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Contents

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* The Journal Publishes original articles, case reports and review articles related to Dental/Medical science. The style of the papers should be in the modified Vancouver style (Ref. New England Journal Medicine 1997; 336:309-15).

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4. Jarett RJ. Insulin and hypertension (Letter). Lancet 1987; 2: 748- 749
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Editorial

It is a pleasure for us to announce that we, the teachers, students, administration and well-wishers of Sapporo Dental College, Dhaka, have crossed yet another milestone by fulfilling one of our most cherished dreams, that is to say, by publishing our own journal. The name of the journal is "Journal of Contemporary Dental Sciences (JCDS)". The journal is expected to attract a significant readership both nationally and internationally and will be contributed by authors from home and abroad.

The most amazing thing of the 21st century is that we are now in the era of sophisticated information technology. The whole world is now only a click away from any PC. The ultimate objective of any education and research is to publish and share them globally. In this manner we can disseminate our knowledge and research findings to the informatics streamline.

This journal will be published twice a year in the month of January and July. The papers to be accepted for publication in this journal will be related to the basic and clinical subjects of dentistry, and of other disciplines which may be pertinent thereof.

This issue of JCDS contains original articles one of which evaluated the effect of cycling pH change on biofilm-induced demineralization of enamel and dentin, a case report of "An unusual presentation of Bell's Palsy", a paper on color-coded pH status of plaque and its association with 'deft' in a pediatric population, a presentation of clinical technique on posterior resin composite, a cross-sectional study regarding dental caries in slum population, and a few more contemporary topics in dental sciences.

It is my earnest request to my colleagues and friends to enrich the journal by valuable contribution of their original articles, clinical studies, critical reviews, laboratory technique and relevant case reports.

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Original Article

Effect of cyclic pH change on (multi-species) biofilm induced enamel demineralization; an assessment by Quantitative Light-induced Fluorescence (QLF)

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Abstract

Purpose: *The aim of this study was to assess the effect of cyclic pH change on multibacterial biofilm induced human enamel demineralization by using Quantitative light-induced fluorescence (QLF).* ***Methods:*** *Square shaped unpolished human enamel coupons were prepared to form artificial biofilms by using freshly cultured Lactobacilli casei, Streptococcus mutans, Streptococcus mitis, Streptococcus gordonii suspended in phosphate buffered saline (PBS), heart infusion(HI) with 1% sucrose and PBS for 20, 30, 40 hrs at 37°C inside a Oral Biofilm Reactor (OBR). Hanks balanced salt solution (HBSS, pH=8) was used to raise the pH in experimental group (HBSS group). The demineralization of enamel coupons from both groups were quantified by a QLF device (InspektorTM, The Netherlands) after the time intervals as mentioned above. The data were analyzed by one-way ANOVA and Dunnett T3 test (p>0.05).* ***Results:*** *Dunnett T3 test revealed significantly more demineralization depth (△F) after 40 hours in the PBS group (-10.79±1.52) compared to 20 (-6.48±3.1), 30 (-7.57±.56) and 40 hours △F of experimental group (-9.12±1.22). In case of demineralization volume (△Q), 30 hours PBS group (-6.67±7.02) had significant differences from 20 hours experimental group (-.95±.53), whereas 20 hours PBS group (-1.12±.89) showed significant differences from both 30 hours (-5.26±4.69) and 40 hours experimental (-10.03±5.51) groups.* ***Conclusion:*** *Lesion volume, rather than demineralization depth, was affected more in the process of enamel lesion induction by multi-bacterial biofilms due to cyclic pH change.*

Keywords: White enamel lesions, cyclic pH change, biofilm, Quantitative light-induced fluorescence

(J Cont Dent Sci 2013; 1(1): 1-5)

Introduction

White enamel lesions are the first clinical expression of demineralization occurring on the surface of enamel, resulting from successive pH alterations in the tooth-biofilm interface provoked by bacterial metabolism. The lesion is clinically characterized by a whitish color and by a rough and opaque appearance.[1] This opacity is perceived through the optical phenomenon occurring when the tooth is dried, with increased porosity of enamel resulting from mineral loss and from the subsurface layer generating light dispersion and loss of the normal translucence of the enamel. A biofilm is an assemblage of microbial cells that is irreversibly associated with a surface and enclosed in a matrix of primarily polysaccharide material.[2] Dental plaque is also a biofilm that coats tooth surfaces.

Due to the advances in the studies of cariology at the present time, the cariologist and clinicians are immensely concerned about the detection of very early carious lesions of enamel that can be reversed or inactivated with minimal interventions or by the modification of the oral environment. The diagnosis of such early lesions is also one of the major research concerns. Quantitative light-induced fluorescence (QLF), designed for clinical application, is a promising method meeting these requirements if it is adaptable to standardized in vitro application.[3]

Several studies showed that QLF is a dental diagnostic tool for both in-vivo and in-vitro quantitative assessment of not only tooth structure mineral loss and gain but also dental

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plaque, bacterial activity, calculus, staining and tooth whitening.[3] In this optical method, demineralized enamel will fluorescence less and this loss of fluorescence can be detected, quantified and longitudinally monitored using QLF.[4]

In recent years, in vitro and in situ biofilm models have gained considerable attention to study the pathogenic mechanisms and to act as aids in the development of efficient antimicrobials or plaque-modifying agents for use as ingredients in caries-preventive oral care products. The pH is an important parameter in oral microbial ecology. With the pH-cycling procedure, the artificial enamel lesions been cycled between a demineralizing and a remineralizing solutions to observe the mineral loss during demineralization, and mineral uptake during remineralization in several in vitro studies.[4]

The conditional change in biofilm environment thought to have effect on tooth demineralization, and quantitative assessment records are rare. Therefore, the aim of this study was to assess the effect of cyclic pH change on multibacterial biofilm induced human enamel de-mineralization by using QLF.

Materials and methods

Tooth Sample preparation

Enamel coupons (EC), 4 4 2 mm^3 in size, were cut from the buccal surface of extracted human premolars and third molars. EC were cut by a diamond saw (Isomet, Buehler, Lake Bluff, IL, USA). Surface of the EC were covered with paraffin wax (GC. 1.4 mm) and 2 mm window was prepared to expose enamel surface at the center of each EC.

Preparation of bacterial suspension

Suspension of S. mutans MT8148, Streptococcus mitis 903, Streptococcus gordonii DL1 in phosphate buffered saline (PBS) at OD500=4.0 (approximately 4.0×10^7 colony-forming units/ml) were prepared separately from a 16 hours fresh culture in Brain Heart Infusion broth (BHI, Becton Dickinson, Sparks, MD, USA) after washing three times with PBS. Lactobacilli casei IAM12473 was also prepared in PBS from 40 hours fresh culture in MRS broth (Becton Dickinson, Sparks, MD, USA). All suspensions were stored at 4^0 C with continuous gentle stirring until to be used. For nutrition of the cultivating bacterium, a solution of Heart Infusion broth (HI, Becton Dickinson, Sparks, MD, USA) with sucrose (1% final concentration) was prepared.

Specimen assembling and biofilms formation in Oral Biofilm Reactor (OBR)

The bacteria were subjected to grow to form a biofilm on surfaces of ECs placed inside of a water jacket-encircled chamber of the OBR (Fig.1). ECs were positioned on the two

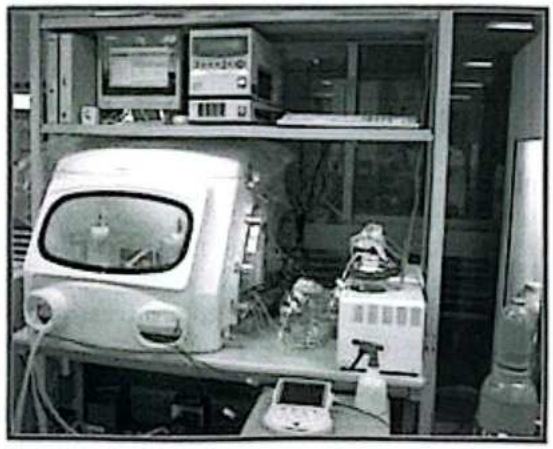

Fig.1: The OBR.

holders around the flat bulb pH electrode using red utility wax (GC, Tokyo, Japan). Therefore, one side of the surface of the respective EC was subjected for biofilm attachment. The open surfaces of ECs were kept horizontal. A holder bearing the ECs was set on a silicon plug, at the bottom of the chamber. Pooled sterile human saliva was then poured onto the coupons, followed by incubation for 30 minutes in order to obtain a coat of salivary pellicle on the EC surface. The top of the chamber was sealed with a silicon plug so that the chamber itself served as an incubator with a 37^0C of inside temperature. The top silicon plug was equipped with five stainless steel tubes (21gauge) which were respectively connected via silicon tubes to computer controlled roller pumps (EYELA EPC-2000, Tokyo Rika, Tokyo, Japan).The tubes were allotted to supply either one of the solutions; one tube for the bacterial suspension,

two for HI with sucrose and the other two for PBS (in control group) or HBSS (experimental group). All of these solutions were pumped into the chambers at 6 ml per hour so that a solution is persistently supplied to the center of the EC surface. In experimental group, Hanks' balanced salt solution [HBSS {KCl, KH2PO4, NaCl, NaHPO4.7H2O, NaHCO3, CaCl2, MgSO4.7H2O, MgCl2.7H2O (Sigma-Aldrich Co. UK)} (pH=8.0)] was also dropped with other mediums and suspension on enamel coupons in a cyclic order:
8-hours without HBSS=2-hours with HBSS [cyclic order 4 times]

All of these solutions formed a water dome on the surface of the holder which was continuously stirred by falling drops. When the water dome reached the maximum height, the mixture of excess liquid fell down from the edge of the holder. Two chambers were used at the same time to make the experiment in a stable condition. During the experiment, pH on the surface of the holder was continuously monitored to maintain a suitable condition for biofilm formation by a pH meter (Horiba, Kyoto, Japan). After 20, 30 and 40 hours, each EC with artificial biofilms were washed with PBS and the wax was carefully removed with knife and finally washed three times (PBS). Then the specimens were visually examined and digital pictures were taken.

Analysis of QLF images

After washing of the samples with PBS and dried with Kim wipe, analysis of the lesion was first conducted by visual inspection. Then the second analysis was done by QLF, using the QLF software version 2.00 (Inspector Research Systems BV, Amsterdam, Netherlands). The absolute decrease in fluorescence is expressed in the value △F. The program also calculates the area of lesion in mm^2 and from this can calculate △Q. QLF images and white-light digital photographs (Fig.2) were taken by using a standard tooth block position in relation to the mounted camera. For one tooth sample including sound enamel, four images were taken. For both control and experimental regions data were recorded form △F and △Q value (Fig 6).

Fig.2: An enamel coupon after 40 hours in OBR

Statistical analysis

All numerical data were analyzed using the Statistical Package for the Medical Science (SPSS Ver.15 for Windows) for statistical procedures. The data were analyzed by one-way ANOVA and Dunnett T3test ($p>0.05$).

Results

The one-way ANOVA showed that severity of demineralization occurred with the increase of time interval. Dunnett T3 test revealed significantly more demineralization depth (△F, Fig 3) after 40 hours in the PBS group (-10.79±1.52) compared to 40 hours △F of

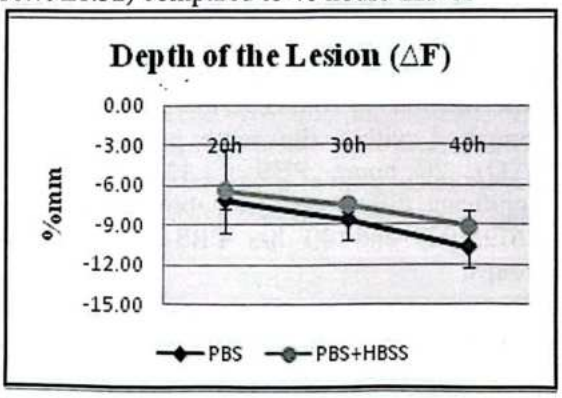

Fig.3: Depth of the lesion after 20,30and 40 hours in both groups (Control-PBS, Experimental-PBS+HBSS).

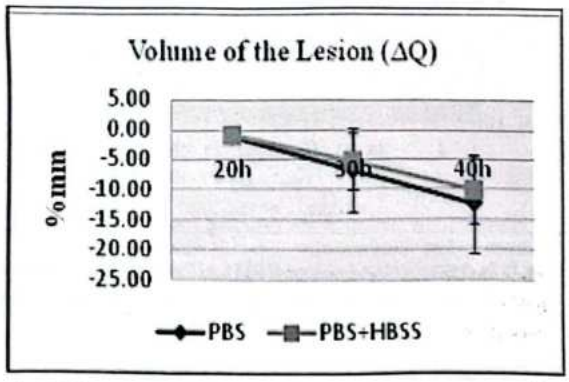

Fig.4: Volume of the lesion after 20,30and 40 hours in both groups (Control-PBS, Experimental-PBS+HBSS).

experimental group (-9.12±1.22). It also showed [40 hours (△F)] significant differences from both 30 hours (-7.57±.56) and 20 hours

(-6.48±3.1) experimental group. 30 hours PBS group (-8.69±1.5) had significantly higher ΔF from 30 and 40 hours ΔF of experimental groups (-7.57±.56, -6.48±3.1). 20 hours ΔF of PBS group (-7.16±.68) showed only significant differences from 40 hours ΔF of experimental group (-9.12±1.22).When compared within the same groups of PBS (ΔF), all (20, 30 and 40 hours) of them showed significant differences from each other. But 20 and 30 hours (ΔF) experimental groups showed only significant differences from 40 hours group when compared within the same groups.

