Sri Lanka Dental Journal

Volume : 37
Number : 01
April : 2007

The Official Publication
of the Sri Lanka Dental Association

ISSN 1391-07280

Sri Lanka Dental Journal

Volume 37 **Number 01** **April 2007**

The **Sri Lanka Dental Journal** is a refereed journal published three times a year by the Sri Lanka Dental Association in collaboration with the College of Dentistry and Stomatology of Sri Lanka, College of General Dental Practitioners of Sri Lanka and the College of Community Dentistry of Sri Lnka.

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ISSN 1391 - 07280

Typeset & Printed by:

AK 2 PRO - Ethul Kotte, Kotte.
Tele: 011 - 5645145 / Tel/Fax: 011 - 2871899

CONTENTS

Sri Lanka Dental Journal 2007; 37(01) 1-2

Sri Lanka Dental Journal

Volume 37 ***Number 01*** ***April 2007***

EDITORIAL

HISTOPATHOLOGY, UNCHALLENGED IN CANCER PREDICTION

Oral cancer is a major global health problem, affecting the quality of life of millions of people worldwide. At least half of these patients will die within five years of diagnosis. Worldwide epidemiology indicates that oral cancer ranks eighth in the cancer prevalence. Over 90% of oral malignancies are squamous cell carcinomas. It is the third most common malignancy in South East Asia. More than 1000,000 new cases are detected annually in this region. In Sri Lanka, one third of all cancers is reported to be oral squamous cell carcinomas (OSCCs). Recent reports show that there is an increase in the prevalence of the disease among younger age groups. WHO predicts a worldwide rising OSCCs incidence in the next decades.

Diagnosis of OSCCs, planning treatment, determination of prognosis, and assessment of treatment responses depend entirely on conventional histopathological typing together with the clinical findings. The starting point of employing histopathology in this context coincides with the invention of the light microscope. However, proper usage of histopathology for diagnosis came up with the development of achromatic microscope in the latter part of the 18th and early part of the 19th century. Proper histopathological grading system for OSCCs (epithelial malignancies) was initially

introduced by Broders in 1920. This system is still used for the purpose of diagnosis, planning treatment and prediction of prognosis of OSCCs. The value of histopathology for cancer diagnosis was further emphasized with the introduction of invasive front grading system by Bryne *et al,* in 1989. This method has been repeatedly validated in studies carried out in the West. This has also been validated for Sri Lank (Dissanayake 2006) where aetiology, onset and prevalence of OSCCs is different to that of West.

With the recent advances in science, attention has been focused on different methodological approaches to identify novel markers for the purpose of diagnosis. With the discovery of the genetic basis of disease, attention was mainly focused on various molecular and genetic markers. Different protein products of either oncogenes (PRAD-1, EGFR, Cyclin D1 etc) and tumour suppressor genes (p53, p16, p21, Rb etc) or both have been investigated using immunohistochemistry. Gene aberrations have also been studied using various molecular biological techniques such as PCR, and gene sequencing. Cell proliferation markers (PCNA, Ki 67) and markers of apoptosis (Bcl2, BAX, Bad) have also been studied using different molecular biological techniques. However, literature reveals that none of these novel molecular and genetic markers is effectively useful in the diagnosis, planning treatment and determination of prognosis of OSCCs, though some of the them may be used as supplementary to the histopathological grading.

The practical usefulness of the majority of proposed molecular/genetic markers are still in doubt. As such, there is no consensus on any of these prognostic indicators. Further, in spite of the vast advances in science and research in the field of prognostic markers, no improvements have been reported on five year survival rate or mortality and morbidity of oral cancer patients. Throughout the 20th Century, diagnosis of OSCCs, planning treatment, determination of prognosis, assessment of treatment responses and prediction of the tumour behavior was determined entirely using histopathological method. **This may well be the scenario for the next decade or two of the 21st century as well.** However, currently attention is being focused with anticipation, on microarray technology or commonly referred to as "DNA chips" where expression levels of hundred to thousands of genes will be determined at the same time providing a unique profile of increased or decreased gene expression of tissues. **However, comparatively speaking, use of histopathological grading and its prognostic value still remains unchallenged in the present context.**

Upul B. Dissanayake
Editor

Leading Article

Sri Lanka Dental Journal 2007; 37(01) 3-5

Competency-based dental education and its implications

N. Wanigasooriya

The training of a dental surgeon has traditionally been considered to consist of the acquisition of an ever expanding body of knowledge and skills. This body of knowledge was imparted according to a discipline. What guided and determined the content were the discipline and the process of teaching. Accordingly the training would be described as consisting of so many hours of lectures, lab work or clinical work in each specific discipline or clinical specialty. The decision of the precise content to teach was left mostly to the discretion of the individual teacher. The response of the profession to the public demand for attention to the outcome of education was by way of specifying learning objectives. These learning objectives thus defined too, were discipline or clinical specialty specific and fragmented when compared to real life clinical work of a dental surgeon. They were also often seen to be rather trivial, perhaps due to difficulty of writing objectives aimed at higher levels of learning

Competencies expected of the graduate are now recognized as a more appropriate way of expressing learning outcomes in dentistry as much as in other health professions. Competency differs from specific learning objectives in that they are broader, and closer to real life situations, and they combine several objectives in various domains. They are also easier for lay, non-professional policy makers to understand, and offers a more rational basis for training.

Competency is essentially to do with ability- the ability to perform in a given capacity- namely that of Dental Surgeon in a given environment. Chambers defines ability as being acquired in a series of stages, and competency as the level which is required for a dental graduate to be able to work independently.[1] Implicit in competency is the idea that, the relevant supporting knowledge and appropriate attitudes, accompany them. Since the acceptance of competency as the more appropriate way of describing learning outcomes, documents describing competencies required of a dental graduate, have been produced, in several different geographical and socio-economic settings.[2,3,4]

Initiatives to formulate competencies for the dentist of the South-East Asian region are already underway. The workshop on dental education held in Chennai in November 2006, by the collaboration of APDF and APRO was the initial step towards this. There is clearly a need for defining the desired competencies of a dental surgeon in Sri Lanka, which should lie within the wider statements for this region.

The establishment of a nationally accepted competency statement for dental surgeons in Sri Lanka, and using it as the basis for the training of dental surgeons, has several important implications for dental education, some of which may be described under the following areas.

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1. Organization of teaching material

Traditional structure for dental education is discipline based. Teaching consists of series of lectures, laboratory or clinical training according to these specialties. This is a very convenient way of organizing content consisting mainly of factual information. Text books and other information resources are also organized in this traditional way. Student learning is guided along different disciplines separately, and not in relation to the context in which it is expected to be used. Students are expected somehow to synthesize and apply content from various disciplines, in a contextually relevant way, subsequently.

In contrast to this, learning content in the relevant context, while being more interesting, has also been shown to be more effective. In a competency- based curriculum it would be logical to organize teaching content around the stated competencies. In such a curriculum, content that is relevant to each competency drawn from each discipline and clinical specialty would be presented in the context of the competency.

This means that the teachers need to work harder, in order to organize the teaching content. They will need to be more discriminating in the selection of content to be included since it will need to be justified in relation to the competencies expected. The inclusion of content for the sake of 'being complete' will no longer be acceptable. They will also need to work out suitable scenarios for their introduction. While individual teachers need to do this, curriculum planners need to come-up with a logical and practical organizational framework in which to present the content.

2. Structure of teaching units

Traditionally dental students learn clinical skills in specialist clinics. This tradition still continues in Sri Lanka. As a result, general competency, which is the ability to work in general dental clinics is expected to be acquired after being exposed to a series of specialist clinics.

Chambers has shown that, general competency in dentistry, demonstrated during early clinical training, is more closely related to general competence at graduation, than discipline specific knowledge and skills.[5] Based on this, one could argue that, giving the opportunity for students to gain general clinical/related skills during their training would be a useful way of helping students to gain overall clinical competence. This would mean a complete change in the training, where the students would be introduced to the clinical setting, management, attitudes etc. in a general way. It is not being proposed that training in skills according to dental specialties is not necessary. Training in general clinical skills before training them in skills related to dental specialties seems to be more appropriate.

3. Student assessment

Traditionally both theoretical knowledge and clinical skills of dental students have been assessed for qualification and certification. Clinical skills were assessed by getting them to perform a particular clinical procedure, and assessing their general clinical ability in relation to one clinical case. The evolution of the assessment process in the Faculty of Dental Sciences, Peradeniya, has been in the direction of increasing the number of disciplines assessed specifically.Currently students are tested separately in nine clinical specialties, between the parts I and II of the final BDS examination, using the Objective Structured Clinical Examination. While assessing skills in specific specialties has its place, assessing general global clinical ability has a higher value as a predictor of later competency.[5] If this is to be given credence to, in addition to the discipline-based assessment, the final examination for dental degrees should also consist of a more global assessment of clinical abilities.

Among the several faculties of study, the University of Peradeniya uses several methods to determine the success or failure of a candidate at an examination. Some faculties adhere to the norm-referenced method strictly, while others follow something related to it. The Faculty of Dental Sciences, has a fixed score of 50% as the level for a pass, and over 60% for honours passes. No attempt is made to relate these scores to specific levels of competency of the candidate. In contrast, according to the criterion-referenced method, the success or failure of a candidate is directly determined according to the demonstrated ability in relation to specified competencies. Implementation of a criterion-based method of assessment would complement a competency-based curriculum.

4. Departmental structure

The development within universities takes place along the lines of teaching activities, namely the disciplines. This has resulted in the creation of separate administrative departments and divisions, which continue to develop further, sometimes giving birth to more departments and divisions. Dentistry used to be one department in the Faculty of Medicine, Dentistry and Veterinary sciences of the University of Peradeniya until 1976.This was later expanded to five departments, and after gaining Faculty of Dental Sciences status in 1986, has now developed into 7 departments with 13 divisions of study.

If teaching and learning is to be guided by competencies the strict demarcation between the administrative structures of departments cannot be maintained, at least when it comes to teaching. This can cause complications for implementation of such curricula.

The concept of competency-based curricula is not something really new, but in the past has had general acceptance in the training of mid-level health workers. This has now been extended to professional training, and a number of medical schools as well as internship training programs are relying on competencies in developing their curricula. In keeping with the accepted advancements in Health Professionals education, the move towards competency-based education is the way forward for Dental Education in Sri Lanka. Competency-based curriculum in dentistry would be more relevant to practice and, by extension, would make learning at the undergraduate phase more meaningful and also interesting to the learner. The profession needs to pay attention to ways of dealing with the envisaged areas of difficulty, if such a curriculum is to be implemented effectively.

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Clinical update

Sri Lanka Dental Journal 2007; 37(01) 6-10

Management of dental patients on anti-platelet and anticoagulant therapy

D. M. Dissanayake, Upul B. Dissanayake

Introduction

A significant number of people in society are on anti-platelet medication or anticoagulant therapy which is either used prophylactically or therapeutically. Usage of anti-platelet and anticoagulant therapies have become more common in today's society as a result of a higher prevalence of ischaemic heart diseases, thrombophilia and strokes than two to three decades ago. As these diseases are common in the latter part of life, the percentage of sufferers would also be increased with the increase in elderly population which is inevitable with the higher expectancy of life. The normal physiological process of haemostasis would be affected in these patients who are under anti-platelet or anticoagulant therapy. As such, there is a higher tendency of bleeding in performing any surgical procedure on them. Further, bleeding from wounds in the oral cavity is comparatively higher even under normal physiological conditions due to hyper-vascularity of the tooth supportive structures and fibrinolytic effect of saliva. Therefore, the risk of bleeding in oral surgical procedures on patients who are under anti-platelet/anticoagulant therapy is much greater and needs very special care. The aim of the present update is to evaluate the current trends in the management of patients who are on anti-platelet/ anticoagulant therapy and need oral surgical treatment.

Anti-platelet therapy

The platelets are basically essential in achieving haemostasis. Platelets are involved in initial sealing off of vascular injuries by formation of a platelet plug. Platelets are the main components of the arterial thrombus. Arterial thrombosis could result in fatal conditions such as myocardial infarctions and ischaemic strokes. Administration of anti-platelet therapy is a significant prophylactic measure in their control and prevention.

Aspirin in low dose, clopidogrel, and dipyridamole are the commonly utilized drugs for anti-platelet medication. Aspirin has been the drug of choice for the secondary prevention of cardiovascular (eg: prophylaxis after coronary bypass) and cerebrovascular diseases. Clopidogrel is widely used for the prevention of atherothrombotic events in patients suffering from myocardial infarctions, ischaemic strokes, peripheral arterial disease and unstable angina or non-Q-wave myocardial infarction in acute coronary syndrome (Microembolism is found to be the basic reason for unstable angina or non-Q-wave myocardial infarction in acute coronary syndrome). Aspirin and clopidogrel irreversibly inhibit the platelet aggregation within an hour of administration.[1] Aspirin exerts its effects by irreversibly inhibiting

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platelet cyclo-oxygenase. This prevents the formation of thromboxane A2 and thereby inhibiting platelet function. The anti-platelet effect of aspirin will last until the bulk of circulating platelets have been replaced (equal to the life span of a platelet). This results in a continuing anti-platelet effect of aspirin for several days after the drug is stopped. Clopidogrel exerts its effect by inhibiting ADP (adenosine diphosphate) receptors of the platelets. It takes 4-7 days to achieve full platelet inhibition and effects last for 5 days after cessation of therapy. Dipyridamol exerts its effects on platelets via number of mechanisms such as inhibition of ADP induced platelet aggregation and release, inhibition of platelet-diesterase and inhibition of uptake of adenosine and glucose.

