giriprasad Nagar, Khammam, Andhra Pradesh, India - 507002. Tel: +91 9963117979 Fax: 091 8742 30862 E-mail: drsudhir_007@hotmail.com

Dr. Rajasekhar Gaddipati

MDS, Professor & Head, Department of Oral and Maxillofacial Surgery Mamata dental college and hospital, Khammam. Tel: +91 9000018128 E-mail: drrajasekharg@gmail.com

Dr. K. Rajeev Reddy

MDS, Associate Professor, Department of Oral and Maxillofacial Surgery Mamata dental college and hospital, Khammam. Tel: +91 9959025029 E-mail: rajivreddy_dr291@yahoo.co.in

Dr. Laxman Roy Chittaluri

Post graduate, Department of Oral and Maxillofacial Surgery Mamata dental college and hospital, Khammam. Tel: +91 9885872225 E-mail: laxman.roy@gmail.com

before they become symptomatic³. Intraductal sialoliths are more common, elongated in shape as compared to intraglandular stones, tend to be oval⁽⁴⁾.

There are currently two theories about the pathogenesis of salivary stones⁽⁵⁾. The first is that intracellular microcalculi develop in normal salivary tissue and are naturally voided through the duct system. If these became impacted during their discharge, they will act as a nidus and help in sialolith formation. An alternative explanation is that a mucous plug forms and calcifies after being exposed to a supersaturated solution⁽¹⁾. Sialoliths may be ovoid or round, smooth or rough with a yellowish color. They are composed of calcium phosphate with small accounts of hydroxyapatite, magnesium, potassium and ammonia⁽³⁾.

The formation is supposed to occur in two phases: a central core phase and a layered periphery phase. The central core is formed by the precipitation of salts, which are bound by certain organic substances followed by second phase of the layered deposition of organic and non organic material⁽⁶⁾. Factors that tend to favour submandibular sialoliths are: longer caliber ducts and slower rates of salivary flow, salivary flow against gravity, increased alkaline nature of saliva and, high salivary mucin and calcium content⁽²⁾.

Diagnosis is usually done clinically by bimanual palpation in a posterior to anterior direction, which in turn reveals a palpable stone in a large number of cases, a uniformly firm gland suggestive of hypofunctional or non-functional gland⁽⁷⁾. Basic imaging like occlusal and panoramic radiographs, to detect deep submandibular or radiolucent or deep parotid stones sialography is useful but is contraindicated in acute conditions and in patients allergic to contrast material⁽⁴⁾. In such cases ultrasonographic examination is considered to be simple and non invasive modality of diagnosing the sialoliths.

Advanced diagnostic aids like Digital sialography and Subtraction sialography have increased sensitivity and specificity over conventional sialography techniques that were considered gold standard. Magnetic resonance sialography (MRS) is a new radiological technique indicated in cases of acute infection where other sialography techniques are contraindicated since it does not require cannulation of the duct. Diagnostic sialadenoscopy is a latest technique in which the complete ductal system can be explored⁽⁸⁾. The CBCT sialogram gives an exposure similar to fluoroscopic sialographic procedures, as performed in C-arm fluoroscopy X-ray units, but higher than an examination performed with plain or static images. It delivers a slightly higher radiation exposure and should therefore be reserved for more difficult cases⁽⁹⁾.

The present case is a giant sialolith reported in the right submandibular duct, measuring about 34mm x 21mm and treated using transoral approach.

Case Report

A 65-year-old patient presented to the department of Oral and Maxillofacial Surgery, with a chief complaint of swelling in the anterior part of the right side of the floor of the mouth (Fig 1). The patient gave history of pain associated with peanut size swelling initially and slowly increased to present size, increase in size during chewing and associated with mild difficulty during intake of food. Patient also revealed a history of calcium supplements usage, secondary to femoral fracture since two years. Extra-oral examination revealed a palpable, firm swelling in the right submandibular region, non tender, non compressible and non reducible in nature. Intra oral examination revealed redness (inflammation) over right posterior region, mesial to right third molar (Fig 2) and bimanual palpation revealed a large, firm, non-tender swelling in the right anterior floor of mouth in the region of the submandibular duct. A paranasal sinus radiograph view showed the

mass to be radiopaque in the posterior region and extending back beyond the mandibular right first permanent molar (Fig 3). Additional investigations like ultrasonography and 3D CT scan were made to confirm (Fig 4,5,6) diagnosis which in turn revealed right submandibular duct sialolith measuring about 34mm x 21mm. No other relevant medical and dental history could be elucidated. A clinical diagnosis of submandibular sialolith was made and subsequently, a treatment plan of surgical excision of sialolith with transoral approach was decided.