In case of demineralization volume (ΔQ, Fig 4), the 40 hours PBS group (-12.29±8.08) showed no significant differences from 40 hours experimental group (-10.03±5.51). 30 hours PBS group (-6.67±7.02) had significant differences from 20 hours experimental group (-.95±.53), whereas 20 hours PBS group (-1.12±.89) showed significant differences from both 30 hours (-5.26±4.69) and 40 hours experimental (-10.03±5.51) groups. When compared within the same groups of PBS (ΔQ), 20 hours PBS (-1.12±.89) showed significant differences from both 30 hours (-6.67±7.02) and 40 hrs PBS (-12.29±8.08) groups.

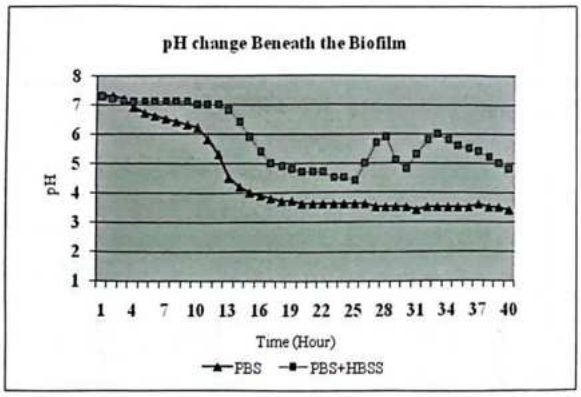

Fig.5: In control group (without HBSS), pH gradually fall down to below 4 after 15hrs and very slowly reached to 3.5 at the end without showing any rise.

But 30 hours and 40 hours PBS have no significant differences from each other. But all experimental (ΔQ) groups (20, 30 and 40 hours) showed significant differences from each other. The pH changes beneath the biofilm showed in the Fig 5. In control group (PBS), pH gradually fall down to below 4 after 15 hours and very slowly reached to 3.5 at the end (40 hours) without showing any rise. But in the experimental group (HBSS), pH fall below 5 around 20 hours but again went up (about 6) around 28 and 34 hours.

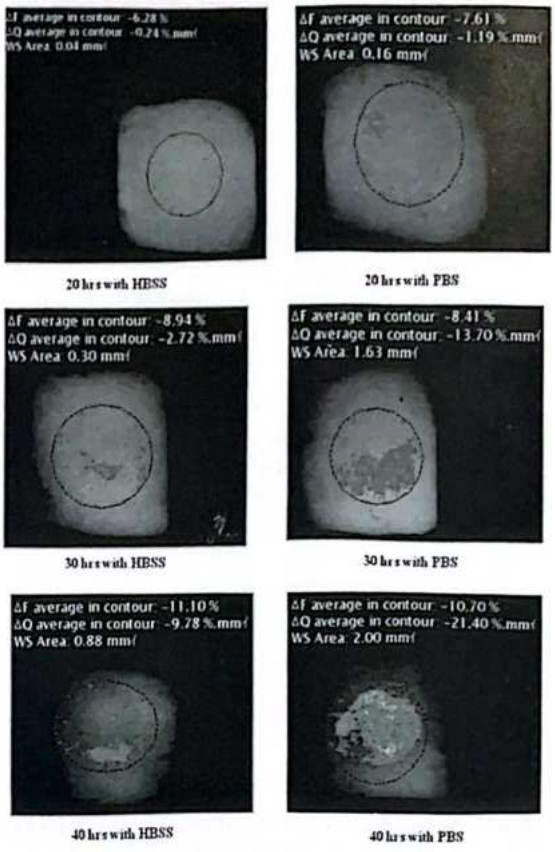

Fig. 6: QLF images of the enamel lesions.

Discussion

Dental biofilm is an organized microbiologic community enclosed in a matrix of extracellular material and attached to dental surfaces.[5] Biofilm models are important tools to evaluate the biochemical and microbiological composition of biofilm formed under different conditions or the changes caused on the substratum surface on which the biofilm is attached. Therefore, the conditions of biofilm formation and the substratum used must be as close as possible to those of real life.[6] Most of the biofilm studies to analyze demineralization use acidic environment though the oral condition is not constantly acidic because of the food, beverages, saliva and other factors. In

addition, the oral environment is a habitat with constant changes in conditions, e.g., nutrient limitation, saliva flow, and oxygen availability. Members of the oral biofilm have evolved to cope with change and compete with other oral bacteria for this ecological niche. To get close to the oral condition, HBSS (pH=8) was used in this study in every 2 hours in the 20, 30 and 40 hours cycles. Such kind of conditional change in the biofilm environment showed significant differences especially $\triangle F$ of experimental group from control (PBS). That is, a lesser amount of demineralization depth in HBSS group. But the lesion volume ($\triangle Q$) showed less significant differences in between the groups.

Dental biofilms harbor cariogenic bacteria are both acidogenic (that is, produce organic acids) and aciduric (that is, can survive in acidic environments). Cariogenic bacteria include S. mutans, Streptococcus sobrinus, Lactobacillus species and Actinomyces species, as well as to a lesser extent Streptococcus mitis, Streptococcus oralis, Streptococcus gordonii and Streptococcus anginosus.[7,8] Perhaps as a response to environmental challenges, the oral biofilm community evolved with individual members assuming specialized functions, (e.g., primary and secondary colonizers) including members that can metabolize excreted products (such as lactic acid) produced by other species.[9] Considering all these factors, multibacterial biofilm were grown on the ECs inside the OBR in the present study.

The technique QLF is based on the auto fluorescence of teeth, which when illuminated with visible blue light (405 nm), emit in the green part of the spectrum.[10] If a sound tooth is illuminated with blue light of a specific wavelength, the tooth starts to fluoresce: substances in the tooth, called fluophores, absorb the blue light and re-emit it as green light. This is also called auto-fluorescence of the tooth. Using a yellow filter, the blue illuminating light can be filtered away so only the green fluorescence remains. A demineralized area is less transparent for the blue as well as the green light compared to the sound enamel. Early lesions are seen as dark spots on a green background with QLF. In normal conditions, when viewed with white light, such areas look white hence they are often called 'white spots'. Even if white spots can be spotted with the naked eye, the contrast between sound enamel and early lesions is much higher with QLF making such lesions much easier to detect. Because the illuminating light is filtered out there are no reflections, resulting in images that are much easier to interpret and to analyze. The software makes it possible to capture in-vivo images of the tooth elements in the computer. Live images are displayed in real-time on the monitor screen of the computer. The most important parameters produced by the software are: Lesion Area(Area in mm2), Lesion depth expressed in percentual fluorescence loss (DF in %), Lesion volume (DQ in mm2%). By early and accurate identification of enamel lesion, preventive therapies and minimally invasive restorative treatments are possible to provide.

QLF makes it possible to follow the emergence and the healing of early lesions with unparalleled detail. By this technology, the white enamel lesions on the ECs produced by OBR were analyzed and it is once again proved that QLF is a suitable tool to analyze the enamel demineralization.

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Original Article

Chemotherapy response of (primary) head and neck carcinomas in relation to Flow Cytometric and Histopathological parameters.

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Abstract

Purpose: *The relationships of chemotherapy response with flow cytometric results and histopathological parameters were evaluated in 55 patients with head and neck carcinoma.* ***Methods:*** *The presence of metastasis was confirmed histologically in 31 lymph nodes of 23 patients. One group of patients was treated with bleomycin and methotrexate and the other groups received a variety of combination of bleomycin, methotrexate, cisplatin and 5-fluorouracil.* ***Results:*** *Sixty four percent of tumors had aneuploid cell populations. Clinically positive response was observed in 67% of the population. Both clinically and histologically primary lesions with diploid cell population, lower S phase fraction, and higher degree of differentiation showed a tendency to better response than their counterparts.* ***Conclusion:*** *The response of primary lesion to chemotherapy was demonstrated in this experiment. Response of metastatic lesion to chemotherapy will be reported gradually.*

Key Words: Chemotherapy, DNA Ploidy, Metastasis, Squamous cell carcinoma

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Introduction

Drug resistance is a major factor in limiting the effectiveness of cancer chemotherapy. There are different kinds of chemotherapy used in the treatment of head and neck cancer. Different anti neoplastic agent show different responses. Moreover, the effectiveness of chemotherapy varies from tumor to tumor. There are some tumors which show less response to chemotherapy from the beginning, while some drug resistance tumor develop resistance to chemotherapy during the course of treatment. Several authors[1,2] have shown that patients with complete clinical response will have a better outcome than those with no or partial response.

Cellular DNA can be measured by Flow Cytometric (FCM). It has been shown in many studies[3,4] that DNA content has been predictive of prognosis. In general, patients with Diploid tumors survive longer than patients with aneuploid tumors. The pre treatment assessment of tumors to chemotherapy is important. DNA analysis by FCM may play an important role in this respect. A meticulously done study[1] did not find correlation between chemotherapy response and DNA content, while another study[5] reported greater response to chemotherapy in diploid tumors than in aneuploid tumors. In those studies selection of patients who would respond to chemotherapy by FCM analysis were not well established because of variation in the chemotherapy regimen used and in the histology of tumors analyzed. So the purpose of this study was to see the influence of cellular DNA content, S phase fraction and histopathological parameters on clinical and histological response to chemotherapy.

Materials and methods

This research study was carried out in First Dept. of Oral and Maxillofacial Surgery. Niigata University, Japan. The effects of induction chemotherapy were evaluated in 55 patients with squamous cell carcinoma (SCC) of the head

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and neck. At the same time FCM was performed on fresh tissue samples of SCC, which were located at the gingiva (18), tongue (13), floor of mouth (9), oropharynx (8), cheek (4), and maxillary sinus (3). The presence of metastasis was confirmed histologically in 31 lymph nodes of 23 patients (Fig. 1). A part of each sample was submitted to conventional histological examination by hematoxylin-eosin staining. The degree of differentiation was graded histologically as well, moderate, and poor. The histological grade of malignancy was classified as grade 1 to 3 according to a modified classification described by Jakobsson et al.[6] by evaluating 4 parameters of the tumor cell population (structure, keratinization , nuclear polymorphism and mitosis) and 2 parameters of the tumor host relationship (mode of invasion and cellular response).

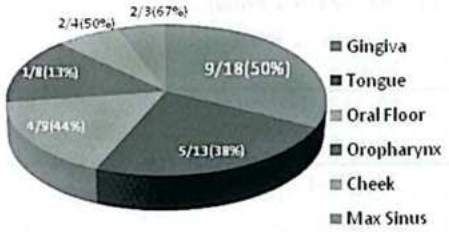

Fig.1: Primary site and incidence of Metastasis (Percentage in parenthesis indicate metastasis)

Thirty patients received a single course of bleomycin and methotrexate and the other patients were treated with two courses of a variety combination of bleomycin(BLM), methotrexate (MTX), cisplatinum(CDDP) and 5-fluorouracil(5-FU)(Fig. 2).

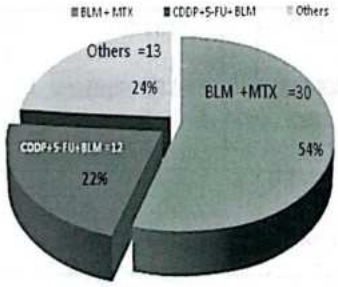

Fig 2: Types of Chemotherapy used

The response to the chemotherapy was assessed 4 weeks after the start of chemotherapy. Complete response (CR) was defined as a complete disappearance for all measurable disease. Partial response (PR) was defined as a reduction of more than 50% in the lesion diameter with no demonstrable disease progression elsewhere. No response (NR) was a response less than PR, stable disease or progression of the tumor while on chemotherapy. Those showing CR or PR to the induction chemotherapy were considered positive responders. The response was assessed separately for primary tumors and metastatic lymph nodes.

The histological response was assessed according to the system devised by Shimosato et al.[7] (Table 1). All surgical specimens were histologically studied on serial sections. It was regarded positive when there was Grade 3 or 4 responses.