Omission of anti platelet therapy prior to any surgical procedures may increase the risk of thromboembolic events by 0.005%.[2] Even though risk is apparently low, the outcome or complications are very serious compared to complications of bleeding. Thromboembolic events, including fatalities, have been reported after anti-platelet drug omission.[3,4,5]

Anticoagulation therapy

Coagulation factors are involved in the formation of fibrin mesh. Fibrin mesh will stabilize the already formed platelet plug. This is a compulsory mechanism in achieving haemostasis under normal physiological conditions. Coagulation is delayed or hindered in those who are on anticoagulation therapy. Oral anticoagulants (Warfarin) are widely used as a prophylaxis for patients with cardiac diseases (atrial fibrillation, prosthetic heart valves) and thrombotic tendencies (deep vein thrombosis, pulmonary embolism, hereditary thrombophilias and antiphospholipid antibody syndrome).[6] Warfarin, a cumarin is the commonly used anticoagulant which blocks the conversion of vitamin K dependent clotting factors namely factor II, VII, IX and X into biological active forms by acting as an antagonist of vitamin K (Vitamin K dependent activation of these clotting factors is necessary for normal process of clotting).[6] As such warfarin hinders both the extrinsic and common coagulation pathways.

It is of utmost importance to assess INR (International Normalized Ratio) of those patients who are on anticoagulant therapy before making any decision on a surgical procedure.[7] It is safer to perform minor oral surgical procedures if the INR is less than 4 or within therapeutic range.[8] The patients on warfarin who have INR over 4 should not be subjected to any surgery without consultation of a haematologist. They need adjustments in the dose to get INR into therapeutic range before surgery. Doctors should not treat these patients in the out patient departments (OPD) as of indoor care is much more essential for them. Assessment of INR only 72 hours prior to surgery is sufficient for patients with stable INR readings. Duration should be reduced to 24 hours in whom the INR readings are unstable. Omission of warfarin for surgeries creates a grater risk of permanent disability or death than the risk of bleeding without omission, especially when the INR value is within the therapeutic range. Omission of warfarin even for 48 hours could lead the patient into rebound hypercoagulable state wherein risk of thrombosis is comparatively much higher.

Literature reveals, no acceptable experimental evidence/clinical reports to show any uncontrolled post-operative bleeding when dental surgical procedures are carried out while a patient is under anti-platelet/anticoagulant therapy.[9,10,11,12] However, the surgeon must take adequate care to prevent excessive trauma. Further, even if bleeding occurs, it can be successfully managed with local haemostatic measures alone.[13] Nevertheless, if post-operative bleeding complications occur, it causes problems for the patient. However, it does not carry as serious a risk as thrombo-embolic complications do.

The new management protocol clearly indicates that ongoing anti-platelet medications/ anticoagulant therapy should only be discontinued in the perioperative period if the haemorrhagic risk is definitely greater than the cardiovascular risk associated with the omission.

Dental surgeons should refrain from treating patients with liver failure, renal failure, chronic alcoholism, thrombocytopenia, haemophilia or other disorder of haemostasis and those who are currently under cytotoxic medications in the out patient dental clinic, if they are on anti-platelet/ anticoagulant therapy.[14]

A haematologist should assess the risk to patients under anti-platelet/anticoagulant therapy when they suffer from above mentioned, second underlying systemic diseases associated with bleeding tendency. The operator must not forget that the age and heavy consumption of alcohol are also contributory risk factors for excessive bleeding. Simple extractions up to three teeth at different sites, gingival surgeries, dental scalings could be performed without omitting or altering anti-platelet/anticoagulant drug therapy. Multiple visits treatment is needed when more than 3 teeth are to be extracted. The treatment may be planned to be done by quadrants at a time, or singly at separate visits. However, any procedure (scaling and gingival surgeries) should initially be restricted to a limited area to assess possibility of bleeding.

Pre-planned surgeries performed at a predetermined time would further facilitate the management of the patient. Selecting early hours of the day and at the beginning or near the beginning of the week would be more preferable for this. Pre-planned surgeries minimize the anticipated problems which can be encounted during the surgery. Surgeries in early hours of the day will save time of the rest of the day for further management of the patient, if any complication occurs.

Administration of local anaesthic injection

Infiltration or intra ligamentary injection with a vasoconstrictor should be used wherever possible. Regional block injection is recommended when there is no other alternative. However, usage of aspiration syringe with care is mandatory. Infiltration of local anaesthetic solution with a vasoconstrictor in and around the site of surgery is recommended as a precautionary measure of bleeding prevention. If the surgery is a tooth removal, post extraction socket should be packed with an absorbable haemostatic dressing such as oxidised cellulose, collagen sponge or resorbable gelatin sponge. Careful suturing with a resorbable material further encourages the haemostasis. Application of all other possible simple local measures would be more valuable. Prescription of tranexemic acid mouth washes can be used on these patients.[15]

Post operative care

It is the duty of the doctor to advise the patient on post operative care. The patient should rest for at least 2 to 3 hours until the effect of local anaesthesia disappears and the clot becomes stabilized. Refraining from mouth washing, disturbing the stabilizing clot with the tongue or foreign objects and abstaining from taking hard foods or hot liquids are very important precautions to follow. Patient should be advised to bite on a piece of folded and clean cloth/gauze for a period of 20 to 30 minutes, if bleeding starts. Patient should seek assistance from a dental surgeon, if bleeding does not stop with local measures. The dental surgeon should consider the necessity of repacking or resuturing the wound depending on circumstances.

Interaction with commonly used drugs

If there is a need of an analgesic paracetamol is the drug of choice. However, they must be strictly advised not to take aspirin for the pain. Non-steroidal anti-inflammatory drugs (NSAID) such as ibuprofen and diclofenac sodium are also not safe. They produce an inhititory but reversible

effect on platelet aggregation and platelet function is restored once the drug is cleared from the circulation.[16] NSAIDs should be used with caution when used in combination with aspirin or clopidogrel. NSAIDs may damage the lining mucosa of the gastro-intestinal tract resulting in bleeding. Aspirin or clopidogrel may aggravate the bleeding.[17] As there are no reports on drug interaction between dipyridamole and NSAIDs, concomitant use of them does not have a risk of bleeding. It has been shown that amoxyciline increases INR and as such there is a risk of bleeding. Drug interaction has been reported between metranidazole and warfarin too. Metranidazole enhances the effect of warfarin. Steps must be taken to reduce the dose of warfarin from 1/2 to 1/3 if the administration of metranidazole is unavoidable.

Conclusion

There is no need of omitting anti-platelet/anti coagulant therapy before any minor oral surgical procedures. However, complete haematological assessment is necessary to evaluate the anticipated problems. Adaptation of local measures will be sufficient to achieve successful haemostasis in these patients. In patients with ongoing anti-platelet/anticoagulant therapy the regime should only be discontinued in the perioperative period, if the haemorrhagic risk is definitely greater than the cardiovascular risk associated with the omission. In such situations a team approach is needed (Physician, Haematologist and Dental surgeon) for decision making.

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Review Article

Sri Lanka Dental Journal 2007; 37(01) 11-17

Oral lichen planus-aetiopathogenesis, clinical features, malignant transformation and management

R.D. Jayasinghe, M.A.M. Sitheeque

Abstract

Oral Lichen Planus (OLP) is a relatively common, chronic mucocutaneous inflammatory condition affecting stratified squamous epithelium. Although cutaneous lichen planus (LP) is self-limiting, OLP is a chronic disease that rarely undergoes spontaneous remission and is difficult to eliminate. It is a potentially malignant condition and causes considerable morbidity. Clinically OLP can present as a symptomatic disease or may present without any symptoms. The characteristic clinical manifestations may be enough to make a diagnosis, especially if the classical features are present or when skin lesions can be identified. An incisional biopsy is recommended to confirm the clinical diagnosis by histopathological means and to exclude any potential for malignant transformation and dysplasia. Management protocol for OLP varies and the main aims are to eliminate ulcerative and erosive lesions and by doing so to reduce the symptoms and the potential for malignant transformation. Asymptomatic reticular lesions do not need any treatment. Corticosteroids are the most commonly used pharmacological agents for treatment of OLP, either in the topical or systemic form. All cases must be followed up for sufficient period of time to identify any malignant changes.

Introduction

Oral Lichen Planus (OLP) is a chronic mucocutaneous inflammatory condition affecting stratified squamous epithelium. It is a relatively common condition in the oral cavity affecting about 2% of the western population.[1] OLP develops commonly in middle-aged patients and mostly in the fifth and sixth decades of life but patients of all age groups can develop the disease. OLP in children is well documented and when present it does not have any difference in clinical presentation from adults. Females are affected twice more than males.

Cutaneous lichen planus (LP) causes itching but is self-limiting whereas OLP rarely undergoes spontaneous remission and is difficult to eliminate. It is a potentially malignant condition and causes considerable morbidity.

Immunopathogenesis

Although many aetiological factors such as systemic immune mediated disease, infections and malignancies have been described in the pathogenesis of LP, no definite aetiological agent is so far identified. There is enough evidence to support the role of immuno-deregulation in the

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pathogenesis of OLP especially the cell-mediated immune system. The inflammatory cell infiltrate in OLP mainly consists of T lymphocytes and macrophages. A small number of plasma cells can be seen infrequently but are not characteristic.[2] Both CD 8+ and CD4+ cells are present in the cell infiltrate of OLP. Majority of cells within epithelium and adjacent to damaged basal cells are activated CD8+ cells. T cell line and clones from LP lesions are more cytotoxic than the lines and clones isolated from the normal skin of the LP patients. CD8+ cells are more cytotoxic then CD4+ cells. Available data suggest that an antigen associated with MHC class I on basal keratinocytes may activate CD8+ cells and those activated cells can induce keratinocyte apoptosis but the nature of antigen is still unknown. CD4+ cells are the main cells in lamina propria in OLP. Recent data suggest that Langerhan cells or basal keratinocytes present antigen associated with MHC class II to CD4+ helper cells. These stimulated CD4+ cells will then secrete Th 1 cytokines and IL 2 and IFN gamma. These will activate the CD8+ cells, which will trigger basal cell destruction.[2]

Oral lichen planus and viruses

A strong association between LP and viruses including herpes viruses, HIV, human papilloma virus (HPV) and hepatitis C virus has been reported in the literature and the commonest virus to be associated is the Hepatitis C virus (HCV). The association between OLP and chronic liver disease is well documented and risk of chronic liver disease in OLP patients is independent of age, sex, alcohol consumption and positive Hepatitis B surface antigen.[2] HCV antigens and RNA have been found in OLP tissues by many authors. HCV may occasionally replicate in the oral mucosa and HCV specific T lymphocytes have been recently demonstrated in oral mucosa of patients with OLP.[3] Some authors recommend testing sera of OLP patients for HCV antibodies to exclude the possibility of HCV infection.[4]

Oral lichen planus (OLP) and oral lichenoid reaction (OLR)

OLR mainly consist of lesions due to adverse contact with foreign materials whereas lesions due to unknown reasons are termed as OLP. Clinically as well as histologically both lesions are almost identical.[5] Some authors have identified amalgam associated oral lichenoid reactions (AAOLR) as a separate entity. Although there is no curative treatment for OLP, replacement of causative agent (mercury or amalgam alloy) or prevention of their contact with oral mucosa results in complete resolution of AAOLR in majority of patients.[6] Identification of AAOLR from OLP is difficult from histological criteria alone so correct correlation of all available data including history, clinical examination and other special investigation results is mandatory.[7]

Clinical features

Oral lichen planus has a characteristic clinical presentation. OLP is more prevalent in females. It occurs at early age in males than females and does not show any familial pattern.[8] Most of the patients with OLP have LP lesions in one or more extra oral sites. About 15% of the patients with OLP develop cutaneous LP that is characterized by flat polygonal papules. A network of fine white lines called Wickham's striae are often associated with the papules. Cutaneous LP can appear in several atypical forms and is then difficult to identify. Careful examination and multidisciplinary approach may be needed in order to exclude other skin diseases.

Apart from the skin in general, the commonest extra oral site in patients is the genital skin and mucosa. Approximately 25% of males with OLP have genital lesions but this value is as high as 57% in females. Genital LP was underestimated in the past and its importance is recognized only very recently.[9] The presence of LP lesions in gingiva, vagina and vulva is referred to as vulvo-vaginal gingival syndrome (VVGS). Symptoms of LP involving the genital mucosa include burning

sensation, pain, vaginal discharge and dyspareunia. In a study done by Belfiore *et al,* in 2006, 60% of patients with LP had genital symptoms compared with 95% with oral symptoms and 33.6% developed genital symptoms after oral symptoms.[9] These highlight the importance of coordination between oral medicine specialist and gynecologist in the diagnosis and management of OLP.

OLP can present as a symptomatic disease or may present without any symptoms. It may present as one of the three major forms, reticular, erythematous (atrophic) or erosive (ulcerative, bullous). Reticular lesions can present in isolation and may be the only clinical presentation but whenever erythematous lesions are present, they are always associated with reticular lesions at their periphery and erosive lesions are accompanied by erythematous and reticular lesions. This is a very important clinical feature and it will help to exclude the other clinically similar conditions like pemphigus and pemphigoid in which lesions present in isolation. The reticular lesions are white lesions, which are arranged as a network of connecting, overlapping lines, papules or plaques. They constitute the commonest presentation and are asymptomatic. Very often symptoms are due to erosive or erythematous lesions. The number as well as the size and location of ulcerations is variable. The erosive lesions rarely undergo spontaneous remission and can cause diagnostic confusion with other vesiculo-bullous diseases.

The posterior buccal mucosa is the commonest site of presentation of OLP followed by tongue, gingiva, labial mucosa and vermilion of the lower lip whereas lesions in the upper lip, palate and floor of the mouth are uncommon. Isolated OLP commonly presents in the gingiva but most of the patients will have lesions affecting multiple sites. About 10% OLP can present on the gingiva. Reticular lesions in gingiva can resemble a keratotic condition like leukoplakia but the commonest forms, erythematous and erosive forms may result in desquamative gingivitis. Desquamative gingivitis is the commonest form of gingival LP. Several other vesiculo-bullous lesions can cause desquamative gingivitis and include pemphigus, pemphigoid, linear IgA disease and foreign body gingivitis. It is very difficult to differentiate OLP from these lesions clinically unless typical LP lesions are present in other parts of the oral mucosa.