Figure 1. Patient with swelling in the right submandibular region



Figure 2. Intra oral showing inflamed area due to penetration of sialolith

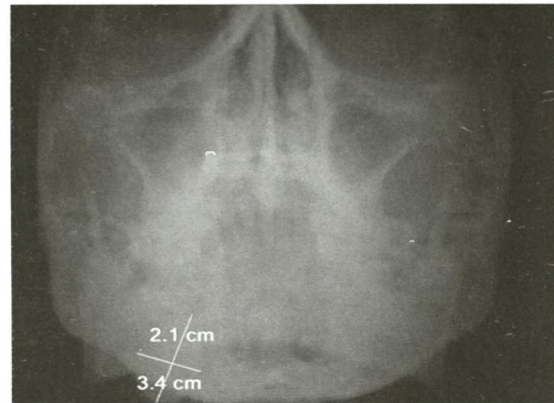


Figure 3. PNS radiograph showing radiopaque mass in the posterior region

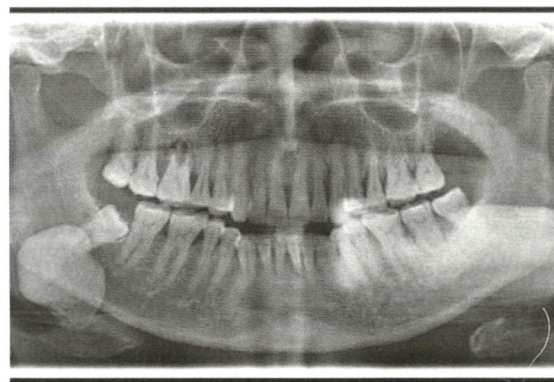


Figure 4. Panoramic radiograph image showing sialolith extending to mandibular right first permanent molar area.

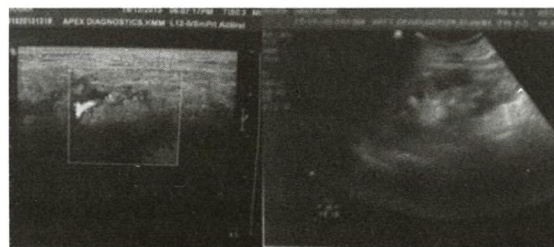


Figure 5. Ultrasonographic image of the sialolith in the submandibular duct.

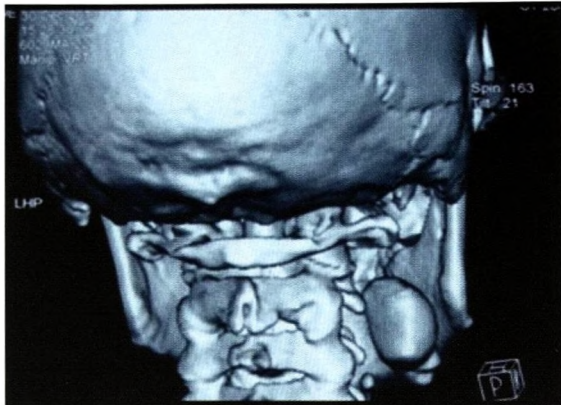


Figure 6. 3D CT image of the sialolith in the submandibular region.

Under general anesthesia, surgical technique was initiated by passing a suture around the duct posterior to calculi (stay suture) to prevent its dislodging or moving into less accessible region. The tongue was retracted towards the opposite side and the needle was passed well behind the calculus to pick up a deep bite of tissue over the duct. The floor of the mouth was lifted up with this suture. Starting beyond the papilla on the other side, the floor of the mouth was infiltrated with local anesthetic and adrenaline solution, to help in local hemostasis. A 4 mm incision anterior to the anticipated position of calculus was then made which bisected the angle between the lateral attachment of the tongue to the floor of the mouth and the plica sublingualis (fig 7). The wound was deepened gently with scissors keeping lateral to the sublingual veins and the duct was identified (fig 8). Once the duct was located, it was mobilized and the dissection was carried anteriorly towards the first molar region; taking into consideration that lingual nerve which passes under the duct is identified and protected (fig 9).