For FCM analysis, fresh tissue samples obtained by biopsy or surgery were immediately transferred into a physiological saline solution at 40C. Single cell suspensions were prepared by a modification of the method described by Peterson[8]. The method for FCM analysis is described in detail in previous papers.[9]

The cell cycle analysis was performed by the program introduced by Kosugi et al.[10] DNA ploidy was determined by the DNA index (the ratio of the modal DNA value of tumor cells to that of the normal lymphocytes) which was calculated as follows:

$$\text{DNA Index} = \frac{\text{Channel no. of G0/G1 peak tumour cells}}{\text{Channel no, of CRBC cells x 2.92}}$$

Diploid tumors were defined as those having a DNA index in the range from 0.95 to 1.05 (mean ± 3SD of normal tissue samples).
For statistical evaluation, the X^2 distribution test or student t test were used.

Results

Response of primary lesions to Chemotherapy

On flow cytometric analysis, 35 tumors (64%) had aneuploid cell populations and the remaining 20 tumors (36%) had diploid cell populations. The mean valus of DNA index and S phase fraction for the aneuploid tumors were 1.48 and 22.7% respectively. The corresponding values for the diploid tumors were 1.0 and

Table 1: Histological grading for evaluation of response to chemotherapy [7]

Grade 1. Characteristic changes are noted in tumor cells but tumor structures have not been destroyed (there is no defects in tumor nests resulting from lysis of individual tumor cells).

Grade 2. In Addition to characteristic cellular changes, tumor structures have been destroyed as a result of disappearance of tumor cells . However, "variable cell" still remain.

Grade 3. Markedly altered, presumably no "viable tumor cells" are present singly or in small clusters and "viable cells" are hardly seen.

Grade 4. No tumor cells remain in any of sections (local cure).

Table 2: DNA Index, S Phase % of primary lesions

DNA Ploidy	No. of Tumors	DNA Index (mean ± SD)		S Phase% (mean ± SD)	
Aneuploid	35	1.48±0.47	**	22.69±10.52	**
Diploid	20	1.0±1.02		15.28±6.63	

**p<0.01

Table 3: Clinical & Histologic response of primary Lesions in terms of DNA Ploidy

Ploidy	No. of Tumors	Clinical Response CR+PR	NR	Histologic Response 3+4	1+2
Aneploid	35	22(63%)	13(37%)	12(34%)	23(66%)
Diploid	20	15(75%)	5(25%)	10(50%)	10(50%)
Total		37(67%)	18(33%)	22(40%)	33(60%)

Table 4: Clinical & Histologic response of primary lesions in terms of S phase fraction.

Clinical Response	S Phase % (mean± SD)	Histologic Response	S Phase % (mean±SD)	
CR+PR	19.4±9.7	3+4	17.1±8.1	*
NR	21.1±10.3	1+2	21.9±10.6	

*p<0.05

Table 5: Clinical & Histologic response of primary lesions in terms of Differentiation.

Differentiation	No. of Tumors	Clinical Response CR+PR	NR	Histologic Response 3+4		1+2
Well	16	11(69%)	5(31%)	11(69%)	**	5(31%)
Moderate	26	19(73%)	7(27%)	8(31%)		**18(69%)
Poor	13	7(53%)	6(46%)	3(23%)		10(77%)

**<p0.01

Table 6: Clinical and Histologic response of primary lesions in terms of Malignancy Grade.

Grade	No. of Tumors	Clinical Response CR+PR	NR	Histologic Response 3+4	1+2
Grade 1	9	5(56%)	4(44%)	5(56%)	4(44%)
Grade 2	35	26(74%)	9(26%)	13(37%)	22(63%)
Grade 3	11	6(55%)	5(45%)	4(36%)	7(64%)

15.3%, respectively, which were significantly lower as compared to those of the aneuploid tumors (Table 2).

Clinically, positive response was seen in 67% of the population. The diploid tumors (75%) showed a better response than aneuploid tumors (63%) (Table 3). Histological response was also better in the diploid tumors (50%) than in the aneuploid tumors (34%) (Table 3). Statistically, however, these values were not significantly different. The mean S phase fractions were lower both clinically and histologically in the responders (19.4% & 17.1%) than in the non responders (21.1% & 21.9%). There was significant difference in the histologic response between the two groups (Table 4).

In terms of differentiation, there were 16 well, 26 moderately and 13 poorly differentiated tumors. Clinically the poorly differentiated tumors (53%) were significantly less sensitive than the moderately differentiated tumors (73%) and the well differentiated tumors (69%) (Table 5). Histologically, positive response was seen in 69%, 31% and 23% of the well, moderately and poorly differentiated tumors, respectively (Table 5). The values for the well differentiated tumors were statistically different from those for the two other groups. Of 9 grade 1, 35 grade 2, and 11 grade 3 tumors , the grade 2 tumors (74%) showed the highest clinical response, whereas the grade 1 tumors (56%) was most sensitive on histological evaluation (Table 6). Statistically, however, significant difference was not noted between any of these values.

Discussion

Induction chemotherapy is well accepted in the management of head and neck carcinomas, because it may improve curability by reducing tumor size before surgery. Another possible advantage is to eliminate microscopic metastatic disease early on. A study by Kokal et al.[11] reported that tumor DNA content give more accurate biological characteristic of solid tumors than can be obtained from routine clinical and histological examinations. Tumor DNA analysis, therefore, may have an important role in the selection of patients for chemotherapy.

Ensley *et al.*[12] in their study reported 79% of the patients with squamous cell carcinoma of the head and neck achieving a CR of aneuploid tumors, whereas the CR rate was only 21% in patients with diploid tumor treated by cisplatinum and radiation. Campbell *et al.* however, did not find any correlation between DNA content and response of head and neck squmous cell carcinomas to induction chemotherapy by nearly similar drugs used in our study, whereas *Masters et al.*[5] reported that response of breast carcinomas to either adriamycin alone or with vincristin was better in diploid tumors (69%) than in the aneuploid tumors (45%). In our study, although there was no statistical difference, diploid tumors tended to respond better to chemotherapy than aneuploid tumors. One possible explanation would be that the chance of evolution of drug resistance cell lines is higher in aneuploid tumors than diploid tumors because the former is consists of more heterogenous cell subpopulations than the latter. According to Chen,[9] the incidence of aneuploidy increased with the decrease of differentiation and the increase of malignancy grade and this finding were analogous with the current study. The drugs used in this study are known to be less effective to tumor of poor differentiation showing diffuse invasion.[13,14]

Histologically, the poorly and moderately differentiated tumors were less sensitive than the well differentiated tumors in our study. The lower incidence of well differentiated tumors may be another possible reason for the lower drug sensitivity of aneuploid tumors. Obviously, response of tumor to chemotherapy depends on the histology, location and size of the tumors as well as the regimen of chemotherapy. Thus, variation of these factors may be responsible for the conflicting results of tumor sensitivity to chemotherapy in terms of DNA content in the literature. Because the chemotherapy in this study was performed as an induction following surgery, a relative short term of drug administration would have been another factor to have made it difficult to obtain definitive results. It may be unlikely; however, that DNA content of tumor cells is a single decisive factor to influence their sensitivity to anticancer drugs.

According to *Masters et al.*[5] sensitivity of breast carcinomas to chemotherapy was not different among tumors with high, moderate and low S phase fractions. In an analysis of 55 primary oropharyngeal squmous cell carcinomas, however, *Feicher et al.*[15] reported that the mean S phase fraction was lower in the CR group than in the PR group, whereas non responders consisted of two groups with higher and lower S phase fractions than that of the CR group. Although cisplatinum is not phase specific, the other drugs used in our study are active on cells in the S phase. Contrary to our expectations, the mean S phase fraction was significantly higher in non responders as compared to responders on histological evaluation. The higher rates of replication of resistance clones than those inactivated by chemotherapy and /or of spontaneous mutation to resistance clones in rapidly growing tumors may be a possible reason for the lack of response of tumors with a high S phase fraction. We only reported results with primary head and neck carcinoma. Results with metastatic lesions will be presented in a future issue.

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Original Article

Caries prevalence among Bangladeshi slum population: a cross-sectional study

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Abstract

Purpose: *The purpose of the study was to explore dental caries status and current trends among the slum dwellers in Tongi, Bangladesh.* ***Methods:*** *A cross-sectional survey using a cluster sampling method was carried out among three thousand nine hundred and four (N=3904) slum dwellers. A close-ended questionnaire using DMFT index ((Decayed, Missing and Filled Teeth) was applied over slum dwellers of all age groups and sexes. Clinical examinations were carried out in different slum settings including slum schools by trained and calibrated examiners.* ***Results:*** *The mean decayed (D) component of the DMFT was considerably higher than filling (F) and missing (M) components while missing component was more than filled part. Both decayed and missing components increased where as filling components decreased as the age progressed (P<0.001). The survey revealed high caries prevalence among Tongi slum dwellers.* ***Conclusion:*** *It was suggested that a comprehensive preventive and interventional program based on the concept of health promotion, motivation and community participation was needed for these underprivileged slum dwellers along with continuous follow up and monitoring system in place.*

Key words: DMFT, slum cluster, caries prevalence ***(J Cont Dent Sci 2013;1(1):11-14)***

Introduction

Bangladesh is a densely populated country in South-Asian region with a population of about 140 million people.[1] Although the majority (>75%) of her people reside in the rural areas, internally population density is highest in urban, followed by peri-urban and rural areas.[2] According to UN-HABITAT (2006) report 79% of these urban populations live in slums in Bangladesh.[2] The slum is usually inhabited by the very poor and socio-economically disadvantaged population. Slum structures ranges from simple shacks to semi-permanent structures, lacking basic household utilities.[3]

Tongi which is an industrial "suburb" near the capital city Dhaka, has got one of the largest slum in Bangladesh. Most of its inhabitants are migrant population coming from rural areas, who live on low wages and often are underemployed for long time. The households are overcrowded having poor water and sanitation system. About fifty percent of working slum dwellers missed an average of 10 working days in a month because of sickness.[4] Illiteracy rate and chronic malnutrition is high among this population. The health of this underprivileged population is largely neglected because of lack of awareness and low purchasing power.[4]

Oral health is an integral part of general health and well being. Some sporadic studies on oral health identified high caries prevalence with DMFT ranging from 1.0 to 4.7 among both urban and rural population in Bangladesh.[5,6] This indicates high prevalence of oral diseases, particularly dental caries in Bangladesh.

Urban planning to accommodate increasing large slum areas requires study to determine

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demographics and determinants of improved quality of life, such as health status, health services, financial stability, education and security. However, scarcity of basic oral health status data among the slum population is hindering comprehensive oral health planning for this population.

The aim of the present study, therefore, was to explore baseline oral health status of inhabitants of Tongi slums like dental caries data, with an objective to find out oral health problems and their current trends among slum dwellers to plan a comprehensive dental preventive program for future.

Materials and Methods:

This Cross-sectional Survey was conducted in the twelve slum clusters of Tongi Municipality to find out baseline oral health status. The sampling method used in this survey was cluster random sampling technique, which included participants of all age groups and both sexes. The survey had the sample size of 3904 subjects.

A simple close-ended questionnaire was used to collect information on demographics and oral health status like caries, missing and filled teeth status. Standardized caries indicator, the DMFT (Decayed, Missing, and Filled Teeth) index was used in the questionnaire to record the data. The questionnaires were filled up by trained survey personnel following oral examination. Survey proposal was submitted earlier to the Tongi municipality authority to get permission. Verbal consent was taken from the subjects prior to examination and data collection. The purpose of the survey was explained to them beforehand. In addition, subjects were assured about the confidentiality of the collected data.

Survey personnel were trained both theoretically and practically about the accurate data collection procedure before going to the operation area. The training included examination procedures, maintenance of uniform scoring criteria for DMFT and data recording methods during survey. Examiners were calibrated to minimize intra examiner and inter examiner variability.

Data were collected during dental camp. At first twelve clusters of slums were selected randomly. After that, subjects were chosen from each cluster regardless of any particular age group and sex. Following consent from the subjects, detailed oral examination was carried out to measure DMFT score using caries explorer, tweezers, cotton rolls and pellets, headlamps, gloves, masks, antiseptic solution. All instruments were cleaned with soap and water before disinfecting in antiseptic solution. All subjects were positioned upright and examined by using headlight/torch light and other necessary dental instruments. Aseptic precautions were taken during each examination. However, subjects requiring emergency dental care were referred to nearby hospital.

Data gathered from the Survey were entered into the SPSS statistical software, version 12.0. Data analysis include cross tabulation of DMFT in relation to sex and different age groups. Chi-square test, analysis of variance (ANOVA) and 95% confidence interval were used to show the statistical significance.

Results:

This survey was conducted among the people living in the twelve slum areas of Tongi Municipality including all age groups and both sexes. The survey had the sample size of 3904. Among the sample analyzed majority of the survey participants were female (59.5%) in comparison to male (40.5%). Young and early adult age group (from 11 to 40 years) is the predominant proportion of the sample, which is about seventy-two percent of the total sam-ples. On the other hand, older population (over 60 years) was a minority group in the survey.

Mean DMFT of the surveyed sample according to the gender and age are illustrated in Table 1 and 2. Mean DMFT of the sample is 3.01 which is higher among male (3.11) in comparison to female (2.94). Mean decayed component of the DMFT is considerably higher than filling and missing component while missing component is more than filled part.