Malignant transformation

OLP is a potentially malignant condition but the reported malignant transformation potential varies between 0.4% to over 5%. Ingafou *et al,* (2006) reported a 1.9% malignant transformation rate with a median time of 7 years between initial presentations of OLP to malignant development in British patients.[10] Malignant transformation potential seems to be independent from the type of OLP or type of treatment administered.[11] Although there is controversy over the malignant transformation potential of OLP, several studies have shown a statistically significant risk of development of squamous cell carcinoma in patients with OLP.[11] The term lichenoid dysplasia is sometimes used to describe lesions that resemble OLP but are dysplastic.[12] It is very important to examine the patients with OLP carefully and over a period of time in order to detect any malignant transformation.

Diagnosis

The characteristic clinical manifestations of OLP may be enough to make a diagnosis, if the classical features are present especially the skin lesions. An incisional biopsy is recommended to confirm the clinical diagnosis by histopathological means and to exclude the malignant potential and dysplasia. Essential histopathological features include superficial band like T lymphocyte infiltrate, basal cell liquefactive degeneration and normal epithelial maturation. Additional features are the saw tooth like jagged rete morphology, civatte bodies (degenerated epithelial cells) and separation of epithelium from lamina propria. The importance of direct immunofluorescence is well

documented and will help in diagnosis specially to exclude other vesicular bullous disease in gingival OLP.

Management

Management protocol for OLP may vary and available treatment options do not provide total cure. Hence the main aim of treatment must be to eliminate ulcerative and erosive lesions and by doing so to reduce the symptoms and the malignant transformation potential. Asymptomatic reticular lesions do not need any treatment but need to be kept under observation in order to identify clinical changes and malignant transformation. Elimination of mechanical trauma, improvement of oral hygiene and correction of malnutrition states if present are important in overall management of the patients with OLP as these steps can enhance the healing.[13] Many treatment modalities have been used to treat OLP and include topical, intra-lesional and systemic corticosteroids, topical immunosuppressive drugs, topical and systemic retinoids, antimicrobials, photo chemotherapy, laser, cryosurgery and surgery. Although there are many ways of managing OLP, there is a lack of strong evidence supporting the effectiveness of any treatment for OLP.[14] It is important to note that some remissions are spontaneous and not related to any drug treatment.[15]

Corticosteroids administered either in topical or systemic form are the mainstay of treatment. Many forms of steroids have been used and that includes mid-potency steroids such as triamcinolone, potent steroids such as fluocinolone acetonide and fluocinomide with reported successes rates of 30 to 100%. Many forms of corticosteroids have been used including mouthwashes, ointments, gels and tablets. Oral rinses especially of triamcinolone acetonide are more effective than creams and ointments and the risk of fungal overgrowth is less.[15] Major problem of the use of topical corticosteroid treatment in oral cavity is the lack of adhesiveness to oral mucosa. Serious side effects with topical corticosteroid treatment are few. Topical corticosteroids can effectively penetrate the squamous epithelium. More than 30% of the patients treated with topical corticosteroids can develop oral candidosis that need treatment or require treatment before they begin the use of topical corticosteroids as a preventive measure but this rate is much less for the cases treated with mouth rinses especially triamcinolone acetonide.[15] Use of chlorhexidine mouthwash can reduce the risk of candidal super infection in OLP patients treated with corticosteroids.[16] Topical corticosteroid treatment is safe when administered for a short period up to 6 months, but with prolonged use, there is a risk of adrenal suppression. Therapy should be started with a potent corticosteroid to achieve rapid improvement followed by one of lower potency as soon as the lesions heal and become asymptomatic. It should be terminated as soon as the disease becomes inactive or only reticular lesions are present. The most suitable therapy for OLP is the topical high potency steroid therapy than systemic therapy because of the effectiveness, low cost and less frequent side effects. Clobetasole in adhesive medium is very safe and effective in this regard.[17]

Intra lesional corticosteroid injections of hydrocortisone, dexamethasone, triamcinolone acetonide or methylprednisolone can be used to manage OLP especially for erosive cases and can be repeated every 2-4 weeks.[2] It is considered as an effective and a simple method of introducing sufficiently high concentrations of drug locally for its immunosuppressive activity with reduced systemic side effects. Although they are effective, repeated injections are very painful, and the effectiveness is not guaranteed. Repeated injections also have localized effects such as mucosal atrophy and can give rise to unwanted systemic doses.[13] If the lesions do not respond to two intra lesional injections, it is unlikely that they will respond to further injections. It is therefore recommended to introduce other combined treatment modalities.[18]

Systemic corticosteroids such as prednisolone and methyl prednisolone are very effective but due to the possible serious complications, their use must be restricted to the severe erosive/ erythematous cases where topical corticosteroid treatment had failed or for systemic mucocutaneus cases such as vulvogingival LP. But newer immunosuppressive topical agents like tacrolimus can reduce the need of systemic steroid therapy even for resistant cases.[17] Although some consider systemic corticosteroids as the major treatment modality in the management of OLP, literature on their use is minimal and a study done by Carbone *et al,* (2003) did not find any significant difference in response to systemic prednisolone, clobetasol propionate in adhesive base or only topical clobetasol.[17] Systemic corticosteroids should probably indicate in high doses. Forty to 80 mg of prednisolone daily in divided dose is recommended. Side effects of systemic corticosteroids are dose dependent. Corbone et al in 2003[17] reported adverse reactions in 32% of patients treated with 50mg of prednisolone that is less than the rates reported for higher doses (61%). The side effects may be minimized if a regimen in which prednisolone is administered on alternate days is adopted.

Several other treatment modalities are described for the treatment of OLP and include immunosuppressive therapy, griseofulvin, antimalarial drugs and retinoids. As the immunopathology of LP is a cell mediated immune response, treatment with immunosuppressant or immunomodulatory drugs appear reasonable. Clobetasol and cyclosporin are known to be two most effective drugs in the management of OLP. Although a good response is reported with cyclosporin mouthwash, it is too expensive to use in routine cases and must be restricted to very severe cases and patients with lesions resistant to other forms of treatment. According to Conrotto *et al,* (2006), 1.5% cyclosporin in hydroxyethyl cellulose gel gives a better result than 0.025% clobetasol in the same medium with fewer side effects.[19] But due to the high cost of topical cyclosporine therapy, clobetasol in 4% hydroxyethyl cellulose gel is more cost effective for treatment of severe OLP patients. Topical cyclosporin can be used as a valuable second line therapy.[19] Recently an immunosuppressive drug tacrolimus that belongs to the macrolide family, which includes cyclosporin, rapamycin and acromycin, is proposed as a very effective treatment for OLP.[20,21] Isotretinoin, a Vitamin A derivative in 0.05% as well as 0.18% concentrations when applied topically has been reported to produce a good response and is an important method of controlling symptoms in patients with erosive OLP. It may also reverse the dysplastic changes if present.[22]

Although several non-pharmacological approaches have been used to treat patients with OLP, their effectiveness is still not very clear. Surgical treatment either with excision, excision followed by free grafts, CO_2 laser, 308-nm UVB excimer laser,[23] photo chemotherapy with ultra violet irradiation combined with psoralen (PUVA),[24] methylene blue mediated photodynamic therapy[25] or cryosurgery can be used for the cases with epithelial dysplasia. Although healing of lesions after cryosurgery without complications has been reported, it can recur in healing wounds or scars producing even worse symptoms.[26]

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Research Article 1

Sri Lanka Dental Journal 2007; 37(01) 18-28

The expression of *Candida parapsilosis* secretory aspartyl proteinase, a putative virulence factor

G. J. Panagoda, P.H.P. Fernando, L.P. Samaranayake

Abstract

Objective: The aim of the current study was to evaluate the production of secretary aspartyl proteinase (Sap) by 24 *C. parapsilosis* isolates.

Material and methods: A total of 24 *C. parapsilosis* isolates were used in the study and their Sap production was evaluated by using Enzyme Linked Immunosorbent Assay (ELISA).

Results: Results indicated that all *C. parapsilosis* isolates were positive for the production of Sapp. These isolates showed a mean Sapp production of 9.47 ng/ml with a range of 1.21 to 43.29 ng/ml. It was observed that the isolate which produced the highest amount of Sap (43.29 ng/ml) was a superficial oral isolate while the least quantity (1.21 ng/ml) was produced by a systemic isolate.

Conclusions:. All the isolates of *C. parapsilosis* produced Sap and superficial isolates were more prominent in the production of Sap than their systemic counter parts.

Key words; *Candida parapsilosis*, secreted aspartyl proteinases

Introduction

A number of investigators have demonstrated the potency of the extracellular products of *Candida* species during the initiation and progression of yeast infections.[1] Further, Odds in 1988 reported some 40 enzymes of *Candida* which have a significant role in the metabolism and also in yeast pathogenicity.[2] For instance, secreted aspartyl proteinases (Sap) and phospholipase, in particular, are considered as prime candidates in *C. albicans* pathogenicity.[3,4] Staib (1965), Odds (1985), Ruchel *et al,* (1986) and Sono *et al,* (1992) investigated the production of Sap by different species of *Candida*.[5-8] Further, they have shown that the production of Sap by species such as *C. tropicalis* and *C. parapsilosis* to be less than that of *C. albicans*. Studies have demonstrated that the Sap of *Candida* is associated with the pathogenicity of the organism.[9,10] With regard to its role in virulence the enzyme may function in two ways. First, the enzyme may be associated with the ability of the organism to initially colonize the host tissues.[11] Second its proteolytic activity may be associated with tissue invasion. It has been demonstrated that the *C. albicans* proteinase degrades both macromolecular

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substrates as well as low molecular weight substrates.[12,13] However, Fusek *et al* in 1994 have demonstrated different substrate specificities of proteinases from *C. albicans*, *C. tropicalis* and *C. parapsilosis.*[14]

There are a number of studies on Sap activity of *C. parapsilosis.*[7,14,15,16] These investigators have studied the properties of *C. parapsilosis* Sap and demonstrated there to be of lower molecular weight (33Kb) than that of *C. albicans* (42 Kb). Further, the Sap of *C. parapsilosis* has also been characterised as hydrophobic and is thought to potentiate its virulence.[16,17] Although there are many reports of *C. parapsilosis* Sap, most of these investigations have utilized only a few isolates. Further, there is no information of the influence of the source of the isolate on the Sapp production. Hence, the aim of this investigation is to evaluate Sapp production by 24 *C. parapsilosis* isolates.

Material and methods

Candida isolates and growth conditions

A total of 24 *C. parapsilosis* isolates were used in the study. A loopful from the stock culture of each isolate was streaked onto Sabouraud's Dextrose Agar (SDA) and incubated at 37 ^{0}C for 18 h. The resultant culture was washed twice with phosphate buffered saline (PBS; pH 7.2, 0.1 M) at 3500 g for 10 min and inoculated onto YCB-BSA broth (23.4 g of yeast carbon base, 2.0 g of yeast extract, 4.0 g of bovine serum albumin) to obtain a cell suspension of 1×10^{6} cells/ml. These inoculated media were incubated for 24 to 48 h, under agitation of 150 rpm at 37 ^{0}C. The cultures were centrifuged for 10 min at a speed of 10,000 rpm for 10 min to obtain a supernatant.

Enzyme-linked immunosorbent assay (ELISA) for Sapp antigen level

The Sap of 24 *C. parapsilosis* isolates was detected by an indirect ELISA method, using the antiserum raised against purified Sap from *C. parapsilosis* in a Guinea pig.[18] The method employed an enzyme labeled second antibody to react with the bound (primary) antigen-antibody complexes which were attached to a solid matrix. Sapp antisera and purified Sapp were kind gifts from Professor Patrick Sullivan (University of Otago). A secretary aspartyl proteinase positive strain of *C. albicans* (Sapc) and a Sap negative strain of *C. krusei* (Sapk) were also used as the controls. Each experiment was conducted on two separate occasions with duplicate determinations on each occasion. The details of the method are given below.

Binding antigen to solid support

In brief 100 µl of the native antigen, 10 µl of coating buffer (concentrated ten times) were added to a microtitre well (Costar, USA) and incubated for 2 h.

Blocking unbound plastic matrix

The culture was removed and blocked with 100 µl of 1 % BSA-PBS (pH 7.4) at 4 ^{0}C overnight. SAP-2 of *C. parapsilosis* was used to obtain the standard curve. After the overnight incubation, the culture was removed and washed three times with PBS (0.1 M, pH 7.4, 0.05%, Tween 20).

Incubation with the primary and secondary antibodies

The Guinea pig anti-Sap serum (100 µl) was added at 1:10000 dilution in 1% BSA-PBS (pH 7.4) to each well and incubated for 2 h at 37 ^{0}C. Then the plates were washed three times with PBS containing 0.05% Tween 20. Thereafter, 100 µl of the secondary antibody, i.e Phosphatase-conjugated goat antiguinea pig IgG (1:1000 dilution in 1% BSA-PBS, pH 7.4) was added to each well and incubated for 2 h at 37 ^{0}C, then washed three times. Wells with no primary antibody were used as negative controls.

Addition of enzyme substrate and reading the results with an ELISA spectrophotometer

The level of antigen concentration is indicated by the amount of bound secondary antibody to the wells. The concentration of the antigen was

detected by reacting with the phosphatase-substrate para-nitrophenyl phosphate (Sigma, St Louis) and the reaction was terminated by addition of 20 µl of 3 M NaOH after 10 min. The plates were read with an automated microreader (Bio-rad, USA) at 405 nm, blanked against air. The amount of proteinase in each well was calculated with the help of a standard curve (1-100 ng) determined using purified Sapp as coating antigen, under the conditions described above.