The duct was incised longitudinally over the sialolith and it was removed. The posterior stay suture round the duct was removed and saliva was expressed by pressure over the lower pole of gland. The remaining stay sutures were subsequently removed and the floor of the



Figure 7. Planned incision anterior to the anticipated position of sialolith.



Figure 8. Exposure of submandibular duct through floor of mouth.



Figure 9. Intra operative picture showing excision of sialolith.

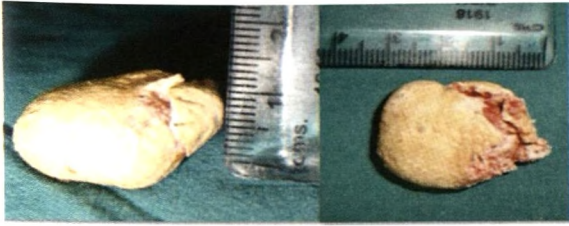


Figure 10. Excised sialolith measuring 34 x 21 mm.

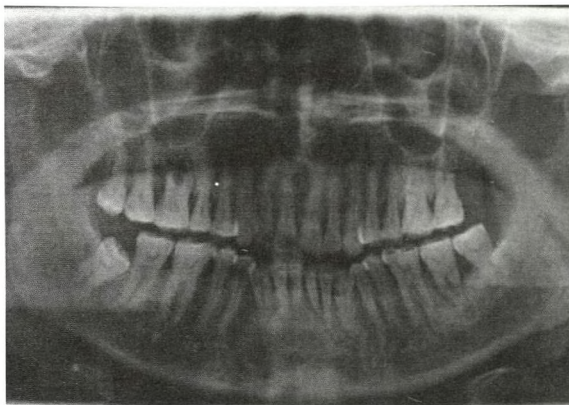


Figure 11. 1 year post operative panoramic radiograph.

mouth is closed with 3'0 silk black silk.. It was measured to be 34mm x21mm long along its greatest length (Fig 10). Due to the swelling in the right submandibular region and the size of the sialolith, the patient was reviewed alternate weeks post operatively to check salivary function of the gland. On review the right submandibular gland was palpable and clear saliva could be expressed from the duct on massage. These post operative sialochemistry revealed presence of calcium phosphate with small accounts of hydroxyapatite, magnesium, potassium and ammonia, reviews showed uneventful healing with absence of post-surgical complications till a follow-up period of one year (Fig 11).

Discussion

Salivary sialoliths are usually small and measure about 1mm to less than 1cm. Giant sialoliths measuring size more than 3cm are rare, with few reported cases⁽⁵⁾. Messerly⁽¹⁰⁾, Brusati and Fiamminghi⁽¹¹⁾ removed a giant sialoliths from

the submandibular duct measuring greater than 30mm^(4,6). Thus, the present case, reports the history and successful management of such a rare giant sialolith of the submandibular duct.

In the present case, ultrasonography and 3D CT scan were used along with bimanual palpation and conventional radiographs to exactly locate the sialolith. Yoshimura Y⁽¹²⁾ et al. found that the detection rate of sialoliths using ultrasonographic images was higher when compared to sialography^(6,8). Sialoliths less than 3mm cannot be detected using ultrasonographic examination⁽⁸⁾.

The algorithm for the management of sialolithiasis depends upon the size and location of sialolith. In the management of small sialoliths, conservative methods such as proper hydration of the patient, application of moist warm heat and massaging the gland in conjunction with sialogogues can be done^(8,13). Since the sialolith in this case was larger measuring 34mm x 21mm, surgical excision using transoral approach was considered to completely expose and remove it. Zenk KJ *et al*⁽¹⁴⁾ suggested that transoral removal is the treatment of choice for submandibular sialoliths, which can be bimanually palpated and localized by ultrasonography. The submandibular sialoliths which are located close to the orifice of Warthin's duct can be excised by Sialodochoplasty⁽¹⁶⁾. If the sialolith is too large or located in the proximal duct, piezoelectric extracorporeal shock wave lithotripsy (ESWL) or surgical removal of the stone or gland may be required.