Mean DMFT showed and increasing trend with the progression of the age. However mean DMFT is lowest (1.51) in the 0-11 year's age group and highest (10.61) in the older age group (over 60 years). This difference in mean DMFT according to age is highly statistically significant. Both decayed and missing components are increasing with age which is also statistically significant. On the other hand, filling components decrease as the age progresses.

Table 1: Mean DMFT according to gender

DMFT	Sex	Mean (95% Confidence Interval for Mean)	Standard Deviation
Decayed teeth	Male	2.11(1.97-2.25)	2.90
	Female	2.07(1.96-2.19)	2.81
	Total	2.10(2.01-2.19)	2.84
Missing Teeth	Male	0.98(0.85-1.12)	2.71
	Female	.85(0.74-0.97)	2.85
	Total	.91(0.82-0.99)	2.79
Filled Teeth	Male	.01(0.00-0.02)	0.17
	Female	.02(0.01-0.03)	0.29
	Total	.02(0.01-0.02)	0.25
Decayed, Missing & Filled Teeth	Male	3.11(2.90-3.31)	4.21
	Female	2.94(2.77-3.11)	4.17
	Total	3.01(2.88-3.14)	4.18

Table 2: Mean DMFT according to age

Distribution of age	Decayed teeth*		Missing Teeth*		Filled Teeth		Decayed, Missing & Filled Teeth*	
	Mean (95% Confidence Interval for Mean)	Std. Deviation	Mean (95% Confidence Interval for Mean)	Std. Deviation	Mean (95% Confidence Interval for Mean)	Std. Deviation	Mean (95% Confidence Interval for Mean)	Std. Deviation
0-10 years	1.42 (1.24-1.59)	1.70	.09 (-0.01-0.18)	.94	.01 (-0.01-0.02)	.10	1.51 (1.30-1.72)	2.02
11-20 years	1.44 (1.34-1.55)	1.70	.11 (0.08-0.14)	.47	.01 (0.00-0.01)	.13	1.56 (1.45-1.67)	1.80
21-30 years	1.90 (1.77-2.03)	2.13	.39 (0.32-0.46)	1.11	.01 (0.00-0.02)	.16	2.31 (2.15-2.46)	2.47
31-40 years	2.38 (2.18-2.57)	2.80	1.0 (0.85-1.15)	2.14	.03 (0.01-0.05)	.30	3.41 (3.16-3.67)	3.67
41-50 years	3.06 (2.66-3.46)	4.18	1.91 (1.54-2.29)	3.94	.02 (0.00-0.04)	.22	5.0 (4.45-5.54)	5.68
51-60 years	3.58 (2.79-4.37)	5.37	3.45 (2.64-4.26)	5.52	.06 (-0.05-0.17)	.74	7.08 (5.96-8.21)	7.63
Over 60 years	3.63 (2.70-4.55)	4.87	6.98 (5.53-8.43)	7.62	.00 (0.00-0.00)	.00	10.61 (8.97-12.25)	8.65

*P= 0.000

Discussion:

It is evident that Populations of this slum area were deprived of any basic dental treatment or preventive interventions and no survey was ever done to evaluate their oral health status. However, this survey was the first ever done to assess the oral health condition of the slum dwellers. Overall, mean DMFT is quite high in both male and female and this is almost similar with the national average level (mean DMFT 1.1 to 4.7).[6]

Both decayed and missing components were increased whereas filling components decreased as the age progresses. Moreover, components like filling and missing teeth which are related to the dental treatment are lower than decayed teeth component. This gives us an indication that people in those slum areas do not have any access to any oral treatment and neither have any preventive program to arrest the progression of dental caries. Additionally, this supports the fact that slum dwellers living in deprived condition have high level of dental diseases and poor oral health status.[7-11]

Untreated dental caries leads to eventual loss of teeth. Early loss of teeth causes impaired function of mastication leading to impaired digestion and mal-absorption of nutrients and affects the general health of the individual.[12] However, majority of the Tongi slum dwellers have been suffering from chronic malnutrition which might be due to severe dental diseases identified in this survey.[4]

The poor slum dwellers are in a continuous state of struggle for subsistence. Moreover, if they face serious health problem including oral disease, their existence is further jeopardized. Furthermore, high prevalence of dental diseases and associated pain significantly hamper the quality of life and hinder their daily activities. Eventually, this result in a loss of important working hours of this poor community, who often are unemployed and live from hand to mouth.

A comprehensive preventive and interventional program is needed for these underprivileged slum dwellers for a longer period. These will include successful implementation of oral health promotion, motivation and creation of awareness among the slum dwellers, increase community participation along with continuous follow up and monitoring system in place.

In conclusion, high level of dental caries was identified among Tongi slum dwellers which confirm previously reported data in socially deprived area in other parts of the world. The result of the survey could be used as a mini model and scaled up for other urban and peri-urban slum areas in Bangladesh and all over the world.

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Original Article

Evaluation of plaque pH with Plaque-Check +pH kit (GC Corporation, Japan) in 3-6 years old children with carious primary teeth

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Abstract

Purpose: *Dental plaque would change colour to its corresponding pH when exposed to Plaque-Check+pH solution developed by GC Corporation, Japan, and thus indicate the resting pH of the collected plaque.The objective of the study was to evaluate the pH status of 3-6 years children suffering from early childhood caries (ECC) in their primary teeth.* ***Method:*** *Sixty-two children suffering from a varying number of dental caries participated in the study. Demographic data were collected with a pretested structured questionnaire and a checklist. For each child a clinical examination for deft status (decayed, extracted and filled teeth) and plaque sample collection were carried out after parental consent. The collected plaque sample was allowed to ferment in the Plaque-Check+pH solution for 5 minutes and a change in color was compared with the corresponding pH scale.* ***Result:*** *Majority (79.2%) of the participants demonstrated a resting plaque pH value between 6.0-6.6, indicating a slightly acidic nature of the plaque and eight children had plaque pH ranging between 5.0-5.8. The overall mean deft status of the study population was recorded at 5.3; with 5-6 years old children demonstrating the lowest caries state (mean deft 4.2). The study also revealed only 38.7% children were brushing twice a day and 72.8% participants had been taking extra sugar with milk. Parental assistance during teeth cleaning of the children was also very low (38.7%).* ***Conclusion:*** *As this study provided only with descriptive results it was concluded that further study for statistical significance be carried out for more precise results and patient-parent motivational purposes.*

Key Words: Plaque pH, Plaque check+ pH,

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Introduction

Primary teeth can be affected by dental caries as soon they erupt.[1] Early childhood caries (ECC) is attributed to added sugar in milk, sugary food and drinks, sleeping with dinky bottle, prolonged and unrestricted breast feeding and most importantly failure to clean the teeth regularly and methodically by children as well as lack of parent supervision during teeth cleaning.[1,2] Plaque is one of the most important factors in caries initiation and its progression. Dental plaque is also responsible for periodontal disease such as gingivitis and periodontitis.[3]

The acid-alkaline properties of saliva and plaque are measured by a graduated scale called pH. The scale values of 1.0 to 6.9 are considered acidic, 7.0 is neutral, and 7.1 to 14.0 are alkaline.

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The average salivary pH is 6.7-7.4 which in presence of sucrose and acidogenic bacteria can produce a fall in pH of saliva in no time.[4] Salivary components contribute to plaque formation and form much of its matrix. Sucrose dissolves in saliva and is readily taken up by the plaque. The buffering power of saliva may limit the fall in plaque pH caused by acid formed in plaque. The resting saliva and stimulated saliva plays an important role in affecting the plaque pH. The resting salivary pH (RS) if less than 6.6 can transform a healthy biofilm into a cariogenic biofilm.[5,6] Numerous studies have demonstrated that tooth decalcification begins at a pH as low as 5.5 pH.[7] At this pH calcium and phosphates ions are released from apatite structure of the tooth into plaque and saliva.

Dental plaque is colonized by acidogenic bacteria such as Streptococcus mutans (MS) and Lactobacillus species along with more than four hundred other species residing in oral cavity.[8] The acidogenic bacteria in presence of refined carbohydrate can reduce the pH of plaque and start decalcification and destruction of both inorganic and organic structures of tooth, setting out the disease process of dental caries, which affects a major portion of world population today.[9] A Classical Stephen-curve would

demonstrate that a 10% sucrose solution diluted in 10ml of water if rinsed inside the mouth can reduce the plaque pH to an acidic level within 5 minutes of consumption before returning to the baseline.[10] The frequency of sugar consumption is also an important factor for caries as the more frequent a refined sugary food or drinks is ingested, the longer the pH of plaque remains acidic. Soft and sticky confectionary like cake, biscuits and lozenges, toffees and chocolates remain stuck on the vulnerable surfaces of the teeth for an extended time period if they are not rinsed out or brushed away and thus become a continuous supply source of fermentable carbohydrate for cariogenic bacteria.[11]

Small children cannot clean their teeth properly or even fail to clean their teeth at all because of parental negligence or ignorance and suffer from dental caries from a very early age. Research shows caries is highest among developing countries, particularly those with low parental education level and an insufficient oral hygiene practice.[12] Very young children would often lack proper motor skills and dexterity for tooth cleaning, as tooth brushing is an acquired skill, and this skill needs to be properly taught by the parents. Unfortunately most parents are not aware of the causes of early childhood caries.[11] In a developing country like ours dental pain is one of the foremost causes for which children are often taken for dental checkups. An educated parent is expected to educate the offspring about the importance of personal hygiene and hence proper oral hygiene. In countries where oral health get the lowest of all health priorities, the responsibility automatically goes to the attending dental health professional to teach the patients about the role of dental plaque in caries initiation and progression.[10] The identification of the type of plaque (neutral, acidic or alkaline) present on tooth surface is an important factor when it comes to patient education. So the characteristic of the plaque in relation to its pH would determine its harmful, neutral or beneficial effect on dental tissues. If the acidic plaque could be identified in an easy-to-do method this would help in patient education and oral hygiene motivation.

This clinical study was designed to investigate the pH of plaque in children aged 3-6 years suffering from carious primary teeth with Plaque-Check +pH kit. The Plaque-Check + pH kit developed and marketed by GC Corporation, Japan, is an in vitro patient motivation test tool, contains a commercial plaque disclosing solution among other motivational components, and can determine the pH of plaque when subjected to a sucrose challenge.[13,14]
The objective of the study was to evaluate the resting pH of plaque by observing a visible change in plaque color in vitro, and thus educating patients and parents about the role of dental plaque in tooth decay.

Methods and Materials

The clinical study was carried out at the Paediatric OPD of Sapporo Dental College and Hospital during May 2010-June 2010. Sixty-two (62) Children between 3-6 years of age with carious teeth were selected by convenient sampling for the plaque pH check. A structured pretested questionnaire regarding socio-demographic status and a clinical exam for decayed(d), extracted(e) and filled(f) teeth (deft) followed by a pH check of plaque were performed by the investigators with Plaque-Check+ pH kit, (GC, Asia, Pve. Ltd., Product of GC Corporation, Japan). The Plaque-Check+ pH kit is an in vitro patient motivation test kit which helps in communicating with the patients by visually demonstrating the cariogenic potential of dental plaque.[14] The Kit is recommended to -

1. Check plaque cariogenicity,
2. Demonstrate plaque acid production to a patient as part of their education about dental caries, and
3. Assess the effect of dietary changes on plaque pathogenicity, regardless of age.[14]

Verbal consent was taken from the parents of the children. The questionnaire was filled up by the attendants of the children. Some children also participated in answering the questionnaire. The subjects were comfortably seated on dental chair, in a slightly reclined position and oral examination was performed to assess their deft status. Immediately prior to collection of plaque sample, the gingiva, mucosa and teeth were air dried with triple syringe as precaution against

salivary contamination of the plaque sample. Plaque samples were collected from lower molar and also from upper anterior teeth with disposable plaque collection instruments supplied with the kit. To achieve an accurate result, sufficient plaque filling at least half of the tip of each instrument were harvested. The collected plaque sample was dipped in a Plaque-Check + pH solution for 1 second only. The plaque-filled instruments were placed at the grooves of the dispensing dish and left for 5 minutes for plaque fermentation. As the greatest production of acid occurs at 5 minutes (Stephen Curve) the plaque pH was measured at this time by checking and comparing the color of the plaque with the chart supplied with the dispensing dish.

1. A green colour indicated a neutral pH around 7.2, a low risk for caries,
2. A yellow or orange colour indicated a final pH of 6.0-6.6, a moderate risk for caries,
3. A pink or red colour indicated a final pH of 5.0-5.8, a high risk for caries.

Of the two samples taken from each of the participant, the one showing the lowest pH as indicated by the change in color was recorded for each individual patient. The results were collated and tabulated for analysis using Excel spreadsheet.