Statistical analysis

The data were first checked for normal distribution using the Statistical Package for Social Science (SPSS) programme. Thereafter, the results were analyzed by a non-parametric statistical test, (Mann-Whitney U test), in particular to determine the differences in Sap production between superficial and systemic isolates of *C. parapsilosis*. The analysis of variance (ANOVA) was used to determine the intra-species variation in the production of Sap by *C. parapsilosis* isolates.

Results

Production of Sapp by *C. parapsilosis* isolates

A total of 24 *C. parapsilosis* isolates were investigated for their production of Sapp. The results of this experiment are given in Table 1 and a summary comparison of their mean values in Table 2. Results indicated that all *C. parapsilosis* isolates were positive for the production of Sapp. These isolates showed a mean Sapp production of 9.47 ng/ml with a range of 1.21 to 43.29 ng/ml. It was observed that the isolate which produced the highest amount of Sapp (43.29 ng/ml) was a superficial oral isolate while the least quantity (1.21 ng/ml) was produced by a systemic isolate. On evaluation of the results a significant intra-species variation in the production of Sapp was demonstrated by the *C. parapsilosis* isolates ($p < 0.0001$).

Production of Sapp by superficial and systemic isolates of *C. parapsilosis*

The superficial (13) and systemic (11) *C. parapsilosis* isolates were compared for their production of Sapp, and a summary comparison of these results is given in Table 3. The superficial *C. parapsilosis* isolates demonstrated a mean value of 14.83 ng/ml while the systemic isolates showed a mean value of 3.14 ng/ml in production of Sapp. A minimum of 3.65 ng/ml and a maximum of 43.29 ng/ml of Sapp were noted in the superficial isolates while these values ranged from 1.21- 6.41 ng/ml for the systemic isolates. Comparing the results of these two groups, the superficial isolates demonstrated almost a four-fold (372 %) increased ability to produce Sap ($p = 0.007$, Fig. 1).

Discussion

The postulate that there is an association between extracellular secreted aspartyl proteinase (Sap) and virulence in *C. albicans* has been known for some time. The Sap activity was first described in 1965 by Staib and subsequently purified from culture filtrates of *C. albicans* by ion-exchange chromatography using cellulose and affinity chromatography by pepstatin-linked sepharose.[5,19] Varying levels of Sap activity in the culture supernatants of individual *C. albicans* isolates have recently been shown by Western blotting.[20] Sap is known to consist of three separate enzymes, depending on the strain used as the source. One of these enzymes is a complete proteinase and the other two are partially proteolytic. Although *C. albicans* Sap activity was first described in 1965, it took a few more years for the discovery of Sapp activity in *C. parapsilosis*.[5,7]

It should be noted that a varying terminology has been assigned to aspartyl proteinase of *Candida*, such as *Candida* acid proteinase (CAP), acid extracellular proteinase (AEP), acid proteinase (ACP)[21,22,23] *Candida albicans* acid proteinase (CAAP) and *Candida* proteinase (CP).[24] At present secreted aspartyl proteinase of *C.*

albicans is abbreviated as Sap while the proteinases of *C. parapsilosis* and *C. tropicalis* are termed Sapp and Sapt, respectively (American Society for Microbiology, 1993). The mode of action of the Sap is far from clear; however, it is possible that the enzyme uncovers sites on epithelial surfaces where the cells adhere, or that it has a cytopathic effect, assisting in spread of infection.[11,25] For instance, the latter group has shown Sap activity associated with blastoconidia attached to excised human epithelium with germ tubes penetrating the epithelial surface.[11] Such reactions were observed when the *C. albicans* penetrated epithelial cells treated with antiproteinase antibody, suggesting *Candida* Sap may be directly involved in fungal invasion and tissue destruction.[11] Ray and Payne (1990) also investigated the role of the Sap in the adherence of *C. albicans* to human epidermal corneocytes and found that pepstatin (a proteinase inhibitor) did not inhibit the attachment of the *Candida* but prevented the formation of cavitations implying that the latter is a result of Sap activity.[26]

Ruchel *et al,* (1986) were the first to isolate and purify Sapp from a clinical isolate of *C. parapsilosis*.[7] They found to it be a lower molecular weight compound (33 Kda) with an increased hydrophobicity compared with Sap of *C. albicans*. Subsequently, Fusek *et al,* (1994) isolated Sapp also from a clinical isolate of *C. parapsilosis* and Staib (1965) demonstrated extra cellular Sap activity in other medically significant species such as *C. tropicalis* and *C. glabrata*.[5,14] Further, a number of researchers have demonstrated that the *Candida* species are not uniform in their production of Sap. For instance, Odds and Abbott (1983), demonstrated that 69.2% of *C. tropicalis* and 71.4% of *C. parapsilosis* isolates were positive for production of Sapt and Sapp.[27] These workers however, were unable to demonstrate a strict correlation between extra cellular proteolytic activity and virulence for *C. parapsilosis* isolates.

Although a number of investigators have studied the aspartyl proteinases of yeasts they have primarily studied their molecular properties.[7,14,16] Only a few researchers have attempted to investigate the degree of Sapp production of a large number of *C. parapsilosis* isolates.[27,28,29,30] It is important to evaluate the Sap activity of a large number of isolates from a given species in order to characterize the species-specific Sap profile. Therefore, in the current study a total of 24 *C. parapsilosis* isolates were evaluated for their production of Sapp.

The results of the current study have shown that all the *C. parapsilosis* isolates are positive for Sapp. Further, these isolates demonstrated a significant intra-species variation in the production of Sapp ranging from 1.21-43.29 ng/ml. These results agree with those of Ruchel *et al,* (1983) and Tobgi (1989).[28,29] Ruchel *et al,* (1983) studied the proteolytic activity of 11 *C. parapsilosis* isolates using a semi-quantitative method.[28] They used bovine serum albumin as the sole nitrogen source and reported that all the *C. parapsilosis* isolates tested to be Sapp positive. Further, Tobgi (1989) also investigated the production of Sapp by five clinical isolates using two semi-quantitative experiments.[29] In the first experiment bovine serum albumin, and in the second, IgA was used as the sole nitrogen source. He showed that all five *C. parapsilosis* isolates expressed Sapp under these two conditions. However, studies of Odds and Abbott (1983) and De Bernardis *et al,* (1989) have shown 71.4% and 91% proteolytic activity in 35 and 11 *C. parapsilosis* isolates, respectively.[27,30] In these studies, Sapp production was ascertained by semi-quantitative methods, serum agar plate and sodium dodecyl sulfate-polyacrylamide gel electrophoresis.[27,30] Therefore it is likely that these investigators may have not detected the Sapp of the reported proteinase negative variants of *C. parapsilosis* isolates due to the poor sensitivity of the techniques used.

There are no previous investigations on the relationship between the origin of the isolate and

the production of Sapp by *C. parapsilosis*. The results of the current study highlights, for the first time, a possible link between the origin of the isolate and production of Sapp as the superficial isolates of *C. parapsilosis* demonstrated a significantly higher ability to produce the enzyme than the systemic isolates.The biological implications of this finding is interesting as it is likely that survival on a host surface requires the proteinase to a greater extent than survival within the host, especially for attachment purposes.

Conclusion

To conclude, the current study demonstrated a significant intra-species variation in the production of Sapp by *C. parapsilosis* isolates ($p<0.0001$). The superficial isolates of *C. parapsilosis* demonstrated a significantly higher ability to produce Sapp than their systemic counterparts ($p=0.01$) implying a possible role of the yeast habitat in modulating this attribute.

Table 1. The production of Sapp by 24 clinical isolates of *C. parapsilosis*

C. Parapsilosis Isolate no	Sap production (ng/ml)		
	Assay1	Assay 2	Mean ± SEM
N1	6.49	5.81	6.26 ± 0.21
	6.74	6.00	
N2	5.90	3.29	5.41 ± 0.73
	6.67	5.81	
N3	4.79	1.62	2.65 ± 0.81
	1.14	3.07	
N4	1.97	4.03	4.54 ± 0.98
	6.09	6.09	
N5	4.86	3.67	3.65 ± 0.79
	1.39	4.69	
N6	1.46	5.40	3.75 ± 1.21
	1.85	6.23	
N7	1.49	1.03	1.25 ± 0.51
	0.00	2.48	
N8	0.00	0.81	1.21 ± 0.64
	1.01	3.03	
N9	3.83	1.01	1.57 ± 0.81
	0.00	1.46	
N10	21.71	24.08	23.89 ± 1.30
	22.27	27.50	
N11	2.23	1.03	2.55 ± 1.11
	1.16	5,81	
N12	1.27	3.99	2.19 ± 0.61
	1.55	1.97	
N13	5.79	7.48	6.41 ± 0.98
	3.95	8.43	
N14	1.62	4.33	4.99 ± 1.76
	4.07	9.95	
N15	3.91	1.52	3.45 ± 0.69
	4.79	3.59	
O1	6.46	8.42	7.22 ± 0.84
	5.22	8.79	
O2	35.70	42.40	33.90 ± 3.28
	28.00	29.50	
O3	5.22	5.25	5.21 ± 0.24
	4.58	5.79	
O4	11.33	7.01	9.33 ± 1.11
	11.13	7.85	
O5	6.67	5.43	5.85 ± 0.34
	5.15	6.17	
O6	7.68	5.81	7.34 ± 0.67
	6.89	9.01	
CI-1	32.19	35.83	34.17 ± 1.61
	30.83	37.83	
CI -2	7.00	8.38	7.37 ± 0.47
	6.24	7.86	
CI-3	39.48	46.23	43.29 ± 1.42
	44.30	43.18	

Table 2. A summary table showing the mean Sapp (ng/ml) production by 24 *C. Parapsilosis isolates*

Candida parapsilosis isolate	Production of Sapp (ng/ml)
N1	6.26 ± 0.21
N2	5.41 ± 0.73
N3	2.65 ± 0.81
N4	4.54 ± 0.98
N5	3.65 ± 0.79
N6	3.75 ± 1.21
N7	1.25 ± 0.51
N8	1.21 ± 0.64
N9	1.57 ± 0.81
N10	23.89 ± 1.30
N11	2.55 ± 1.11
N12	2.19 ± 0.61
N13	6.41 ± 0.98
N14	4.99 ± 1.76
N15	3.45 ± 0.69
O1	7.22 ± 0.84
O2	33.90 ± 3.28
O3	5.21 ± 0.24
O4	9.33 ± 1.11
O5	5.85 ± 0.34
O6	7.34 ± 0.67
CI-1	34.17 ± 1.61
CI-2	7.37 ± 0.47
CI-3	43.29 ± 1.42
Overall mean ± SEM	9.47 ± 0.50

Table 3. A comparison of the mean values of the Sapp production by 13 superficial and 11 systemic isolates of *C. parapsilosis*

Candida parapsilosis isolate	Superficial (ng/ml)	Systemic (ng/ml)
N1	6.26 ± 0.21	*
N2	5.41 ± 0.73	*
N3	*	2.65 ± 0.81
N4	*	4.54 ± 0.98
N5	3.65 ± 0.79	*
N6	*	3.75 ± 1.21
N7	*	1.25 ± 0.51
N8	*	1.21 ± 0.64
N9	*	1.57 ± 0.81
N10	23.89 ± 1.30	*
N11	*	2.55 ± 1.11
N12	*	2.19 ± 0.61
N13	*	6.41 ± 0.98
N14	*	4.99 ± 1.76
N15	*	3.45 ± 0.69
O1	7.22 ± 0.84	*
O2	33.90 ± 3.28	*
O3	5.21 ± 0.24	*
O4	9.33 ± 1.11	*
O5	5.85 ± 0.34	*
O6	7.34 ± 0.67	*
CI-1	34.17 ± 1.61	*
CI-2	7.37 ± 0.47	*
CI-3	43.29 ± 1.42	*
Overall mean ± SEM	$14.83^{a} \pm 3.81$	$3.14^{b} \pm 0.50$
p value: a vs b = 0.007		

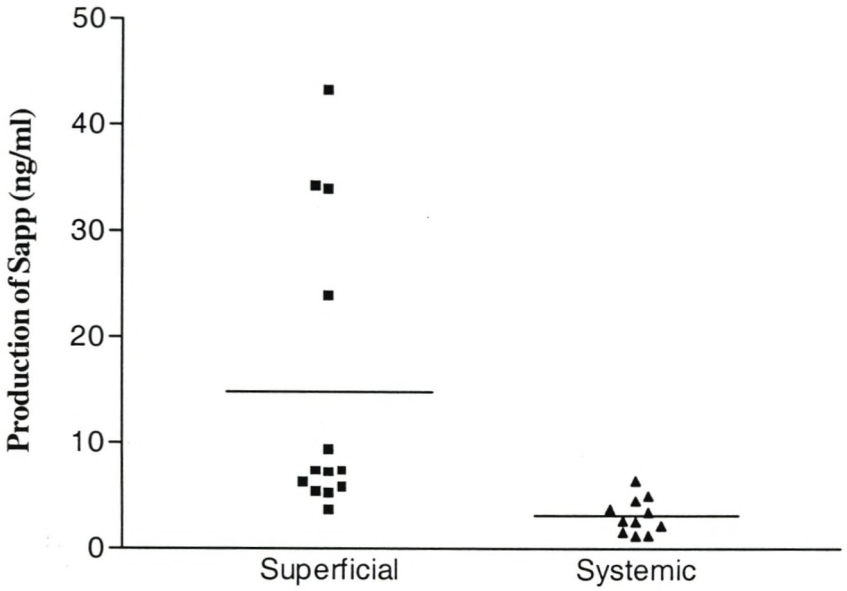

Figure 1. A scattergram showing the production of sapp by superficial and systemic isolates of *C. parapsilosis*

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Research Article II

Sri Lanka Dental Journal 2007; 37(01) 29-36

Validation of a modified version of the Oral Impacts on Daily Performances (OIDP) index for use among adolescents in Sri Lanka

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Abstract

Objective: to test the psychometric properties of a Sinhala translation of a modified version of the Oral Impact on Daily Performance index (OIDP) among adolescents in Sri Lanka.