Sialoendoscopy is a new treatment modality and minimally invasive technique for treating obstructions of the ductal system and for removal of large salivary stones^(5,6,9,11). Co2 laser technique have the advantage of less bleeding, minimum scarring with minimal post operative complications⁽¹⁷⁾.

Conclusion

Although various advanced diagnostic and treatment modalities have emerged in the management of sialoliths, the conventional techniques retain their popularity to date. A case of giant submandibular sialolith (34mm x 21mm) is reported which was diagnosed clinically and radiographically and treated with no postoperative complications.

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Hemi section and Orthodontic Management of a fused tooth A review and case report

V.S.N. Vithanaarachchi, E.M.U.C.K. Herath, S.P.N.P. Nagarathna

Abstract

Fusion and gemination of teeth are considered as developmental anomalies of the dental hard tissues. Since abnormal tooth morphology can predispose to caries, periodontal diseases and occlusal irregularities, careful management of fused teeth seems to play a vital role. In this article management of a lateral incisor fused to a supernumerary tooth is discussed in details while reviewing similar cases reported elsewhere.

Keywords: Fusion, Gemination, Supernumerary, Hemi section, Orthodontic treatment

Key Messages: Fusion and gemination of permanent teeth are developmental anomalies of dental hard tissues. Since these conditions can predispose to caries, periodontal disease and occlusal irregularities, careful management of such teeth seems to play vital role.

Introduction

Tooth fusion is defined as a union between the dentine and / or enamel of two or more separately developing teeth. The fusion may be partial or total, depending on the stage of tooth development at the time of union^{1,2}. Prevalence of tooth fusion is estimated as 0.5 - 2.5% in the primary dentition and it is less than 1% in the permanent dentition³.

The aetiology of the fusion is still uncertain, but many different views have been put forward. Shafer *et al* (1974) discussed the influence of pressure produced by physical forces prolonging the close contact between two tooth germs resulting in fusion⁴. Lowell and Soloman (1964) suggested that fusion results from union between enamel organ and dental papilla as a result of close contact between two tooth germs leading to necrosis of intervening tissue⁵. However genetic predisposition has also been reported in the literature, as an aetiological factor⁶.

Fusion seems to be mainly associated with anterior teeth and could be either unilateral or bilateral⁷. Furthermore, fusion may occur between two normal teeth or between a normal tooth and a supernumerary tooth. Clinically, fused anterior teeth frequently show groove or a notch in relation to the incisal edge and which may extend bucco-lingually. There may only be one pulp chamber dividing into two root canals or two independent endodontic systems can be identified with fused teeth⁸.

There may be joining of two adjacent teeth by cementum which is referred to as concrescence. Concrescence can occur at the time of development of two teeth or following complete development of teeth due to hyper cementosis¹.

Dr. V.S.N.Vithanaarachchi	Senior Lecturer, Division of Orthodontic, Faculty of Dental Sciences, University of Peradeniya
Dr. E.M.U.C.K. Herath	Senior Lecturer, Division of Paedodontics, Faculty of Dental Sciences, University of Peradeniya
Prof. S.P.N.P.Nagarathna	Professor in Orthodontics, Division of Orthodontic, Faculty of Dental Sciences, University of Peradeniya

Gemination is also a similar dental anomaly in which two completely separated crowns are associated with a single root^{1,2}. It is important to differentiate fusion and gemination especially when fusion is associated between normal tooth and a supernumerary tooth³.

Fusion of teeth can give rise to crowding of the region affecting dental aesthetics. Therefore, a multidisciplinary approach in the management of fused teeth becomes important to achieve a successful outcome. In this context a team of specialists consisting of an orthodontist, endodontist, maxillofacial surgeon, periodontist, prosthodontist and paedodontist have to combinely play a major role in the improvement of dental esthetics as well as function, in affected individuals.

The following case report describes the orthodontic and surgical management of a patient with unilateral fusion between maxillary lateral incisor and a supernumerary tooth, who had been referred to the Division of Orthodontics of the Faculty of Dental Sciences, University of Peradeniya, from a peripheral dental unit.

Case History

A 13-year old boy attended the Division of Orthodontics at the Faculty of Dental Sciences, University of Peradeniya, requesting treatment for his irregularly arranged upper front teeth including presence of a large tooth in front. His medical history was not significant.