Results

Table 1: Distribution of the study population according to age and sex (N=62)

Age in years	Male No.	Female No.	Total No. %
3-4	13	4	17 (27.6%)
4-5	20	8	28 (44.8%)
5-6	7	10	17 (27.6%)
Total	**40 (64.5%)**	**22 (35.5%)**	**62 (100%)**

Table1: *show that among 62 participants of the clinical study 64.5% were male. Children between four and five years participated more than the other two age groups with female children dominating only the 5-6 year-old age group.*

Table 2: Distribution of the study population according to deft score (N=62)

Age in years	deft 1-3	deft 4-6	deft 7-9	deft> 10	Total deft	Mean deft
3-4	3	7	4	3	96 (28.4%)	5.65
4-5	4	12	9	3	170 (50.3%)	6.1
5-6	7	6	4	0	72 (21.3%)	4.2
Total	**14**	**25**	**17**	**6**	**338 (100%)**	**5.3**

Table 2 demonstrate that 338 units of decayed, missing (extracted) or filled teeth were identified among the 62 participants. A little more than fifty percent (50.3%) of the decayed, missing and filled teeth were found in 4-5 age group. The overall mean deft was 5.3. However, five to six years children had the lowest mean deft status (4.2) while 4-5 years old children had the most of number of carious lesion (mean deft status 6.1).

Table 3: Distribution of the participants according to baseline plaque pH (N=62)

Age in years	Color: green pH score >7	Color: Orange pH 6.0-6.6	Color : Red/Pink pH 5-5.8	Total No.
	No.	No.	No.	
3-4	2	12	3	17
4-5	2	24	2	28
5-6	1	13	3	17
Total	**5 (7.9%)**	**49 (79.2%)**	**8 (12.9%)**	**62 (100%)**

Table 3 *shows almost eighty percent (79.2%) of the children had a baseline plaque pH between 6.0-6.6. Eight children in all demonstrated a high acidic pH of 5-5.8. Interestingly five children had neutral or alkaline pH (resting pH value >7).*

Table 4: Distribution of participants according to brushing, fand sugar added milk consumption

Characteristics	Frequency	Percentage
Parental assistance in tooth cleaning		
Needs parental help	24	38.7
Cleans own teeth	38	61.3
Frequency of teeth cleaning		
Once daily	34	54.8
Twice Daily	24	38.7
None	4	6.5
Takes extra sugar in milk		
Yes	45	72.6
No	17	27.4

Table 4 *shows that 38.7% of the children were assisted by parents while brushing their teeth. Only twenty-four children (38.7%) brushed twice a day while four children (6.5%) did not brush regularly at all. Majority of the children (72.6%) consumed extra sugar-added milk.*

Discussion

The baseline plaque pH of children with multiple tooth caries were persistently slightly acidic and this finding was similar with the study by Hayes.[15] A resting plaque pH between 6.0-6.6 was recorded in 79.2% of the participants, a moderate risk factor for further caries activity, while eight children had lower acidic pH (pH less than 5.8). It can be assumed that the children with lowest pH were going through an active decay process of their teeth during the clinical examination as tooth demineralization starts at a pH below 5.5.[10] Nevertheless, studies have shown that a slightly acidic plaque is conducive for aciduric bacteria as fermentable carbohydrate rich diet creates a favorable environment for such bacteria, thus a vicious cycle is initiated, leading to further lowering of plaque pH for demineralization of tooth structure.[15] So both these groups need attention for more extensive preventive and operative measures.

The study found a mean overall deft status of 5.3 among the participating children, with 5-6 years age group showing the lowest decayed, extracted (missing) and filled teeth (deft 4.2). Studies have shown that caries prone primary dentition could predict risk of caries in permanent dentition.[16] A continuous follow-up schedule would be an integral part of preventive measure for such children.

The results of this study also showed that 72.6% of children had extra sugar added to their milk, 54.8% children brushed once daily and only 38.7% parents assisted their children brush their teeth. These facts point to parental negligence or ignorance of oral hygiene measures for their children as 3-6 years children would not be expected to take the best care of their teeth as a slightly acidic pH of dental plaque could be the cumulative effect of extra sugar added milk intake with improper and irregular tooth cleaning habits.

Conclusion

The Plaque-Check +pH kit readily demonstrated the pH of the collected plaque within minutes and it showed potential to be a very effective patient education tool. This kit has other components which were not used for this clinical study and a more elaborate study is needed to demonstrate the effectiveness of this tool for protection of oral tissues from harmful plaque and for prevention of oral diseases including caries, gingivitis and peridontitis. As this study was not designed to find any statistical significance or association among the variables, only descriptive results could be tabulated from the clinical findings. It is recommended that a comparative study for evaluating the plaque pH of caries-free and caries- prone children be taken up for patient-parent motivational purposes.

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DNA platination in cisplatin-sensitive and cisplatin-resistant oral squamous carcinoma cells

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Abstract

Purpose: *We recently established HSC-3 cell and BHY cell as cisplatin-sensitive and cisplatin-resistance respectively and reduced cisplatin accumulation is the main reason for this resistance. The purpose of this study is to find out the relationship between drug accumulation and platinum DNA adducts formation and or DNA repair.* ***Methods:*** *After treatment with different concentration of cisplatin in both the cisplatin-sensitive and cisplatin-resistant cells, DNA was separated. Subsequently DNA platination and DNA adduct repair were measures by ICP-AES.* ***Results:*** *Cisplatin-resistant BHY cells express highly reduced levels of DNA platination compared to cisplatin-sensitive HSC-3 cells. However, there was no significant differences in DNA adduct repair in both the cells.* ***Conclusion:*** *This study suggested that reduced DNA-platinum adduct formation was a consequence of the reduced drug accumulation. DNA adduct repair have little or no effect in cisplatin resistance.*

Key words : DNA platination; Cisplatin; Oral squamous carcinoma cell

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Introduction

Squamous cell carcinoma comprise >90% of all malignancies diagnosed in the oral cavity and the head and neck region and is a tremendous public health challenge around the world. The 5-year survival rate for the patients with head and neck cancer is one of the lowest of any major cancers and has remained un-improved over the last 20 years.[1-3]

Cisplatin (cis-diamminedichloroplatinum II) a relatively simple compound consisting of a platinum atom complexed by two ammine groups and two chloride ions was introduced into the chemotherapeutic armamentarium and has been a cornerstone chemotherapy treatment for patients with oral squamous cell carcinoma. Despite the importance of cisplatin in the treatment of head and neck cancer and a broad range of other malignancies, there are many uncertainties about its molecular pharmacology and ultimate mechanism of action. Cisplatin has been most extensively characterized as a DNA damaging agent by binding to DNA and forming adducts. Its cytotoxicity is mediated mainly through interactions with DNA and inhibition of DNA synthesis and replication by formation of bifunctional interstrand and intrastrand crosslinks.[4,5] Its efficacy is limited due to acquired resistance and dose-limiting side effects, mainly nephrotoxicity.[6]

Based on the unique ability of cisplatin as anti-cancer agent, we were interested in investigating the signaling pathways of cisplatin by studying the DNA platinization, DNA adducts repair in the cisplatin-sensitive variant HSC-3 cells and cisplatin-resistant variant BHY cells of oral squamous carcinoma cells. Moreover, we wished that the studies might further help us to understand the molecular mechanism and relationship between drug accumulation and platinum DNA adducts formation and or DNA repair.

Materials and methods Drugs and reagents

Cisplatin (Sigma, St Louis, MO); DNAzol, Trypsin-EDTA (GibcoBRL , Life Technologies, Canada); Dulbecco's modified eagle's medium (D-MEM) (ICN Biomedicals Inc., Aurora,

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Ohio); Fetal bovine serum (FBS) (HyClone, Perbio, Canada); Kanamycin sulfate (Meiji, Japan); All other drugs and chemicals were obtained from Wako Pure (Osaka, Japan).

Cells and culture conditions

Two different types of human oral squamous carcinoma cells were used in this study and were provided by the Japanese Cancer Research Resources Bank (JCRB, Tokyo, Japan). The cells were cisplatin-sensitive HSC-3 cell (squamous cell carcinoma of tongue, low metastatic type) and cisplatin-resistant BHY cells (epithelial like squamous cell carcinoma of mouth, non- metastatic but highly invasive to muscle and bone).

Cells were grown in a humidified incubator gassed with 5% CO2 - 95% air at 370C. The media used for the cells were D-MEM supplemented with 1 mM L-glutamine, 4.5 g/l glucose, 3.7 g/l NaHCO3, 66.5 mg/l kanamycin sulfate and 10% FBS. The cells were maintained in 100 x 20 mm tissue culture dish (Falcon , Becton Dickinson Labware, Franklin Lakes, NJ) and media were renewed every second day. Subcultures were obtained by trypsin-EDTA treatment. Experiments were performed when the dishes became at least 80% confluent.

DNA extraction

Cells were washed twice by ice cold PBS and collected in 1.5 ml tube using cell scraper. Then centrifuged at 1000 rpm for 2 minutes at 40 C. The precipitated cells were incubated with 1 ml DNAzol (Gibco-BRL) at 370 C in hybridization oven overnight and centrifuged again at 10,000 rpm for 10 minutes at room temperature. The supernatants were taken in a new 2.0 ml tube and add 0.5 ml of 100% ethanol and mixed by inversion for about 10 to 15 minutes. DNA was then visible as a cloudy precipitation. The DNA was transfer to a new tube by spooning with a sterilized pipette chips followed by centrifuged at 5,000 rpm for 2 minutes at room temperature. The liquid over DNA pellets were removed and DNA were air dried for 2 to 3 minutes.

Measurement of DNA platination

DNA platination was measured by using a previously published method adapted to tumor cells with some alterations.[7,8] Briefly, both the cells, 2 x 10^6 cells were seeded and exposed to different concentrations of freshly prepared cisplatin (0 - 1 mM) dissolved in respected medium for 3 hours and DNA were separated following the same procedure as described above. Afterwards, DNA were lysed with 200ml concentrated nitric acid for 20 min on the water bath at 60 degree C. The lysed samples were then diluted to 3.5 ml of distilled water and platinum (Pt) concentrations were measured by inductively coupled plasma atomic emission spectrometer (ICP-AES) (Hitachi P-4010, Tokyo, Japan) following the same procedure as we described recently. Pt levels were expressed as nM / 2 x 10^6 cells,[9] with cell number determined by counting (measured by Burker-Turk cell counter, SLGC, Tokyo) in parallel cultures. Experiments were performed in duplicate and the values are the means SD of the three independent experiments.

In order to study DNA repair the cells were incubated with 1 mM concentrations of cisplatin for 3 hours. The cisplatin concentration was chosen based on previous results in order to obtain a comparable extent of DNA platination. Subsequently, the cells were incubated up to 3 hours with drug free medium. The further procedure was the same as described before.

Results

DNA platination

After incubation with increasing cisplatin concentrations there was a proportional increases of DNA platination both in sensitive HSC-3 and resistant BHY cells **(Figure 1)**.

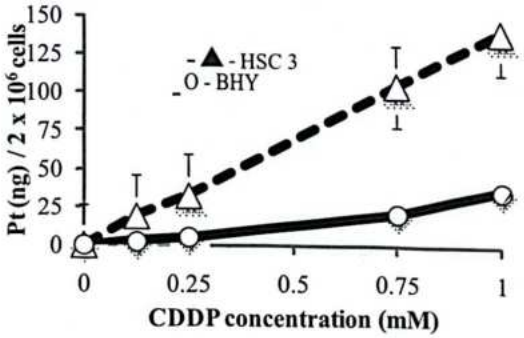

Figure 1: HSC-3 (r) and BHY (™) cells were seeded and exposed to different concentrations of freshly prepared cisplatin for 3 hours and DNA were separated and platinum (Pt) concentrations were measured by inductively coupled plasma atomic emission spectrometer (ICP-AES). Pt levels were expressed as nM / 2 x 10^6 cells, with cell number determined by counting in parallel cultures. Experiments were performed in duplicate and the values are the means SD of the three independent experiments.

However, in the cisplatin resistant BHY cells the amount of DNA platination was significantly lower than in the corresponding cisplatin sensitive HSC-3 cells. The resistant BHY cells formed on average 4 fold less Pt-DNA adducts than sensitive HSC-3 cells.

To investigate whether the differences in DNA platination are due to an increased DNA repair the alteration of DNA platination after incubation with cisplatin and subsequent incubation with drug-free medium was investigated. The extent of DNA platination at the end of the cisplatin incubation was set to 100% and the subsequent decline was monitored **(Figure 2).** There was no obvious difference in DNA repair between sensitive and resistant cells were observed.

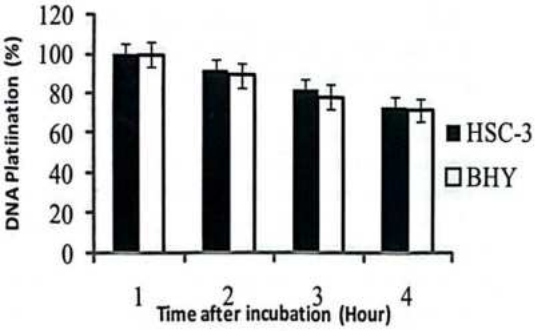

Figure 2: *HSC-3 cells (¢) and BHY cells (£) were incubated with 1 mM concentrations of cisplatin for 3 hours. Subsequently, the cells were incubated up to 3 hours with drug free medium. DNA were separated and platinum (Pt) concentrations were measured by ICP-AES. The extent of DNA platination at the end of the cisplatin incubation was set to 100% and the subsequent decline was monitored. Experiments were performed in duplicate and the values are the means SD of the three independent experiments.*

Discussion

We recently established HSC-3 and BHY cells as cisplatin sensitive and resistant respectively.[9] Compared with the sensitive HSC-3 cells, resistant BHY cells exhibited an approximately threefold increase in resistance to cisplatin. The dominant factor in cisplatin resistance in this cells is reduced uptake of cisplatin due to lower Na+,K+-ATPase activity.