Material and methods: A cross-sectional study was conducted among 227, 15-year-old children drawn from five randomly selected schools from the Panadura educational area of the Kalutara district in Sri Lanka. The psychometric properties considered for evaluation were reliability in terms of internal consistency and validity analyses included the assessment of face, content, criterion and construct validity. Reliability of the translated index was assessed in terms of the internal consistency using Cronbach's alpha. Criterion validity was determined by examining the association between OIDP scores and self-rating of oral health. To test the construct validity, associations between OIDP scores, perceived severity of symptoms and perceived need for dental care were assessed.

Results: Cronbach's alpha of the translated index was 0.94. Corrected item-total correlation coefficients ranged from 0.63 to 0.86. The significant associations between OIDP scores and self-rating of oral health, perceived severity of symptoms and perceived need for dental care support the criterion and construct validity of the translated index.

Conclusion: The Sinhala translation of the modified version of the OIDP is a valid and reliable index to assess the oral health related quality of life in adolescents in Sri Lanka.

Key wards: OIDP adoliscents validation

Introduction

It is well known that oral conditions have a considerable impact on the quality of life of individuals. Thus assessment of psychosocial implications of oral health/illness and quality of life is now considered a priority area in oral health research.[1] Several oral health-related quality of life measures have been developed and used in oral health surveys in recent times. These range from a single-item global oral health rating to multi dimensional instruments.[2,3]

The Oral Impacts on Daily Performances (OIDP) index is an 8-item oral health related quality of life indicator with acceptable psychometric properties.[4] Its theoretical framework is based on the International Classification of Impairments, Disabilities and Handicaps of the World Health

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Organization and amended for dentistry by Locker.[5,6] The OIDP index measures the serious oral impacts on a person's ability to perform daily activities, such as physical, social and psychological performances.The main advantages of the OIDP index over other oral health-related quality of life measures are that being short it reduces the response burden and it measures behavioural rather than feeling state impacts.

Most oral health-related quality of life indicators have been primarily developed for use in adults or the elderly and the OIDP index is no exception in this regard. Psychometric properties of the OIDP index were first evaluated among 34-44 year old Thai adults. However, it is one indicator that has proved to be valid and reliable in assessing the impact of oral conditions on the quality of life of adolescents from different socio-cultural backgrounds.[7,8,9,10] Continuing this process of cross-cultural adaptation of the OIDP index is important, as it will allow for international comparisons and multi-centre research studies on oral health related quality of life of adolescents. The aim of this study was therefore to assess the psychometric properties of a Sinhala translation of a modified version of the index of OIDP.

Material and methods:

The data for the present paper were obtained from a pilot study that was conducted to determine the sample size and validate several indicators for a comprehensive study on social inequalities in oral health among adolescents in Sri Lanka. Only the methodology and the results associated with assessing the psychometric properties of the OIDP index will be presented here.

The OIDP index in its original form assesses oral impacts associated with 8 daily performances. They include: eating and enjoying food; speaking and pronouncing clearly; cleaning teeth; sleeping and relaxing; smiling, laughing and showing teeth without embarrassment; maintaining usual emotional state without being irritable; carrying out major work or social role; and enjoying contact with people. An OIDP score for an individual is calculated by multiplying the frequency and severity scores for each item and then summing the 8 items and is finally presented as a percentage. As the multiplication scores did not show any significant improvement over using the frequency or severity scores alone, it has been suggested that either the frequency or the severity score could be used for simplicity.[4]

The psychometric properties considered for evaluation were reliability in terms of internal consistency and validity analyses included the assessment of face, content, criterion and construct validity. For this purpose the first step included the translation of the OIDP index to Sinhala language by a professional translator. An independent person who was not involved in the study, subsequently back translated the Sinhala version to English in order to ensure accuracy and comparability of the translation.

Face validity refers to whether the instrument appears to measure what it intends to measure and is determined by the judgment made by an investigator or a non-expert on the surface appearance. To assess face validity of the translated index, a discussion was held with a group of 20, 15-year-olds attending the orthodontic clinic of a dental hospital. Content validity is similar to face validity expect that it refers to judgements made by a panel of experts about the extent to which the contents of the instrument measures what it intends to measure. Content validity of the translated index was assessed by conducting a discussion with a panel of experts consisting of consultants and registrars in community dentistry and a sociologist. They assessed the comprehensiveness of the items of the translated OIDP index, the clarity and relevance in the Sri Lankan context. Based on the comments made by the children and the expert panel, it was decided to make certain modifications to the index. They included changing the wording of

some items, considering only the frequency score for the purpose of calculating the OIDP score and also simplifying the criteria for reporting the frequency of impacts.

The Sinhala translation of the modified OIDP index included the following items: eating and enjoying food; speaking and pronouncing clearly; cleaning teeth; sleeping and relaxing; smiling and showing teeth without embarrassment; maintaining good emotional state; carrying out school- and other extra curricular activities; and enjoying contact with friends. Also it was considered to record the frequency of impacts experienced over the preceding 6-months on a 5-point Likert scale: never=0; hardly ever=1; occasionally=2; fairly often = 3 and very often =4 rather than what had been recommended in the original index, which was found to be confusing for the children.

Criterion validity is the extent to which an instrument agrees with a "gold standard". Since there is no "gold standard" for an oral health-related quality of life index, criterion validity was established by examining the association between ODIP scores and self-rating of oral health status (4-point scale). Construct validity is the extent to which the instrument tests the hypothesis or theory it is measuring. To test the construct validity of the translated index, the associations between the OIDP scores and perceived severity of symptoms (4-point scale) and perceived need for dental care (3-point scale) were examined. In order to determine the clarity of the Sinhala version of the modified OIDP index, it was pre-tested among a group of 30, 15-year-olds attending a dental hospital. Very minor modifications were made at this stage.

The sample of this pilot study consisted of 227, 15-year-old children drawn from five randomly selected schools in the Panadura educational area of the Kalutara district in Sri Lanka. As it was necessary to include children from varying socio-economic backgrounds, the schools selected included three state- and two private schools. All children who had completed their 15th but not the 16th birthday from these 5 schools were included in the sample. Data for the pilot study was collected by means of a self-administered structured questionnaire to the students, which included the translated OIDP index as well. Informed written consent was obtained from both students and their parents. Permission to conduct the study was obtained from the provincial and zonal directors of education and also from the principals of the selected schools. Ethical approval for the study was obtained from the Ethical Review committee of the Faculty of Medicine, University of Colombo.

SPSS ® 13 software package was used for data analysis. The OIDP score for a student was calculated by summing the response codes for the 8 items. The OIDP score for a student ranged from 0-32 with higher scores denoting poor oral health-related quality of life. The prevalence of oral impacts was determined by calculating the percentage of students who reported at least one impact item fairly often or very often. Internal consistency of the translated index was assessed using Cronbach's alpha statistic, which measures the overall correlation between items within the scale. In relation to the internal consistency, inter-item and corrected item-total correlation coefficients for the different OIDP index items were also calculated. As the OIDP scores were not normally distributed, it was necessary to use non-parametric tests (Krushkal Wallis test) to determine the associations between OIDP scores and self-rating of oral health, perceived severity of symptoms and perceived need for dental care.

Results

The prevalence of oral impacts (OIDP>0) among the sample was 60.4%. The median OIDP score was 1 with a range of 0-32. The correlation matrix for the 8-item OIDP index is presented in Table 1. Inter-item correlation coefficients ranged from 0.46 (the relationship between smiling, laughing and eating and enjoying food) to 0.85 (the

relationship between enjoying contact with people and carrying out schoolwork). No inter-item correlation was negative indicating the homogeneity of the items. Moreover no inter-item correlation was high enough for any item to be redundant. The corrected item-total correlation coefficients ranged from 0.63 (eating and enjoying food) to 0.86 (maintaining usual emotional state).The Cronbach's alpha coefficient of the index was 0.94. The alpha coefficient was also calculated after having deleted one item at a time. For all 8 items, the value of alpha did not increase beyond the original value of 0.94 when an item was deleted (Table 2)

Table 3 shows the results of the assessments of criterion and construct validity of the OIDP index. There was a highly significant difference between OIDP scores and self-rating of oral health status. Compared to those who rated their oral health as good or satisfactory, the median OIDP score of those who rated their oral health as poor was higher. Also there were significant differences between OIDP scores and perceived severity of symptoms and perceived need for dental care. There was a trend for the median OIDP score to increase significantly with the increase in the intensity of the perceived severity of symptoms. Moreover, those who perceived a need for dental care had a higher median OIDP score compared to those who did not perceive a need for dental care. All 227 subjects had responded to all 8-items of the OIDP index and there were no missing values.

Discussion

Oral health-related quality of life instruments are culture specific. Thus if an instrument is to be used in a population that is culturally different to that of the original population for which it was developed, the instrument needs to be translated and validated before it could be applied to other cultures. The present study assessed the psychometric properties of a Sinhala translation of a modified version of the OIDP index in order to ensure its appropriateness to assess oral health-related quality of life among adolescents in Sri Lanka.

The results indicate that the Sinhala translation of a modified version of the OIDP index has excellent psychometric properties. When the back translated and the original English version of the OIDP index was compared, it was evident that they were very similar. This indicates the appropriateness of the Sinhala version of the index. The face and content validity of the index were established at the pre-pilot stage. Based on the comments made by the groups of adolescents and experts during this stage, it was decided to make certain modifications to the index. The item "carrying out major work or social role" in the original index was replaced by the item "carrying out school work and extra curricular activities" which was, considered more relevant in the context of adolescents. Moreover, minor alterations to the wording of certain items were made and the frequency of impacts was recorded according to a modified scale.

Internal consistency is a measure of the extent to which items of a scale provide consistent information. It was assessed in a number of ways and all assessments provided evidence for very satisfactory internal consistency of the translated index. First, no inter-item correlation coefficient was negative denoting the homogeneity among the items of the index. Second, the Cronbach's alpha of the index was 0.94 thus indicating a high internal consistency of the index. For an instrument, a Cronbach's alpha value of 0.7 is deemed minimally reliable.[11] In previous studies where translated versions of the OIDP had been validated for use among adolescents, alpha values ranging from 0.70 to 0.84 have been reported.[7,8,10] Third, the fit of a specific item to the index was assessed by deleting the item and observing the change that the omission caused to the alpha value. It was evident that deleting any of the 8-items did not increase the alpha co-efficient. If an item were well fitted to its scale, the value of

alpha would decrease when the particular item is deleted from the scale. Fourth, the corrected item-total correlation coefficients ranged from 0.63 to 0.86 and were all above the minimum recommended corrected item-total correlation of 0.20 for including an item in a scale.[12]

Criterion validity of an instrument has to be determined by comparing with a "gold standard". However as there is no "gold standard' indicator for oral health-related quality of life, a single-item global rating of oral health has been suggested as a proxy gold standard.[13] The significant association between self-rating of oral health and OIDP scores thus provided evidence for criterion validity of the translated index. The Sinhala version of the OIDP was shown to be a valid index in terms of construct validity as well. Significant associations between OIDP scores and perceived severity of symptoms and perceived need for dental care demonstrated this aspect of the scale. It is noteworthy that all 227 subjects had responded to all 8-items of the index giving a 100% response rate for all items. This indicates the very high acceptability of the index. The fact that the index is short thus reducing the response burden and the wording of the items was simple and clear may have contributed to the high acceptability of the index.

In conclusion, the results revealed that the Sinhala version of the modified OIDP index has excellent psychometric properties in terms of reliability and validity. Thus this index could be considered as an appropriate tool to assess oral health-related quality of life among adolescents in Sri Lanka.

Table 1. Reliability analysis: OIDP inter-item correlation matrix

ODIP impact item	1	2	3	4	5	6	7	8
1. Eating and enjoying food	1.00							
2. Speaking & pronouncing clearly	0.57	1.00						
3. Cleaning teeth	0.52	0.70	1.00					
4. Sleeping & relaxing	0.57	0.64	0.69	1.00				
5. Smiling, laughing & showing teeth with out embarrassment	0.46	0.56	0.62	0.64	1.00			
6. Maintaining usual emotional status with being irritable	0.57	0.64	0.68	0.74	0.74	1.00		
7. Carrying out school work & other activities	0.60	0.58	0.62	0.72	0.68	0.80	1.00	
8. Enjoying contact with friends & other people	0.51	0.63	0.64	0.71	0.66	0.82	0.85	1.00

Table 2. Reliability analysis: corrected item-total correlation, alpha if item deleted and Cronbach's alpha

ODIP impact item	Corrected item-total correlation	Alpha if item deleted
Eating and enjoying food	0.63	0.94
Speaking and pronouncing clearly	0.74	0.93
Cleaning teeth	0.76	0.93
Sleeping and relaxing	0.81	0.93
Smiling, laughing and showing teeth - without embarrassment	0.74	0.93
Maintaining usual emotional status - with being irritable	0.86	0.92
Carrying out school work and other activities	0.84	0.92
Enjoying contact with friends and - other people	0.83	0.92
Cronbach's alpha	0.94	

Table 3. Criterion and construct validity: associations between OIDP scores and self-rating of oral health, perceived severity of symptoms and perceived need for dental care

Variable		OIDP score		
		mean rank	Median	(range)
Self-rating of oral health				
Good	(82)	102.1	0.0	(0- 32)
Satisfactory	(92)	109.3	1.0	(0-28)
Poor	(16)	178.1	16.0	(0-26)
Don't know	(37)	124.5	2.0	(0-30)
		P=0.001		
Perceived severity of symptoms				
No symptoms	(117)	103.2	0.0	(0-32)
Mild	(83)	115.7	2.0	(0-30)
Moderate	(24)	150.3	6.5	(0-22)
Severe	(3)	198.5	14.0	(12-24)
		P=0.001		
Perceived need for dental care				
Yes	(92)	129.1	9.0	(0-30)
No	(76)	99.9	0.0	(0-30)
Don't know	(59)	108.5	1.0	(0-32)
		P=0.009		

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Research Article III

Sri Lanka Dental Journal 2007; 37(01) 37-47

The survival of immediate placement resin retained fixed prostheses

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Abstract

Objectives: This paper describes the fabrication and use of an immediate resin bonded fixed prosthesis to replace single missing tooth in a Sri Lankan general dental practice. The aim of this study was to investigate the survival of these immediate placement resin retained bridges placed between 1992-1996 which were reviewed in 1999.