The extra - oral examination revealed no obvious asymmetry of face with slightly convex facial profile. With regards to the assessment of mandibular path of closure, there was a forward displacement of mandible with premature contact on the left upper and lower central incisors.

Intra - oral examination revealed that patient was in late mixed dentition and maintaining adequate level of oral hygiene to commence orthodontic

treatment. Overjet was 3mm and positive on right side and it was 1mm and negative on the left side due to presence of a cross bite in relation to left upper and lower central and lateral incisors. (Fig. 1) Overbite was normal in right side but reversed in left side due to a cross bite. There was an over retained upper left deciduous canine with labially erupting permanent canine. Upper right lateral incisor was abnormally large due to fusion of two clinical crowns, which has erupted into cross bite. (Fig. 2) The large clinical crown was contributory to the development of severe crowding seen in the labial segment.



Figure 1. Preoperative photograph: labial view



Figure 2. Preoperative photograph: Upper occlusal view showing abnormally large upper right lateral incisor due to fusion.

Radiographic investigations confirmed the clinical findings and revealed that the upper lateral incisor was fused with a supernumerary tooth which showed two separate endodontic systems. (Fig. 3) Lateral cephalometric analysis

Hemi section and Orthodontic Management of a fused tooth
A review and case report

showed mild Class III skeletal pattern with normal vertical dimension. Pulp testing gave normal response in relation to both upper lateral incisors.



Figure 3. Preoperative radiograph revealing irregular morphology of tooth

Orthodontically the case was diagnosed as a class III malocclusion on a mild class III skeletal base with fusion of upper lateral incisor to a supernumerary tooth. The patient was given priority for orthodontic treatment on functional grounds to eliminate premature contact in order to correct mandibular path of closure and to relieve severe dental crowding in order to prevent deterioration of periodontal status as well as development of caries. Comprehensive dental management commenced with extraction of over retained upper left deciduous canine after obtaining informed consent from patient and his parents.

At the beginning it was decided to use an upper removable appliance to correct the left central and lateral incisor cross bite. Following correction of forward displacement of the mandible, patient was reassessed by the team of specialist to decide about the management of fused tooth. For orthodontic and restorative reasons a decision was made to remove the supernumerary tooth which was displaced mesio - palatally.

Surgical removal was planned to perform under general anesthesia as the surgical separation of twin tooth is a time consuming procedure.

With the surgical exposure it was confirmed the union was between two roots as observed in the radiograph. The root of the distal portion of the twin tooth was closer to the line of the arch but the mesially placed tooth was more palatally displaced and mesio- palatally rotated. Therefore the twin tooth was separated and mesio - palatally placed supernumerary tooth was removed with minimum trauma to the upper lateral incisor. The flap was replaced and sutured and patient was reviewed after one week period and sutures were removed.

Prior to plan further treatment upper lateral incisor was investigated radiologically and evidence revealed that presence of slight vertical bone loss in relation to mesial aspect of the alveolar crest of upper right lateral incisor. (Fig,4) However the sound periodontium was observed in relation to the rest of the root surface area. The right lateral incisor gave positive response with the vitality test.



Figure 4. Postoperative radiograph

Three months later full fixed appliance was provided to correct the remaining local dental irregularities and to camouflage the underlying mild Class III skeletal discrepancy. (Fig.5) Before commence fixed appliance treatment, upper right lateral incisor was recontoured with composite to compensate the abnormal morphology. Esthetically as well as functionally stable occlusion was achieved following 23 months of total treatment period. (Fig.6) Upper and lower bonded retainers were fitted from canine to canine to maintain class III correction.



Figure 5. Fixed orthodontic appliance during treatment



Figure 6. Postoperative photograph: labial view

Patient was reviewed for any occlusal changes in six months intervals for three years until completion of the growth. In addition, special attention was given for the assessment of the periodontal health and vitality of the right upper lateral incisor during periodic assessments.

Discussion

Several treatment options have been discussed in literature depending on the morphology and functional impairments of fused and geminated teeth⁹. However prior to treatment planning it is important to differentiate fusion from gemination because it is often confusing. However these two conditions can be differentiated with the help of following parameters¹⁰.