Upon entering a cell, all platinating agents become aquated, losing chloride or oxalate ions, and gaining two water molecules. This positively charged molecule is then able to interact with nucleophilic molecules within the cell, including DNA, RNA, and proteins. It is generally agreed that DNA is the preferential and cytotoxic target for cisplatin and other platinating agents.[10] When binding to DNA, platinating agents favor the N7 atoms of the imidazole rings of guanosine and adenosine. Three different types of lesions can form on purine bases of DNA: monoadducts, intrastrand crosslinks and interstrand crosslinks. Monoadducts are first formed as one molecule of water is lost from aquated platinating agents; however, greater than 90% of monoadducts then reacts to form crosslinks. Almost all of these crosslinks are intrastrand crosslinks. Additional DNA lesions include interstrand crosslinks. All crosslinks result in contortion of the DNA[11]

A reduction in uptake has consequently great influence on the degree of DNA platination. Therefore it is not surprising that we also found reduced formation of Pt-DNA adducts in resistant cells. As the cytotoxic activity of cisplatin is based upon DNA platination, the increase of EC50 values in resistant cells is the consequence of this alteration. Other authors reported reduced DNA platination in cisplatin resistant cells as well[12-16] except one.[17] This data and the data presented in figure 1 along with our recently published data[9] strongly suggested that intracellular cisplatin accumulation plays a vital role in cisplatin sensitivity in tumor cells and Na+,K+-ATPase plays major role in intracellular cisplatin accumulation.

In order to interpret the quantitative relationships between uptake, DNA platination and cell death it is important to know whether DNA repair is different between sensitive and resistant cells. Platinating agent adduct repair occurs primarily through nucleotide excision repair. However, the extent of repair did not correlate completely with the extent of resistance to cisplatin, suggesting that enhance DNA repair was one of the several determinants of the level of resistance. Several groups are conducting studies of the relationship of DNA repair to cisplatin resistance in mammalian cells. In our study we did not determine different DNA repair rates. The results of other

studies concerning DNA repair are contradictory: some authors reported a faster DNA adducts repair rates in resistant cell others did not observe differences between sensitive and resistant cells.[13,14,18-20] Further investigations concerning DNA adducts repair are necessary to explain these conflicting data.

Based on results describe in this article and our recently published report[9] we can come to a conclusion that intracellular cisplatin accumulation plays a key role in cisplatin resistance. In cisplatin-resistant cells reduced intracellular cisplatin accumulation leads to reduce DNA platination. Reduced DNA-platinum adduct formation was a consequence of the reduced drug accumulation and vice versa. DNA adduct repair have a little or no effect in cisplatin resistance.

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Original Article

Isolation of AmpC-ß lactamases in Gram-negative bacteria from wound infections.

MF Karim[1], ANI Satter[2], MZ Hossain[3], A A Saleh[4], M R A Miah[5]

Abstract

Isolation of AmpC-β lactamase producing Gram-negative bacteria was performed in BSMMU and DMCH. Among 300 wound swabs, 182(60.67 %) Gram-negative organisms were isolated. Majority of bacterial isolates were Esch.coli (35.71%), followed by Pseudomonas (30.77%), Klebsiella (12.09%), Proteus (8.24%), Enterobacter and Acinetobacter (6.6 % each) respectively. Antimicrobial sensitivity test was done in all Gram-negative isolates by Kirby-Baeuer disc diffusion method and AmpC- β lactamase producers were identified by disc Approximation method. Among 182 Gram-negative isolates 15.93 % were AmpC- β lactamase producers. Highest number of AmpC- β lactamase producers were Esch.coli (18.46 %),followed by Enterobacter (16.67 %), Pseudomonas (16.07 %), Klebsiella (13.64 %), Proteus (13.33 %) and Acinetobacter (8.33 %).All the AmpC- β lactamase producers were higher in burn wound than in surgical wound infections. A large number of AmpC- β lactamase producers were isolated from wound infections which indicates that AmpC- β Lactamase is a major threat of antibiotic resistance .

Key words: AmpC-β lactamase, Gram-negative bacteria, antibiotic resistance

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Introduction

Over the last two decades, many ß -lactam antibiotics have been developed that were specifically designed to be resistant to the hydrolytic action of ß-lactamases. Bacterial resistance to ß-lactamase drugs and the mechanism leading to this resistance is gaining importance as a field of interest to medical researchers throughout the world. The most common mechanism of resistance to ß- lactam antibiotic in Gm-negative bacilli is the expression of ß- lactamases which are responsible for therapeutic failures. AmpC ß -lactamases are cephalosporinases that are not inhibited by clavulanic acid and hydrolyze cephamycins as well as other extended-spectrum cephalosporins. A simple disc diffusion test was described[1] for the laboratory detection of AmpC ß -lactamase resistance. Disc approximation test is a simple test, easy to perform, low cost can easily be incorporated into the routine laboratory practices for detection of AmpC-gene.

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Methods

A total of 300 wound swabs were examined, of which 140 were burn wounds and 160 were surgical wounds. Wound swabs were collected from patients of indoor and outdoor department of Bangabandhu Sheikh Mujib Medical University (BSMMU) and Dhaka Medical College Hospital (DMCH). All the swabs were inoculated on Blood agar and MacConkey's agar media and incubated at 370oC for 16-18 hours and suspected colonies were identified by colony morphology, staining character and necessary biochemical tests. All isolated Gram-negative bacteria were subjected to Disc-approximation test.

Method of Disc approximation test:

For each strain, 3-5 isolated colonies of the organism were suspended in 5 ml of sterile normal saline. A sterile cotton wool swab was dipped into the bacterial suspension and streaked on the dried surface of Muller-Hinton

plate. Then a cefoxitin disc was placed on the surface of inoculated plate. The cefotaxime disc was placed at 2.5 cm from the cefoxitin disc and incubated at 370oC for 16-18 hours and reading was taken on the next day. AmpC ß-lactamase production was positive if there was flattening of zone of inhibition of cefotaxime disc towards cefoxitin disc by >4mm.

Results

A total of 300 specimens consisting of 140 burn wounds and 160 surgical wound swabs were cultured from which 182 (60.67 %) were isolated. Among them 103 (56.59 %) were from burn wounds and 79 (43.41 %) were from surgical wounds.

Table 1: Distribution of AmpC ß - lactamases among Gram-negative bacteria from isolated wound samples (N=182)

Type of sample	No. of Gram negative bacteria	No. of AmpC ß- lactamases Positive strains	% of AmpC ß- lactamases Positive strains
Burn wound	103	21	20.39%
Surgical wound	79	08	10.13%
Total	182	29	15.93%

Table 1 shows that out of 182 isolated Gm-negative organisms 29 (15.93%) were AmpC positive among which 20.39% were from burn wound and 10.13% from surgical wounds.

Table 2: Distribution of AmpC positivity among the strains tested (N=182)

Name of strain	No. of strains tested	No. and %of AmpC ß - lactamases
E.coli	65	12 (18.46%)
Klebsiella	22	3 (13.64%)
Proteus	15	2 (13.33%)
Pseudomonas	56	9 (16.07%)
Enterobacter	12	2 (16.67%)
Acinetobacter	12	1 (8.33%)
Total	182	29 (15.93%)

Table 2 shows the AmpC positivity rate. It was highest in Esch.coli (18.46%) followed by Enterobacter spp. (16.67%), Pseudomonas spp.(16.07%), Klebsiella spp.(13.61%), Proteus spp. (13.33%) and Acinetobacter spp. (8.33%).

Table 3: Distribution of isolated AmpC producing bacteria in different samples (N=182)

Organism	Burn wound		Surgical wound	
	No. of bacteria	No. and % of AmpC +ve strain	No. of bacteria	No. and % of AmpC +ve strain
E.coli	31	8 (25.80)	34	4 (11.76)
Klebsiella	12	2 (16.67)	10	1 (10)
Proteus	10	2 (20)	5	0
Pseudomonas	35	6 (17.14)	21	3 (14.29)
Enterobacter	8	2 (25)	4	0
Acinetobacter	7	1 (20.39)	5	0
Total	103	21 (20.39)	79	8 (10.13)

Table 3 shows that among the isolated AmpC ß - lactamase producing organisms, Esch.coli was found 25.80 % in burn wounds and 11.76% in surgical wounds. Klebsiella spp. was found 16.67% in burn wounds and 10% in surgical wounds. Proteus spp. was found 20% in burn wound. Pseudomonas spp. was found 17.14% in burn wound and 14.29% in surgical wound. Enterobacter spp. was found 25% in burn wounds and Acinetobacter was found 14.29% in burn wounds. No AmpC positive Enterobacter, Proteus and Acinetobacter was found in surgical wounds

Discussion

Nosocomial infection continues to be a serious threat causing adverse outcome and increased costs. There is changing pattern of infective organisms especially in hospital environment. Majority of infections involve initially Gm-positive which are later superseded by Gm-negative opportunists that appear to have greater propensity to invade.[2] Antimicrobial resistance among Enterobacteriacae is increasing worldwide specially in hospitals.[3-5] Failure to detect the enzymes like ESBLs, AmpC ß - lactamases and Metallo ß - lactamases has contributed to their uncontrolled spread and therapeutic failures. This study isolated considerable number of AmpC ß - lactamases producing organisms in wounds. Antibiotics including third generation cephalosporins are being indiscriminently and irrationally used so that there is increasing number of occurrence of antibiotic resistance among Gm-negative bacteria. Antimicrobial sensitivity tests being used now are unable to detect AmpC ß-lactamase mediated drug resistance against 3rd generation cephalosporins which may lead to treatment failure. It may be concluded that there is a large number of AmpC ß- lactamase producing bacteria responsible for wound infections which are resistant to most commonly used antibiotics.

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Clinical Review article

Restoration with AP-X resin composites: A 15-year retrospective observation

MU Chowdhury[1]

Abstract

Clearfil AP-X composite resins are in use for restoration of teeth for many years. The author, who has been using AP-X for more than 15 years, discusses his experience with this specific composite restorative material in this retrospective clinical review paper.

Key words: Clearfil AP-X composite, Tooth degradation, Noncarious cervical lesion

(J Cont Dent Sci 2013;1(1):26-29)

In April, 1995, I returned from Japan after completion of graduate studies at Hokkaido University, Sapporo, Japan. My friends and colleagues did not forget to include a few syringes of Clearfil AP-X composite resins (Fig. 1.) as gift. This is the brand of composite materials which I have been using for more than 15 years to restore teeth of the patients. Meanwhile, I changed my office location twice, but I did not change my brand of resin composite.

Though currently, it is most widely used in posterior teeth, the very name suggests that it was designed as an X-ray detectable material (X) for both anterior (A) and posterior (P) application, and hence the name AP-X. The salient features and advantage of this material are: [1,2] (Table:1)

1. Esthetic quality: It ensures highly aesthetic resin restorations because of its high polishability. Commonly encountered Vita shades are available.

2. Improved physico-mechanical properties: It possesses a better wear resistance[1] and high flexural strength and fracture toughness properties among others. It is classified as a micro-hybrid composite resin with carefully adjusted viscosity that affects the success rate with these materials and its good wear resistance is attributable to barium glass fillers which are softer than quartz. So it can be used for posterior teeth.

3. Ease of handling: Non-sticky formulation prevents the material from sticking to the instruments but not the prepared and treated cavity surfaces, contact matrix and the latest version is supplied as PLT compules with a gun, thereby reducing the chance of cross-infection and at the same time, ensuring better placement.

4. Radiopacity : It is sufficiently radiopaque due its high barium content and facilitates X-ray evaluation

5. Less polymerization shrinkage (1.9%): it is said to be due to high filler loading (85%) that reduces stress on the cavity walls, thereby reducing the chances of post-operative sensitivity.