Design: The records of 50 consecutive patients who had received immediate placement resin retained bridges in a general practice in Sri Lanka and who attended for review during 1999 were analysed and survival data determined.

Results: The mean survival time of bridges was 41±35 months. In the maxilla the mean duration was 38±31months and in the mandible 57±46 months (not significant). The survival time for bridges replacing incisors was 50±39 months, canines 31±9 months and posterior teeth 33±30 months (not significant). Bridges in male patients had a mean survival time of 38±38 months and females 44±32 months (not significant). The mean duration of each bond was 12 months and rebondings up to four times was carried out. Rebonding provided similar bond durations and so extended the life of the restoration. Patient satisfaction was high.

Conclusion: The immediate placement resin retained bridges described in this paper offers a medium-term solution where laboratory facilities are not readily available. The technique can also be used to provide an interim restoration.

Key words: immediate replacement, fixed prosthesis, bridge

Introduction

The first description of an acid-etched composite retained pontic was in 1973. and the first study of a wire-free, composite retained bridge described a survival of 21 out of 31 at 33 months.[1,2] Interestingly the mode of failure was described as cohesive failure of the resin.

In a laboratory study comparing acid etched composite retained bridges the inclusion of a wire in the resin did not improve resistance to displacing forces.[3] However, wire has been used with orthodontic composite retainers.[4]

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Rochette was the first to describe the use of a resin-bonded cast metal prosthesis for the splinting of periodontally mobile teeth using a perforated, cast metal framework that was cemented with acrylic resin to acid etched enamel.[5] Later Howe and Denehy described a technique that used a cast metal perforated bridge framework with clinical success and such restorations have subsequently come to be known as Rochette bridges.[6,7] Wise illustrated an acid-etch retained provisional bridge which avoids the need to prepare unrestored teeth or compromise the anterior occlusion.[8] He suggested that if the occlusion permits, the retainer should be confined to the lingual surface of the abutments with the use of a Rochette bridge so that removal can be readily accomplished by cutting through the resin tags.

Previous studies have shown that the Rochette bridge is more likely to debond than other designs but because of the greater ability to remove and rebond its overall survival time, including rebonds, is comparable.[9] The ability to remove and rebond the bridge during treatment has made it suitable for a provisional restoration during implant placement.[10]

One disadvantage with indirect resin-retained bridges is the need for laboratory support which may not be readily available. Laboratory facilities are required to cast the metal framework and fabricate a ceramic pontic. Clinically, two visits are required. There are also clinical emergencies where an immediate restoration, fabricated at the chairside is required and techniques have been described.[11,12]

In a developing country it can be difficult to obtain access to a dental laboratory with casting facilities. Even if such facilities are available patients may be unable to receive this treatment because of the cost, travel difficulties and other socio-economic factors which discourage two-visit treatment plans.[13] Hence a low cost simple chairside treatment was utilised and the survival of this design was monitored in this study.

The current paper describes a technique loosely based on the Rochette bridge concept but fabricated using wire and a denture tooth rather than the more costly cast framework. This modified Rochette-type bridge was used in Sri Lanka and appeared successful. This study was designed to investigate the longevity of these bridges.

Methods

The immediate technique was developed by one of the authors (BGN) based on previously described techniques using composite-alone and cast metal frameworks. When a patient presented with a missing anterior tooth (Fig. 1) an alginate impresssion was recorded following prophylaxis and caries management (Fig. 2). Casts were mounted on an articulator (Fig. 3) and a wire framework fabricated in 0.7mm stainless steel orthodontic wire (Fig. 4). The wire loops were designed to be away from the occluding areas and the wire ends were incorporated within the body of the denture tooth pontic (Fig. 5). The bridge was cemented using acid-etch retained composite resin [*Spectrum*, Dentsply] (Fig. 6) and the occlusion adjusted. A post-op view shows the restoration in place (Fig. 7&8). While these restorations were fabricated on a model the technique can also be used clinically as a chairside procedure.

All patients receiving this design of bridge between October 1992 and April 1996 were included in the study. Patients were followed-up by recall at intervals of 2 weeks, 3 months and

yearly where possible. A final review was carried out in 1999 when all bridges were assessed.

Assessment at recall included obtaining the following records:

- a caries check under and around the bridge
- periodontal check using pocket probing depths including recording of recession
- debris index
- plaque index
- gingival condition
- evidence of debonding
- damage to bridge such as fracture, exposure of wire
- a bridge categorised as satisfactory or failed.

Analysis was carried out once all data had been collected and statistical calculations were made using *Sigmastat.*

Results

A total of 50 bridges were placed and analysed during the period October 1992 and April 1996. These restorations were followed-up until 1999. Records were kept showing all rebonds and data from each recall visit. Table I shows a summary of the outcomes.

Investigating the mean survival duration of each bonding cycle (Table 2): 1st life of 8.8±14.6 months (n = 37), 2nd life of 17.3±15.8 months (n = 18), 3rd life of 16.9±19.4 months (n = 7) and a 4th life of 7 months (n = 1).

Analysis of the survival data according to patient's age at placement (Table 3) shows that the mean survival was 12±16 months for patients aged 10-19 (n=2), 39±27 months for patients aged 20-29 (n=22), 42±43 months for patients aged 30-39 (n=18) and 53±35 months for patients aged 40-49 (n=8). This was not significant (ANOVA, P=0.485).

A comparison of survival of the bridges according to the patient's gender is presented in Table 4. Bridges placed in male patients had a mean survival time of 38±38 months (n=22) and those in females 44±32 months (n=28). This difference was not significant (t-test, P=0.536).

An analysis of the survival time according to the location of the restoration in the maxilla or mandible was carried out (Table 5). The mean survival time in the maxilla was 38±32 months (n=42) and in the mandible 57±46 months (8). This difference is not significant (t-test, P=0.156).

Comparing the position of the pontic in the arch shows (Table 6) that the survival times for bridges replacing incisors was 50±39 months (n=25), canines 31±9 months (n=3) and posterior teeth 33±30 months (n=22). This is not significance (ANOVA, P=0.229).

Investigating the survival according to number of teeth replaced by the bridge (Table 7) indicates a survival time of 35± 30 months for single pontics (n = 44) and 87±29 months for multiple tooth pontics (n = 6). This difference is significant (t-test, P<0.001) but reflects the use of the multiple pontic bridges only for the replacement of central incisors.

Figure. 9 illustrates the Kaplan-Meier Probability of Survival for the immediate adhesive bridges expressed in months.

Discussion

The overall survival duration, including rebonding where necessary, was 41±35 months. This is less than cast framework adhesive bridges: 2.7

3.25 years.[17] However, the technique is suitable for medium-term definitive restorations or interim, temporary or provisional restorations. Rebonding was a viable means of extending the life of these restorations, as has been shown for cast framework adhesive bridges.[9,18] The majority of patients were satisfied with the treatment, as in other studies.[19]

The duration of service according to each bond was a mean of 12 months again suggesting that the technique would be suitable for use as a temporary replacement. This duration is comparable with cast framework adhesive bridges.[9]

There was no significant difference between restorations placed in either jaw, or the position of the pontic in the jaw, or patients gender or age. This differs from data available for cast framework adhesive bridges.[20,21] However, the smaller number of double pontic restorations replacing incisors did significantly better than bridges taken as a whole. There was an increased accummulation of plaque in agreement with al-Wahadmi *et al.*[22]

Conclusions

The adhesive bridge described in this paper provided a simple, immediate restoration suitable for medium-term use. The survival duration was less than cast frame design but could be provided at a lower cost.

Table 1. Data Analysis for bonded and rebonded bridges

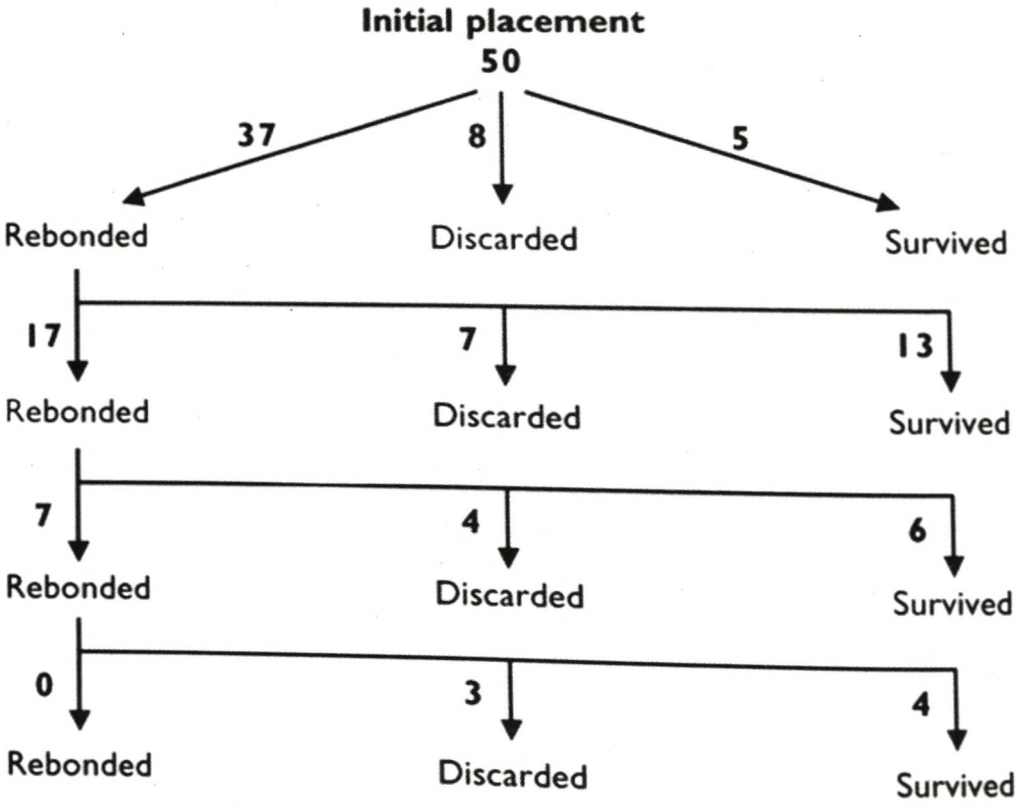

Table 2. Mean survival duration following each of the bonding procedures.

1st life =	8.8 ± 14.6	mo, n = 37
2nd life =	17.3 ± 15.8	mo, n = 18
3rd life =	16.9 ± 19.4	mo, n = 7
4th life =	7± 0	mo, n = 1

Table 3. Mean survival duration for bridges accoording to the age of the patient (ns=not significant) (p>0.05).

Age	N	Mean	SD
10-19	2	11.5	16.3
20-29	22	39.2	27.3
30-39	18	41.6	42.7
40-49	8	53.1	35.0

Table 4. Mean survival duration for bridges in male and female patients (ns=not significant) (p>0.05).

	N	Mean	SD
Male	22	37.7	38.1
Female	28	43.9	31.9

Table 5. Mean survival duration for bridges in maxilla and mandible (ns=not significant) (p>0.05).

	N	Mean	SD
Maxilla	42	37.7	32.1
Mandible	8	57.2	45.8
Total	50	41.2	34.6

Table 6. Mean survival duration for bridges replacing incisors, canines and posterior teeth (ns=not significant) (p>0.05).

	N	Mean	SD
Incisors	25	50.1	38.6
Canine	3	31.4	9.3
Post.teeth	22	32.8	30.2

Table 7. Mean survival duration for bridges according to number of teeth replaced (significant) (p<0.05).

	N	Mean	SD
Single pontic	44	35.3	29.8
Multiple pontic	6	87.1	29.3

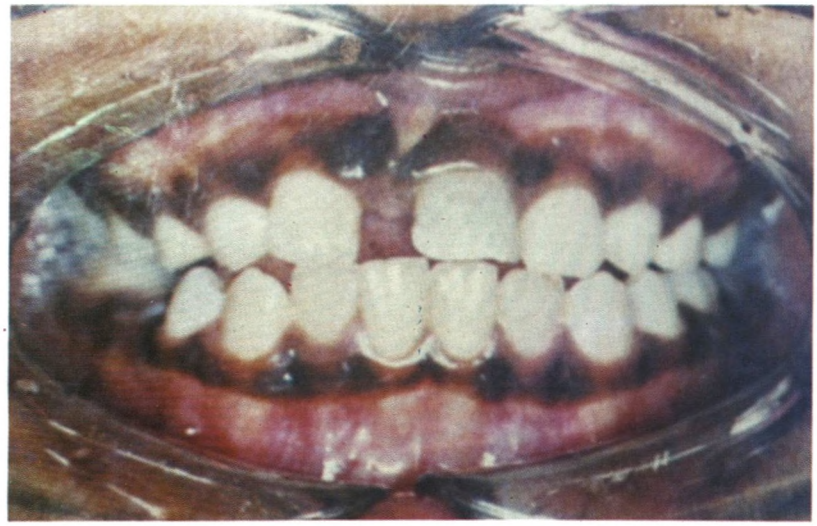

Figure 1. Missing 11 following caries management and composite build-up on 12 to match 22.