Gemination results in mirror images of the coronal halves, whereas fusion takes place at an

angle causing a crooked appearance.

Pulp anatomy is an important guidance in diagnosis of these two conditions. Fused teeth would mostly have two independent endodontic systems but geminated teeth usually have one big pulp canal.

In relation to location of the jaw, fusion is common in mandible and gemination in maxilla but fusion between supernumerary and normal tooth is more common in maxilla.

If two regular teeth have fused, the resulting dental structure occupies less space than two single teeth, which may give rise to diastema and loss of proximal contact. If regular tooth and a supernumerary tooth fuse, crowding or even impaction of neighboring tooth may result. Usually geminated teeth give rise to crowding of that region of the arch. Therefore it is hard to differentiate fusion from gemination if the supernumerary tooth bud is fused to a normal tooth¹¹.

Fusion is counted as one tooth and thus diminishes the number of teeth whereas number is increased in gemination. According to Mader, “Two Teeth Rule” may be useful in differentiating fusion from gemination. If the resulting dental structure is counted as two teeth and normal number of teeth are present in the dentition, that probably represents fusion. If the abnormal dental structure is counted as two teeth and if an additional tooth presence in the dentition that may be a gemination or fusion between a normal and a supernumerary tooth.

However diagnosis of fusion is based on the history of patient as well as on clinical and radiographic findings. Rani (2010)¹² reported that they have used spiral computed tomography as a diagnostic tool for better understanding of the complicated root canal morphology of the fused teeth.

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Different treatment approaches have been practiced by different clinicians for fused teeth and those methods include selective grinding, surgical separation or extraction of the tooth. Stillwell and Coke (1986)¹³ suggested separation of fused teeth and aesthetic restorations when they are retained in the mouth. Clem and Natkin(1966)¹⁴ recommended the removal of one part of fused teeth due to aesthetic, periodontal and orthodontic problems. Some clinicians have indicated endodontic treatment for the remaining tooth following surgical separation. Olsen and Johnston (2002) reported two cases with successful management with a technique of using guided tissue regeneration to promote periodontal healing following a removal of a supernumerary tooth fused to a permanent maxillary lateral incisor¹⁵.

In this case report a fusion between a maxillary lateral incisor and a supernumerary tooth was successfully managed with surgical separation and removal of supernumerary component of the fused tooth while maintaining vitality of the remaining lateral incisor.

Only few case reports have described post operative complications such as hypersensitivity, devitalisation of the retained tooth, ankylosis, external and replacement resorption¹⁶.

The present case was reviewed periodically for 3 years in every 6 months. It is useful to report that the right lateral incisor is maintaining its vitality without any evidence of periapical pathology or external root resorption. However we hope to follow this case for at least another 5-6 years or more depending on the availability of the patient.

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Unpublished article

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Books and other monographs

Pindborg JJ Atlas of diseases of the oral mucosa. 5th edition. Copenhagen: Munksgaard, 1992: 50-66.

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Boyde A. Amelogenesis and the structure of enamel. In: Cohen B, Kramer KH (eds). *Scientific Foundations of Dentistry*. William Heinemann Medical Books Ltd. London. 1976: 335-352.