Evolution of the current generation composite resin systems

With Bowen's (1962 patent) and Buonocore's (1955) inventions, it became possible to restore class IV restorations in a more conservative and predictable way, something that had been impossible before.[3] The first composites changed the scenario of esthetic restorative dentistry dramatically and in effect, eliminated silicates from restorative armamentarium. But the result was far from satisfactory. Researches were extensively done to improve the physico-mechanical properties of the composite resins and clinical techniques. The earlier problems were extensive wear, finishing complexity, filler materials, filler loading, morphology, and surface treatment thereof. The bond strength of current generation resin composites were

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extensively studied in both clinical and laboratory trials and were found to be satisfactory (Fig. 2) and were in excess of 20MPa. Wear resistance was improved by assorting filler particle size and material. Various studies indicated that composite resions with glass fillers had better wear behavior than with quartz[3]. Quartz is very hard and wore away opposing natural teeth. So barium glass/strontium glass replaces part of the fillers. This reduced the damaging effect of CR restoration on the opposing teeth surface. In this evolutionary process, hybrid composite resins were introduced to the profession. As far as clinical techniques are concerned, viscosity, an important physical property of liquid is of prime importance. So very recently, we have packable and flowable composite resins. The high viscosity of bis-GMA based resin is attributed to the two centrally occurring benzene rings. So diluent monomers like, triethylene glycol dimethacrylate (TEGDMA), urethane dimethacrylate (UDMA) are incorporated as viscosity controllers. Considering physico-mechanical properties (Table 1), AP-X is an optimized material intended for various application. In clinical practice, I used this material mainly, as an esthetic restorative material to restore teeth in the appearance zone and to treat the non-carious cervical lesions (NCCLs/Wedge Shaped Defects / WSDs), besides Class I and II preventive resin restorations. But it worked well in all these clinical situations and specially in NCCLs. Here, a review of etiology of NCCLs may be of importance.

Review of mechanisms of non-carious cervical (NCC) tooth degradation

Before any attempt is made to restore a tooth, the operator should have an understanding of the situation, that is to say, how the Non-Carious Cervical Lesions (NCCL) develop. This will not only helpful for selecting the restorative material and technique but also help initiating preventive measures!

Earlier these lesions were attributed to "toothbrush-dentrifice abrasion while others termed it 'erosion' due to acid. Later, more appropriately, it was termed 'bio-corrosion[4]. Grippo[5] is the first to designate these cervical lesions as abfraction in 1991 and amended in 2004[4]. He attributed these surface lesions to stress concentration in the involved areas. The word abfraction is related to 'ab' meaning 'distant/away' and 'fraction' meaning destruction/loss, thus justifying the name; because due to masticatory load in occlusal lesion, stress concentration occuring at the cervical areas, and the tooth damage occurring away from the site of loading.

Grippo et al[6]. , in a recent article, discussed in detail the mechanisms of microstructural degradation of tooth substance not related to caries.

Discussion

In short, this is an account of my experience with composite resin Clearfil AP-X series. The AMS (Anterior Matrix System, Hawe-Neos Dental, Switzerland, Fig. 3) was very useful in the NCCL restorations. The AMS contained transparent cervical matrices which were used in anterior cervical lesions that allowed visible light-curing of the composite resin under pressure and helped initial morphological configuration of the cervical area. Color-coded Al2O3 polishing strips (coarse, medium, fine, and superfine) were very useful for finishing flat surfaces as is needed in diastema closure. Figure 4-7 shows different lesions of teeth treated by AP-X at different times by the author.

Table 1: Physico-mechanical Properties of AP-X series (Kuraray incorporation, Japan)

Physicochemical Properties of AP-X series			
Filler Content (Wt %)	**Flexural Strength (MPa)**	**Vickers Hardness**	**Tooth brush wear (mm3)**
85.5	**208**	**134**	**0.20**

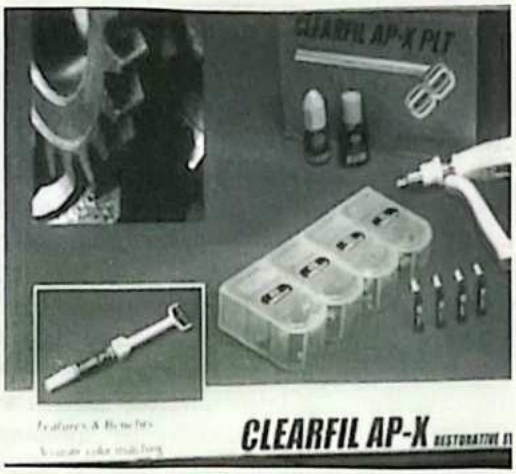
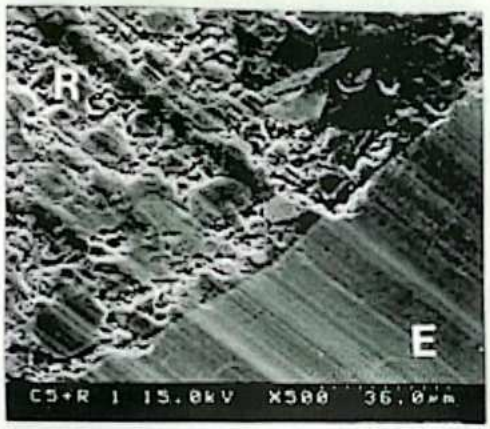


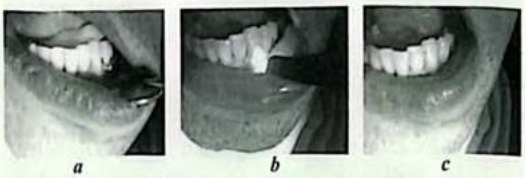
Figure 1: AP-X supplied by manufacturer



b, worn surface, sliding direction : E→R

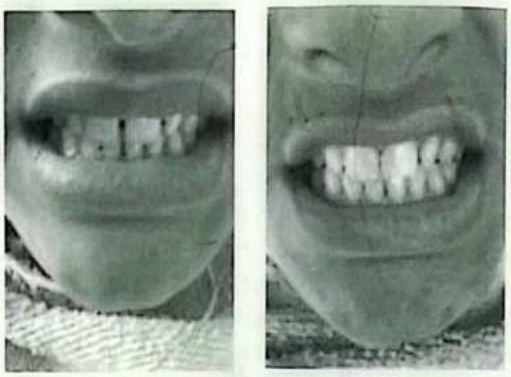
Fig 2: SEM view of composite resin showing favourable bond and wear behavior. (E>Enamel, R> Composite Resin)

Figure 3: The Anterior Matrix System (AMS), Hawe-Neos Dental, Switzerland



a b c

Fig.4 a. Shaping the material with AMS, b. Photopolymerization, c.The finished restoration



a b

Figure 5: Median Diastema Closure, a. Preoperative view, b. Postoperative view

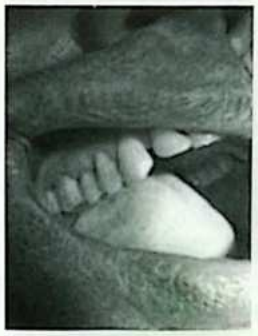


Figure 6: Buccal view of NCCL on first permanent molar, restored with AP-X (In Service >10 years)

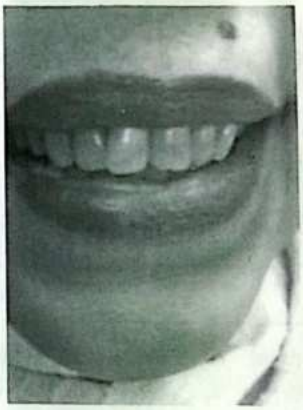


Fig 7: Aesthetically restored median diastema with AP-X (12 years postoperatively, still intact!)

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Review Article

Ingredient of Hot Chili Makes the Local Anesthetics More Pain Specific!

H M Zakir[1]

Abstract

Do the local anesthetics selectively block only pain without causing numbness, impairment of mobility, reduction of proprioception? In this mini review the noble strategy which has been invented recently in pre-clinical research to develop pain specific local anesthetics and the subsequent researches based on this strategy will be discussed. Invention of clinically proven pain selective local anesthetics will extend the opportunity of their clinical utility beyond the current indications.

Key Words: ***Capsaicin, QX-314, Local Anaesthetics, Neuropathic Pain***

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Introduction

Local anesthetics (LAs) like lidocaine are now widely used most powerful method including dental operative procedures to block pain while retaining the consciousness.[1] Research on LAs has demonstrated that LAs block the generation and propagation of action potentials by blocking voltage-gated sodium channel at the intracellular site of a neuron.[2-5]

All LAs including lidocaine has little or no selectivity [6-10] which means they block action potentials in all sensory, motor and autonomic fibers in the region where injected. Therefore, although the goal of topical or regional anesthesia is to block the transmission of signals in nociceptors to prevent pain, the administration of local anesthetics also produces numbness due to blocking of pressure and touch receptors, immobility due to blocking of motor axons, and low blood pressure due to blocking of autonomic (sympathetic) nerve fibers.[11] In particular, inferior alveolar nerve (IAN) block during dental operative procedure, LAs cause numbness of lips, checks and tongue and there is partial immobility of tongue.

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While such an outcome may be acceptable during surgery, however blocking of only pain sensation would be more desirable. Sensory-selective local anesthesia has long been a key goal in local anesthetic development. Invention or improving the sensory/pain selective LAs will clearly extend their clinical utility beyond their current indications. For example, it allows women to be pain free during labor without compromising their ability to push or for musculoskeletal disorders in which it is important to maintain mobility. Block of nociceptors to produce analgesia without a loss of proprioception or motor function would enable early mobilization in patients receiving peripheral nerve block or plexus block, for example, following knee or hip joint replacement.

A further issue with regional injections of local anesthetics is their relative short duration, limited to several hours, which is usually not enough to fully cover the normal duration of post-operative pain. Furthermore, because of lidocaine's action on central neurons and cardiac muscle, it can have major central nervous system and cardiovascular toxicity problems when administered locally at high volumes.[12,13] There is therefore a need for a pharmacological therapy that has more selectivity for nociceptors, a longer duration and a reduced side effect burden.

Recently a strategy has been developed in pre-clinical research, based on targeting local anesthetics to selectively enter into nociceptors and not into motor axons and low threshold sensory axons. In this review, the story of this noble development and consequent researches based on this strategy will be briefly discussed.

Development of a noble strategy to selectively

block pain fibers (combination of a LA with chili's ingredient):

How nociceptors can be selectively blocked? One way would be to selectively target those voltage-gated sodium channels expressed only or predominantly in pain specific neurons. However, till date only a few subtype selective[14,15] sodium channel blockers have been invented and none have been shown to produce local analgesia. In 2007 Binshtok et al.[16] from Harvard Medical School, Boston, MA, USA, developed an alternative procedure. They invent the strategy to make entry of local anesthetics only to the pain specific neurons. Most LAs in clinical use are tertiary amines that under physiological conditions exist in a mixture of protonated and uncharged base forms.[17] It is the uncharged hydrophobic form of LAs that penetrates through the membrane of all neurons, so that in addition to blocking pain signals, LAs produce general numbness, immobility etc.[16,18]

Fig-1(A_E)

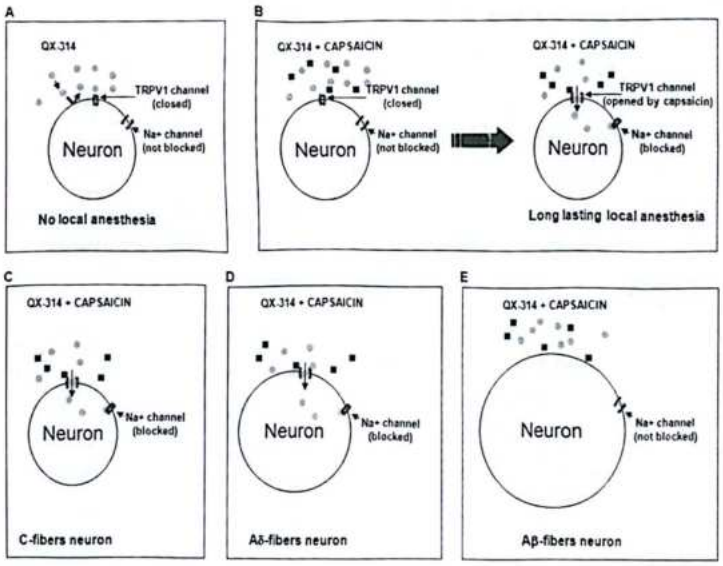

Fig 1. Mechanism of action of combination QX-314 and capsaicin as a local anesthetic (Diagrammatic representation). A. QX-314 is a positively charged derivative of lidocaine which generally can not pass through the membrane of a neuron and thus produce no local anesthesia. B. When QX-314 is applied with capsaicin (TRPV1 agonist), it pass through the membrane due to opening of TRPV1 channel and causes blocking of sodium channel and produce long lasting local anesthesia. C-E. As TRPV1 are expressed mainly in small (C-fibers) and also in medium diameter (A Delta-fibers) neurons, but not in large diameter (A Beta-fibers) neurons the combination of QX-314 and capsaicin causes blocking of nociceptive neurons only (C and A Delta fibers), not other neurons.