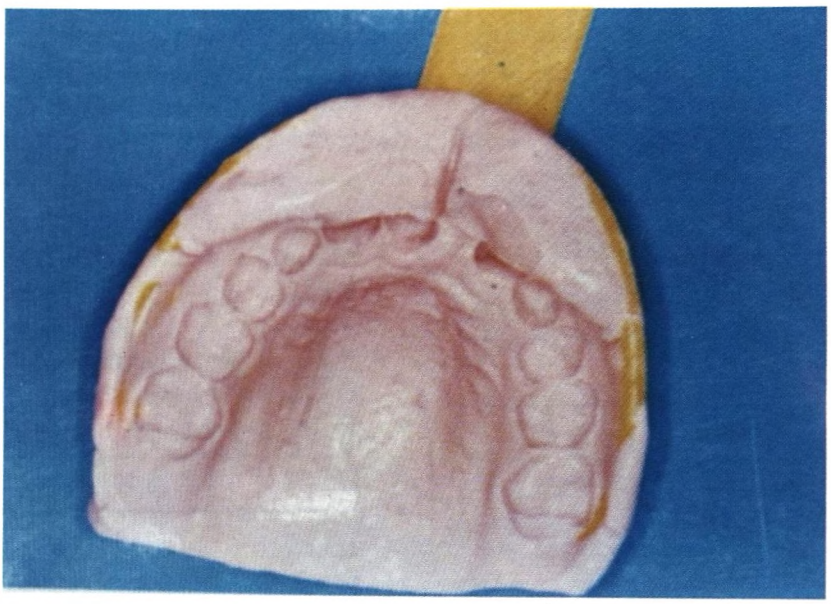

Figure 2. Alginate working impression

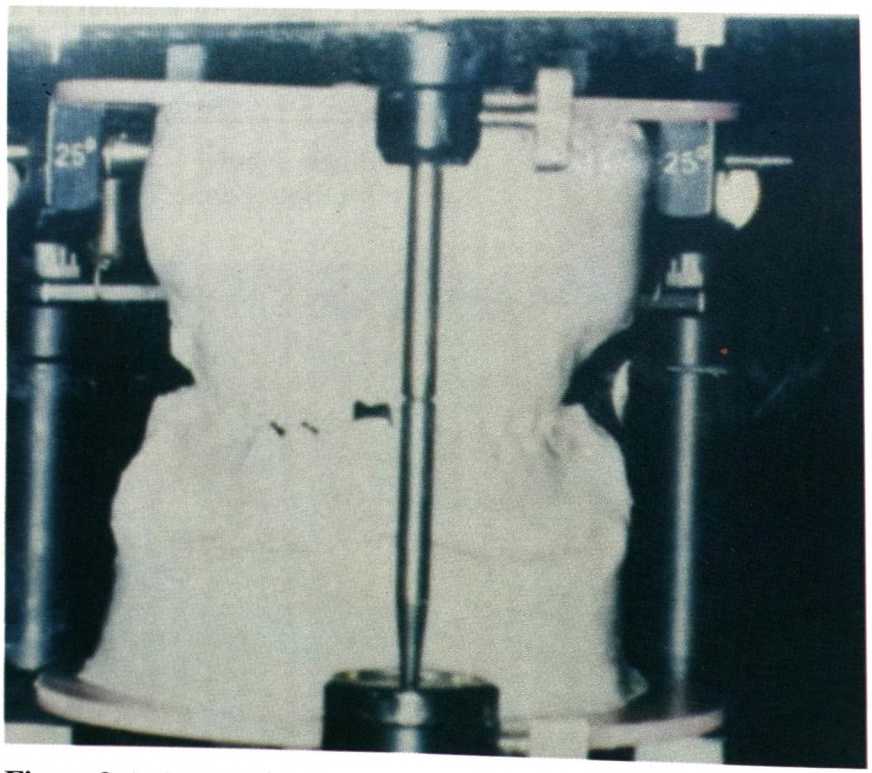

Figure 3. Articulated casts

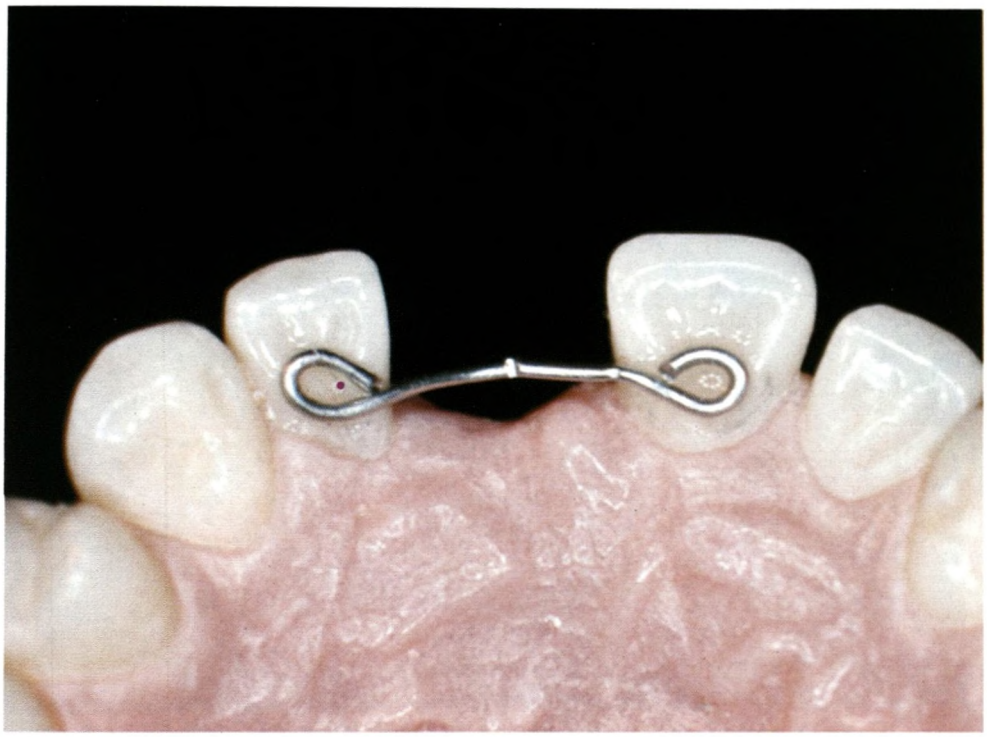

Figure 4. Close-up on model illustrating the wire loops

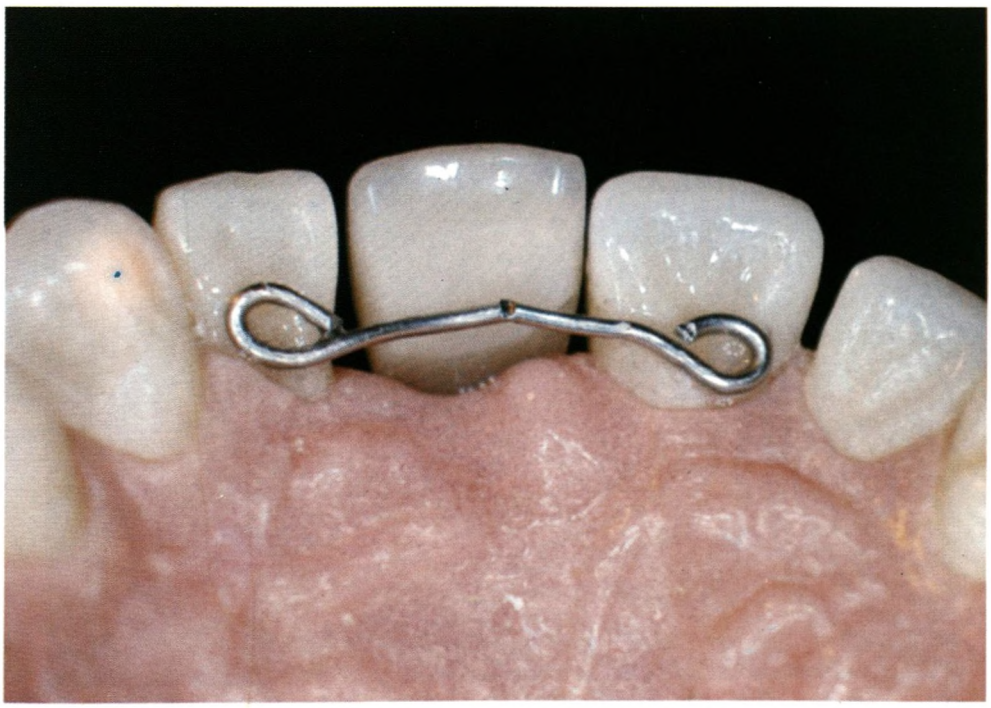

Figure 5. Close-up on showing the wire loops embedded in a denture tooth

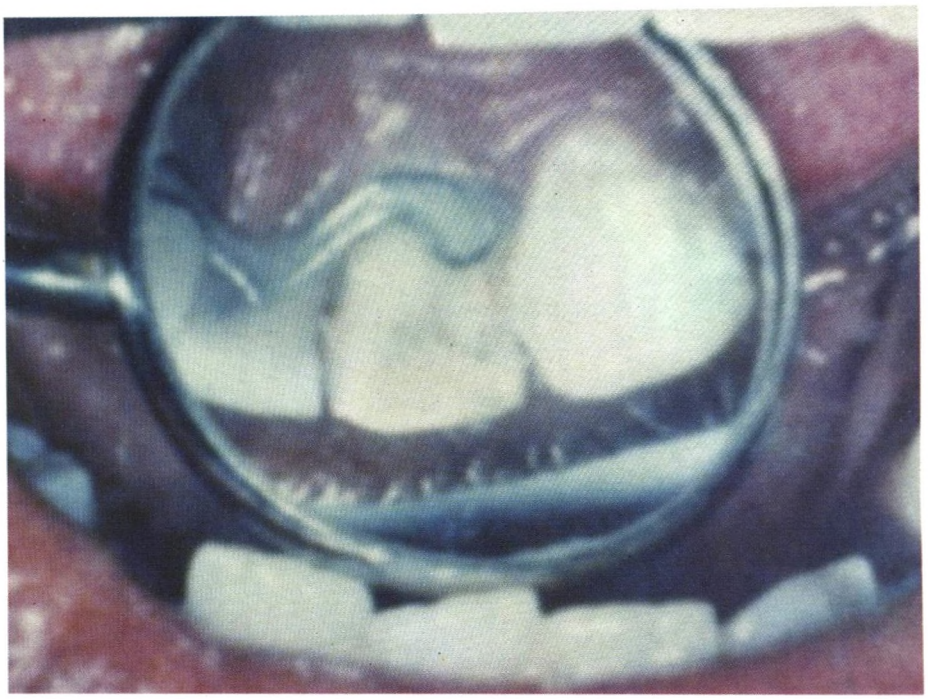

Figure 6. A bridge cemented in place with composite resin

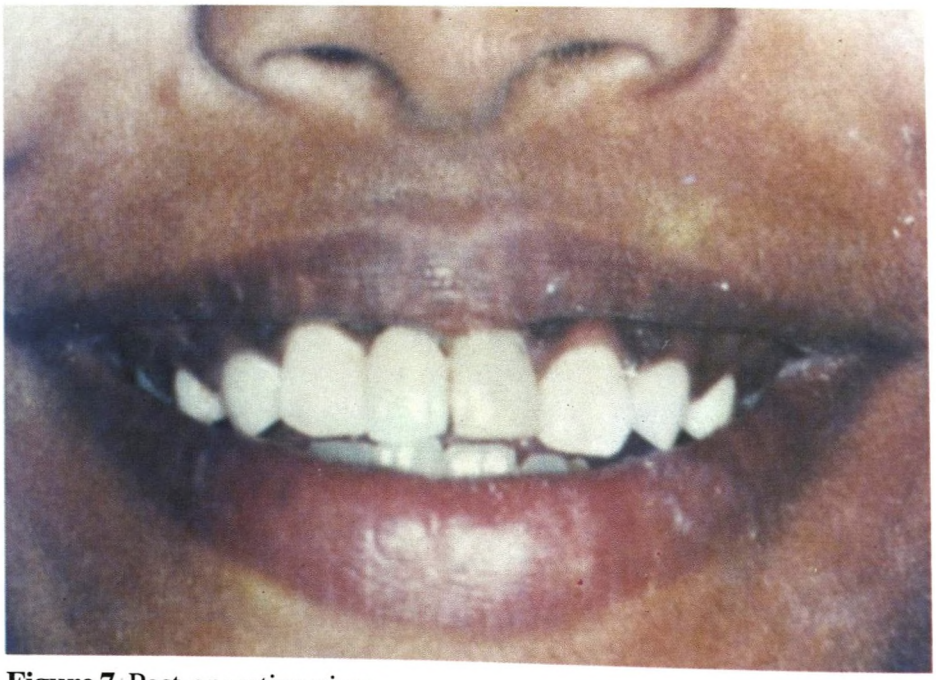

Figure 7. Post-operative view

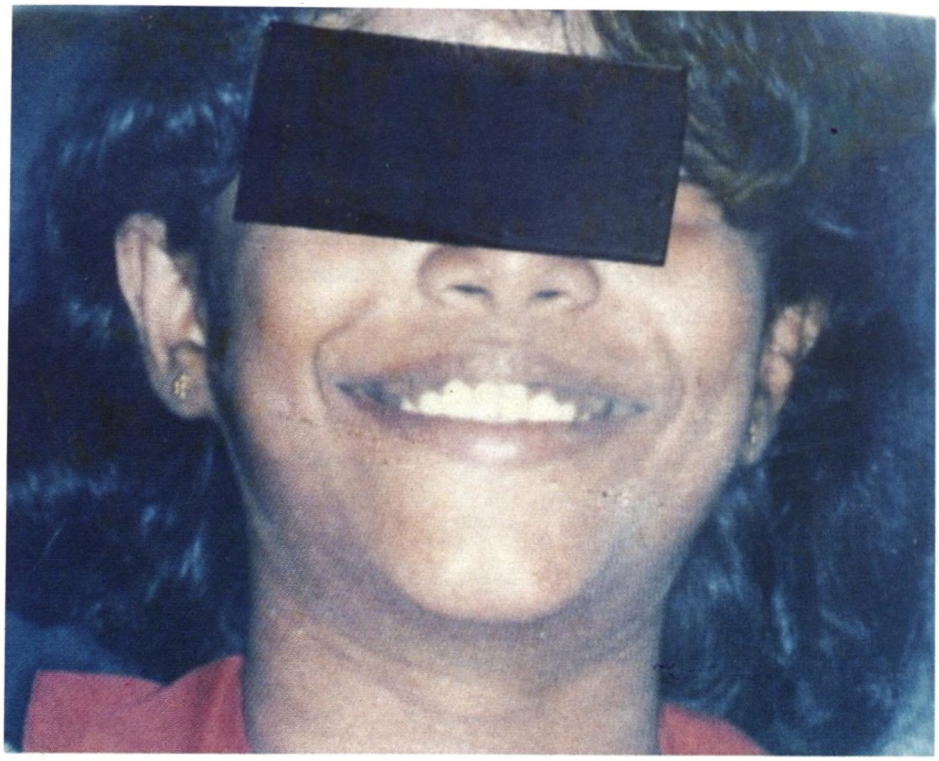

Figure 8. Post-operative view

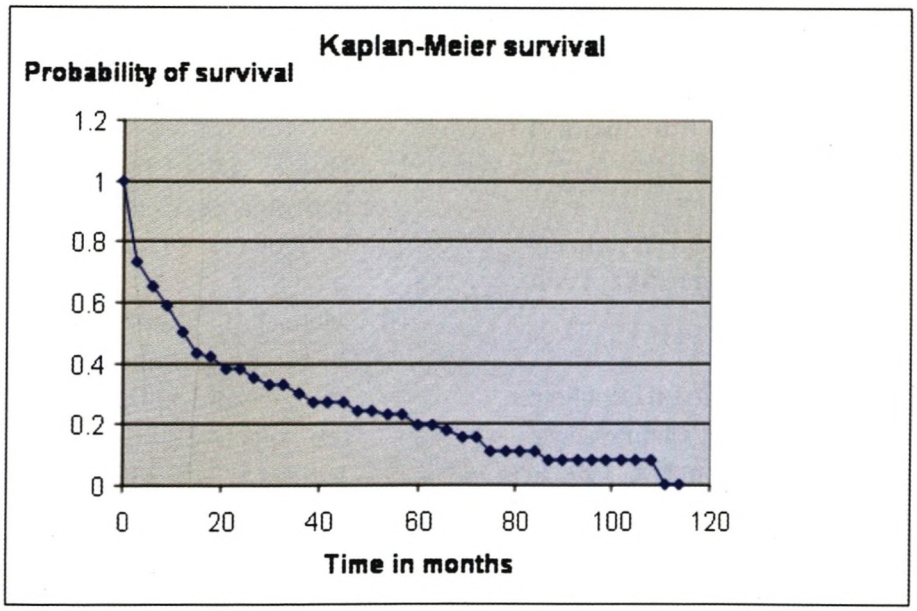

Figure 9. Kaplan-Meier Probability of Survival for the immediate adhesive bridges.

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Case Report

Sri Lanka Dental Journal 2007; 37(01) 48-54

Parotid abscess following inferior alveolar nerve block: A case report and a review of complications of the inferior alveolar nerve block

M.A.M. Sitheeque

Abstract:

The inferior alveolar nerve block (IANB) is one of the commonest local anaesthetic procedures undertaken by a dental surgeon. It is the most technically demanding procedure among all local anaesthetic injections administered by a dental surgeon. It has a failure rate of about 15-20%. The paper reviews the relevant surgical anatomy and the direct and indirect techniques of IANB. Other techniques available for achieving analgesia in the mandibular region are also briefly stated. Complications reported in the literature as sequel to IANB are reviewed. A case of parotid abscess that developed within three days of attempted multiple IANB injections, accompanied by fever and trismus in a 20 years old female is presented together with ultrasonographic evidence. The case was managed by drainage of pus and antibiotic therapy. How this previously unreported complication might have arisen and the measures to prevent it are discussed. .

Introduction

The inferior alveolar nerve block (IANB), more popularly referred to as the inferior dental (ID) block or mandibular block, administered to obtain local anaesthesia in the mandibular region is one of the commonest clinical procedures undertaken by a dental surgeon. It can provide analgesia of all mandibular teeth and the lower labial mucosa of the administered side. In conjunction with lingual and long buccal blocks with which it can be easily combined, it can establish analgesia of all soft and hard tissues of one half of the lower jaw and the tongue. Among all the local anaesthetic injections administered by a dental surgeon, the IANB is the most technically demanding and thus requires considerable skills. Most dental surgeons are however quite adept at performing this procedure and could claim very high success rates. But in comparison to infiltration anaesthesia employed in anterior regions of the mandible and in the maxilla, where success rates approximating 100% are common, the success rate of the IANB is only 80% to 85%.[1] Furthermore it has been estimated that one out of every five patients (20%) receiving IANB block requires re-injection to achieve clinically adequate anaesthesia.[1] It is helpful to review the surgical anatomy related to the IANB in order to understand the reasons for the failure rate of 15-20% and the occurrence of complications.

Surgical anatomy

The inferior alveolar nerve is blocked by the deposition of the local anaesthetic solution in the vicinity of the nerve when it is in the pterygomandibular space just before it enters the mandibular foramen. This space is bound laterally by the ascending ramus and medially by the medial pterygoid muscle, the posterior boundary being the parotid gland containing the branches

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of the facial nerve. The relative position of the mandibular foramen may vary depending on the following factors: [2]

(a) Width of the ascending ramus of the mandible: The greater the width of the ascending ramus, then the farther back the foramen will be situated and thus the deeper the needle will have to be inserted.
(b) Width of the arch of the mandible: The wider the right to left dimension of the mandibular arch, then the farther back the body of the syringe will have to be placed on the opposite side to the site of injection in order to allow the needle to clear the internal oblique ridge and still reach the mandibular foramen.
(c) Obliquity of the angle of the mandible: The more oblique the angle of the mandible then the farther forward and the higher up the mandibular foramen will be.
(d) Age of the individual: The position of the mandibular foramen in children tends to be lower than that in adults.
(e) Edentulous jaw: The mandibular foramen may be located higher than its expected position in relation to the crest of the alveolar ridge as the latter may have lost height due to resorption.

Failure to give due consideration to the above factors may lead to failed analgesia and other complications.

There are several techniques described in the literature for the IANB. Among these the direct and indirect techniques are often adequate to achieve the purpose of establishing IANB.[2] These techniques are well known to most dental surgeons in Sri Lanka from their training at dental school. In addition to these well tested techniques, high mandibular blocks named Clarke and Holmes method,[3] Angelo Sargenti's technique[4] and Gow-Gates technique[5] have been described in the literature. Techniques put forward by Vazirani (1960) and Akinosi (1977) are methods suitable for patients presenting with difficulty in opening the mouth.[6,7] IANB by an external approach is also described, especially designed to deal with a patient presenting with severe limitation in opening the mouth as may occur with ankylosis of the temporomandibular joints.[2] A description of these techniques is beyond the scope of this paper.

Complications

Many complications have been reported following the administration of IANB. Some of these complications are not specific to this particular procedure and are common to any method of local anaesthetic injection. Allergic reactions such as acute angioedema and allergic dermatitis are uncommon with the commonly used lignocaine but have been reported with procaine in the past. Fainting or vasovagal syncope is a frequent problem associated with local anaesthetic injections. Angina pectoris, postural hypotension, seizures, acute asthma, hyperventilation, palpitation due to adrenaline reaction and hypoglycemia are some of the relatively more serious complications.[8]

Very serious complications such as cardiac arrest and other types of collapse may occur in medically compromised patients with ischaemic heart disease or adrenal suppression. However, complications specific to inferior alveolar block injections are often related to the surgical anatomy of the technique.