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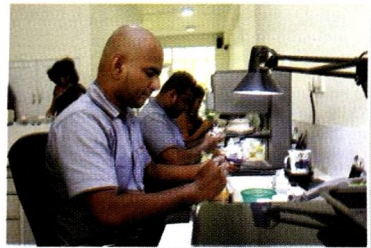
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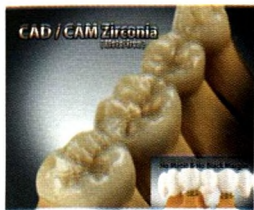
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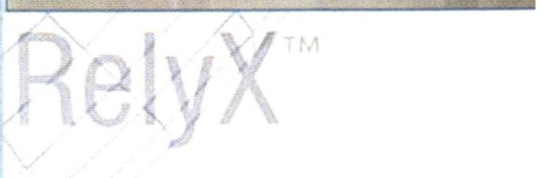
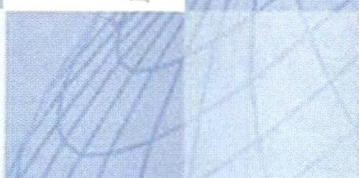
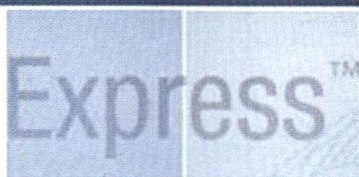
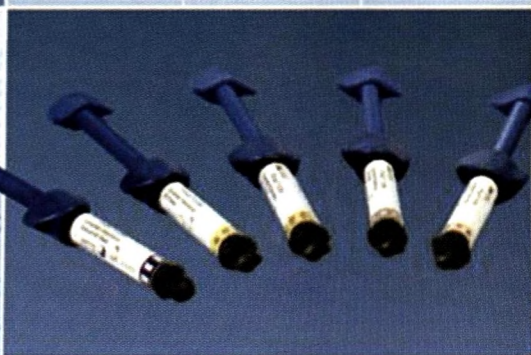
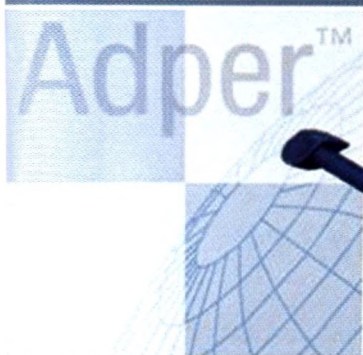
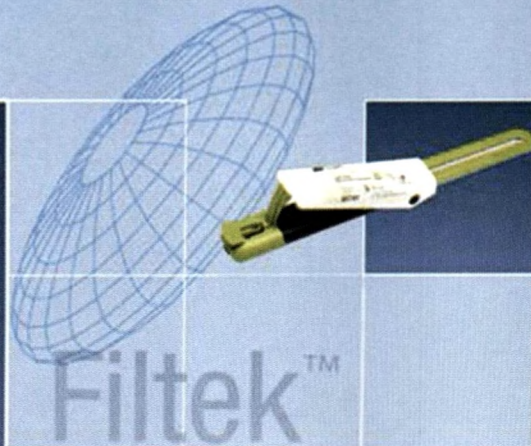
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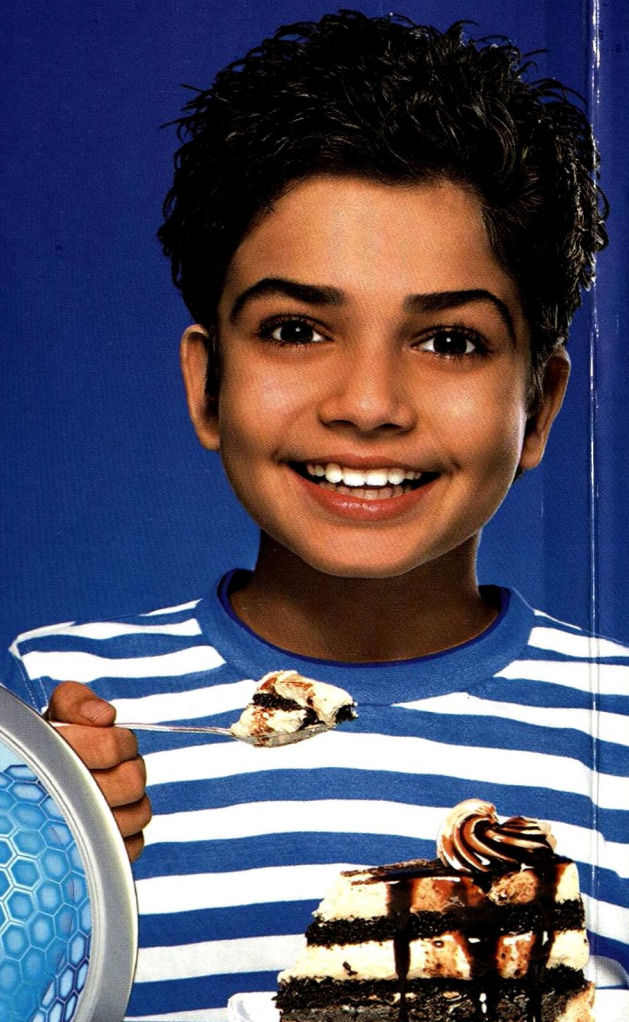
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