Binshtok et al.[16] developed a strategy to deliver a permanently charged sodium channel blocker QX-314 (N-ethyl-lidocaine) into the pain specific neurons by entry through large-pore ion channels (TRPV1, Transient receptor potential vaniloid 1) selectively expressed in pain specific neurons. QX-314 (lidocaine N-ethyl bromide) is a lidocaine derivative which becomes protonated (being charged) in a short time when applied extracellularly of a neuron and can not enter into the cell and produces no or little affect on sodium channel, thereby produces no local anesthetic effect (Fig.1A). Binshtok et al.[16,18] use this QX-314 with capsaicin and they found that the combination of drugs produce long lasting pain specific local analgesia without any impairment of motor function when applied subcutaneously (paw region) or perineurally (sciatic nerve) in rats. Capsaicin, the pungent ingredient in chili peppers (which makes the chili hot) is a TRPV1 agonist which means capsaicin can open TRPV1 channels. It has been found that TRPV1 channels has large pore and they are mainly expressed in the pain specific neurons (c-fiber and A -fiber neurons) (Fig. 1C&D). When the combination of capsaicin and QX-314 was applied capsaicin causes opening of TRPV1 on the pain specific neurons and QX-314 enters through the opening and block the sodium channels from the inside of a neuron

(Fig. 1B), there by produce local analgesia and it has been found that very small amount of this combination produces long lasting pain specific local analgesia.

Implication of this noble strategy in the trigeminal system:

To understand the potential utility in the trigeminal system for treating dental and facial pain, Kim et al.[19] and Zakir et al.[20] tested whether the combination of QX-314 (a derivative of lidocaine) and capsaicin (ingredient of chili) is effective in orofacial region to selectively block pain sensation. In the head region the trigeminal nerve (largest cranial nerve) is primarily a sensory nerve but it is also involved in certain motor functions such as mastication. The sensory function of the trigeminal nerve is to carry the tactile, proprioceptive and pain sensation of the face, teeth, mouth etc.. The cell bodies of incoming sensory nerve fibers for tactile and pain sensation present in the trigeminal ganglion (TG) which is analogous to the dorsal root ganglia of the spinal cord. The proprioceptor fibers have their cell bodies in the trigeminal mesencephalic nucleus. The mandibular branch of trigeminal nerve contains motor branches of the trigeminal nerve that originates in the motor nucleus of the trigeminal nerve. It has been found that combination of QX-314 and capsaicin block the action potential of TRPV1 positive TG neurons but not TRPV1 negative TG neurons[19] and it is well known that TRPV1 is exclusively expressed in pain specific neurons (c-fiber and A Delta -fiber neurons). The efficacy of the drugs was also tested by observing jaw-opening reflex induced by noxious electrical stimulation of the tooth pulp. It has been reported that the reflex was inhibited when the combination was placed on sensory nerve (inferior alveolar nerve) but was not inhibited when the combination was placed on motor nerve (mylohyoid nerve). When this combination of drugs (small amount) was applied subcutaneously on the mental region of rats, the pain sensation was blocked as the rats did not show nocifensive behavior on painful stimulus.[19,20] These data indicates that this combination has the potentiality to use in dental operative procedure to selectively block pain without blocking tactile, propioception or motor activity.

The efficacy of the combination of QX-314 and capsaicin on neuropathic pain condition:

Very recently this drug combination has been tested on neuropathic pain condition in the trigeminal system[20] and in spinal system[21]
Trigeminal neuralgia is a neuropathic condition in the trigeminal system which may develop after extraction of third molar tooth or surgical operative procedure in the orofacial region. Zakir et al.[20] tested this combination on a rat model of neuropathic pain where neuropathic pain was induced by transection and reposition of inferior alveolar nerve. During regeneration of transected inferior alveolar nerve neuropathic pain like behavior developed which was observed by decrease of threshold of pain to stimuli. The neuropathic pain condition was attenuated when the QX-314 and capsaicin combination was used subcutaneously on the region innervated by inferior alveolar nerve (mental region). Zakir et al.[20] also showed that nerve injury causes changes of the pattern of TRPV1 expression on regenerated neurons and that the effectiveness of QX-314 and capsaicin mediated blockade depends on the availability of functional TRPV1 receptors on regenerated neurons.

Use of combination of lidocaine and QX-314 to overcome the use of capsaicin which itself is a pain inducer:

Although the combination of QX-314 and capsaicin has been shown to work, an important problem remained for its clinical exploitation. Capsaicin causes activation of TRPV1 very instantly (<1 s) while enough amount of entry of QX-314 into the neuron takes several minutes. This delay of entry of QX-314 into the neuron is long enough for the capsaicin to produce burning pain for several minutes before long-lasting pain-selective block would manifest.[22]
This problem was solved by using the lidocaine instead of capsaicin with QX-314. It has been reported that lidocaine itself, at clinically administered concentrations, is a TRPV1 agonist.[23]
Binshtok et al.[18] showed that much longer analgesia than lidocaine alone can be achieved with a combination of lidocaine and QX-314. It has been found that lidocaine and QX-314

combination produces initial short duration nonselective analgesia followed by long lasting pain specific analgesia. Therefore, it has been suggested that a lidocaine and QX-314 combination that induces a relatively short motor block followed by a much longer-lasting regional analgesia might be ideal clinically; producing initial immobilization of a surgical area followed by sustained analgesia after motor function recovers.

Conclusions: The strategy which has been invented may lead to the new generation of local anesthetics which only blocks the pain sensation. The next step would be to investigate the strategy in human. If the clinical data match the preclinical findings, the combination may be a useful addition for regional pain control, producing a longer and more selective action than existing local aesthetic agents.

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Case Report

An unusual presentation of Facial paralysis: A case report

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Abstract

Facial paralysis is a neuropathy of the peripheral seventh cranial nerve, usually resulting from traumatic, compressive, infective, inflammatory or metabolic abnormalities. However, in many cases no etiology is identified and the eventual diagnosis is idiopathic. Here, we present one such case of a patient of facial paralysis with slight slurred pronunciation during any recitation, heaviness of the right side of the face and the flu-like symptoms about 1-2 weeks ago.

Keywords: Facial paralysis, Bell's Palsy

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Introduction:

Facial paralysis is a clinical condition which is named after Dr. Charles Bell, who, in 1821, described complete facial paralysis after injury of the stylomastoid foramen.[1] Facial paralysis can be defined as acute peripheral facial nerve palsy usually of unknown cause.[2] It is typically unilateral and can be complete or partial.[3] Although there is agreement on the definition, there is no consensus regarding the etiology, diagnostic approach or management of this enigmatic condition.[4]

Facial palsy is generally a unilateral disease, affecting both sides of the face equally. Acute inflammation and edema of the facial nerve are thought to lead to entrapment of the nerve in the bony canal (especially in the labyrinthine segment), which leads to compression and ischemia[5-7]. Many viruses, such as HIV,[8] Epstein-Barr virus[9] and hepatitis B virus[10] have been suspected in initiating this inflammation, but herpes simplex virus (HSV) is the most frequently implicated.[11,12] Patients generally experience rapid onset of unilateral facial palsy and often describe numbness or stiffness, although no actual sensory loss occurs.[5,7] Affected patients are usually unable to close their eyes. Facial appearance becomes asymmetric, and saliva dribbles down the angle of the mouth. Depending on the site of the lesion, some patients may complain of noise intolerance or loss of taste sensation.[1,5] Treatment of facial palsy is controversial, because as many as two-thirds of patients recover spontaneously. Corticosteroids alone or associated with antiviral agents have been recommended[7]. Adour[13,14] reported that patients with facial palsy treated with acyclovir and prednisolone experience a more favorable recovery and less neural degeneration than patients treated with placebo plus prednisolone. The favorable response to the treatment of facial palsy with acyclovir-prednisolone supports the theory that reactivated HSV causes neuritis. A case of facial paralysis in a young patient with unusual complain is presented in this article.

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Case report:

A 25-year-old patient reported to the department of Oral and Maxillofacial Surgery of Sapporo Dental College and Hospital, Dhaka complaining of slight slurred pronunciation

during any recitation, heaviness of the right side of the face. He denied any numbness, tingling, or weakness in his extremities. He denied recent cold sores, ear discharge, or recent trauma; however, he noted flu-like symptoms about 1-2 weeks ago. He also denied any change in hearing or ear pain. The patient noted no previous medical history. He denied any prior surgeries. He is a smoker but denied alcohol or illicit drug use. On exam, the patient was in no acute distress and in no obvious discomfort. His speech was slightly slurred. His skin was warm and dry with no apparent rashes or lesions. He demonstrated an inability to close his eyelid and to wrinkle his forehead. He was also unable to whistle properly. No ulcerations were noted in his ears. The remainder of his exam including a thorough neurologic exam was unremarkable. All diagnostic tests were essentially normal, including orthopantomogram and labs.

Fig. 1a

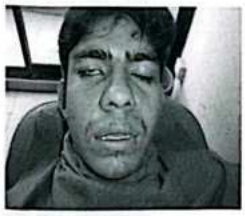

Fig.1b

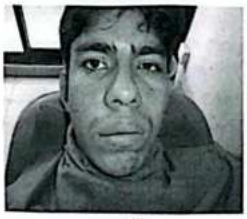

Fig. 1c

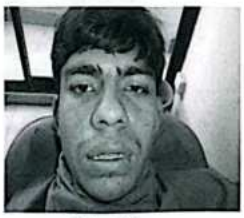

Fig. 1d

Figure 1: Facial aspect of patient showing facial paralysis on the right side. (a) Inability to smile. (b) Incomplete right eye closure. (c) No movement in the upper right eyebrow. (d) No movement in the right portion of the labial orbicular muscle.

Based on the clinical examination, the patient was diagnosed with unilateral (right sided) facial nerve paralysis.

Treatment:

The aims of treatment in the acute phase of facial paralysis include strategies to speed recovery and to prevent corneal complications. Eye care includes eye patching and lubrication. Lubricating drops should be applied frequently during the day and a eye ointment should be used at night.[5] Strategies to speed recovery include physical therapy, corticosteroids and antiviral agents. Accordingly Prednisolone 1mg/kg body weight per day for five days and acyclovir 800 mg per day in divided dose for one week were prescribed to speed recovery. Chloramphenicol eye drops and ointment was prescribed as eye lubricant and he was advised to use eye pad and glass to protect the cornea. The dose of Prednisolone was then tapered 10mg/day for a total treatment time of 15 days. Significant improvement was noted fifteen days later and Vinpocetine 5mg twice daily for 10 days were prescribed to increase peripheral blood circulation for further development. The prognosis was drastic and complete recovery of all facial muscle function was achieved one month later.

Fig. 2a

Fig..2b

Fig. 2c

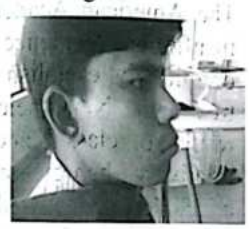

Fig.2d

Figure 2: Facial aspect of patient in case 1 after recovery of facial movement. (a) Normal, symmetric smile. (b) Complete closure of the right eye. (c) Normal right eyebrow movement. (d) Normal movement of labial orbicular muscle

Discussion:

Much pathology can be included in the differential diagnosis of facial paralysis: unilateral central facial weakness, Ramsay Hunt syndrome, Lyme neuroborreliosis, tumours, diabetes mellitus, sarcoidosis, weight loss, visual changes, vertigo and weakness or

numbness. No specific laboratory test confirms the diagnosis of facial paralysis, its assessment remains clinical. It is important to emphasize the fact that we followed this patient weekly and noted that he was recovering very well. Patients should be advised to use artificial tears to keep the eyes moist and prevent exposure keratitis. During the day, sunglasses are indicated, and dirty, noxious fumes should be avoided. During sleep, an ophthalmic ointment should be used. Our patients enjoyed complete recovery after 4 weeks, but clinicians should be aware of possible morbidities. Treatment for Bell's palsy is etiology-driven if a specific case is identified. Most patients with Bell's palsy recover without treatment 71% achieve complete recovery, 84% achieve near normal function. Commonly employed, noncontroversial treatment modalities for facial paralysis include eye patching and lubrication to protect the cornea from drying and abrasion secondary to poor lid closure and reduced tearing.

Controversy remains regarding the therapeutic effectiveness of steroids and acyclovir. According to the Cochrane Reviews, the available evidence from randomized controlled trials does not show significant benefit from treating facial paralysis with steroids alone. Upon completing their own systematic review, The American Academy of Neurology found insufficient evidence in class I studies to definitively establish the efficacy of steroid treatment. Nevertheless, based upon pooled results of class I and class II studies and a relatively benign side-effect profile, they concluded that steroids are safe and probably effective in improving facial functional outcomes in patients with Bell's palsy. Treatment with corticosteroids should begin within 5 days (earlier if possible) after the onset of palsy and should only be used in the first 7 days. Treatment with antiviral seems logical in Bell's palsy as the etiology is often viral. The American Academy of Neurology considers acyclovir safe and possibly effective in improving functional outcomes. However, a recent double-blind, placebo controlled, randomized study demonstrated no evidence of a benefit of acyclovir given alone or an additional benefit of acyclovir in combination with Prednisolone.[15]

Conclusion:

In this case we have used Vinpocetine which is a peripheral vasodilator to increase the blood supply of the facial muscles of the affected side in order to achieve normal muscular function within a short period. Complete recovery was achieved in our case with the above mentioned treatment accordingly

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—— Chief Editor