Permanent alteration in sensation in the area of innervation of the inferior alveolar nerve or the lingual nerve or both have been reported. Pogrel and Thamby (2000) have reported permanent involvement of the lingual nerve in 79% patients among a study population of 55 women and 28 men.[9] In the same group the inferior alveolar nerve was involved in 21% of patients. Ngeow *et al,* (2006) have reported transient loss of power of accommodation in the ipsilateral eye following an IANB injection which resolved after 15 minutes.[10] It has been postulated that

intravascular entry of the local anaesthetic and its subsequent spread to the orbit has resulted in the anaesthetisation of the short ciliary branch that innervates the ciliary muscle.

Intra-arterial injection of the local anaesthetic during IANB has resulted in permanent loss of vision according to a report by Walsh and Hoyt (1969).[11] Rood reported a case in which entry of the local anaesthetic solution into an artery has led to immediate loss of vision in the ipsilateral eye, along with upper-eyelid ptosis and medial strabismus, which resulted in double vision.[12] The patient also developed ischemia of the palatal mucosa. Within 45 minutes, all symptoms disappeared. Blaxter and Britten reported several cases involving transient loss of vision, diplopia and amaurosis (total or partial blindness).[13] They postulated that an intra-arterial injection of the inferior alveolar artery occurred, with the anesthetic traveling to the internal maxillary artery, the middle meningeal artery and, finally, the lacrimal and ophthalmic arteries. Goldenberg reported a similar case following a mandibular injection.[14] The patient developed dizziness, diplopia and partial amaurosis, as well as total blanching of the forehead and upper eyelid ipsilaterally. The author traced the anaesthetic to the lacrimal artery, which affected the sixth cranial nerve that innervated the lateral rectus. This muscle became paralyzed for about 20 minutes. Dryden reported a case following an inferior alveolar block injection using the Gow-Gates technique.[15] The patient felt a burning sensation around the right eye and infraorbital region. Diplopia and ptosis of the right eye developed, as well as blanching of the skin coinciding with the region of supply of the infraorbital artery. In addition, the patient experienced blanching on the right side of the hard palate that followed the distribution of the greater palatine artery. Webber *et al,* (2001) have reported a case that developed very similar features and resolved within 45 minutes.[16]

A survey of the literature reveals that most complications specific to the injection of local anaesthetic are related to the IANB. Most types of complications described above are unpredictable and are only partially avoidable by ensuring that the needle does not enter a blood vessel by aspirating before administering the injection. Indeed some of the authors of the above reports have asserted that they aspirated after entering the injection site and that they deposited the solution only when blood was not aspirated.

A not uncommon complication of the mandibular block injection is a temporary ipsilateral facial paralysis which results in the patient being unable to close the eye on the affected side in addition to paralysis of the facial muscles.[2] This arises due to the local anaesthetic solution reaching the facial nerve which may occur if the needle is inserted through the capsule of the parotid gland by being directed far too deep. Occasionally the needle may become deflected posteriorly producing the same result. Invariably, such a misdirected injection also fails to establish adequate analgesia.

A case report of unsuccessful inferior alveolar block injection leading to the development of not only a temporary facial paralysis but also much prolonged trismus and parotid abscess is presented and the relevant factors are discussed.

Case Report

A 20-year old female patient was referred to the Dental Hospital from Mahaoya Hospital in the Ampara district. The patient presented with a preauricular swelling of three weeks duration on the left side that developed, accompanied by severe pain, fever and trismus, three days after the extraction of a lower left molar tooth. She had been treated at Mahaoya Hospital with antibiotics for nearly two weeks. Fever and severe pain had subsided with the use of antibiotics. However, mild pain, trismus and swelling persisted. On clinical examination a mildly tender and slightly fluctuant left preauricular swelling was found (Fig.1). The patient was able to open

the mouth only to an interincisal distance of 28mm and the mandible deviated towards the left side on opening. There was neither tenderness of the masseter muscle nor other signs of temporomandibular or myofascial problems. The patient denied having any previous temporomandibular joint related symptoms. The patient recalled that she had to be given about four injections to obtain anaesthesia and the analgesia was only partially effective. Furthermore, she confirmed that she had developed a temporary difficulty in closing her left eye after the injection but it resolved within the next two hours.

Intraorally there was no evidence of acute sepsis in the remaining dentition or the periodontal tissues. A full blood count revealed no abnormal findings. A dental panoramic tomography and an occipitomental radiograph failed to contribute any positive findings. An ultrasonogram of the swelling was undertaken and it revealed patchy non-echoic areas in the middle of the otherwise normal echogenic parotid gland tissue (Fig. 2). Suspecting the presence of fluid within the gland, an ultrasound-guided aspiration was performed and it yielded more than 6ml of frank pus. The pus was dispatched for bacterial culture and antibiotic sensitivity test. Intravenous co-amoxiclav was commenced and continued for three days. The bacterial culture report was negative with no growth on the third day. In spite of the negative culture report the co-amoxiclav was continued orally for two more days. The patient's mouth opening improved and the swelling subsided. On the fifth day the swelling was almost imperceptible and her mouth opening measured 38 mm interincisally although there was a residual leftwards deviation of her jaw on opening the mouth. She was discharged on the seventh day with instructions to carry out jaw exercises to counteract the deviation of the jaw to the left.

Discussion

From the history of this patient, it is clear that the local anaesthetic solution in the inferior alveolar block injection had entered her left parotid gland as evidenced by the facial paralysis of short duration she had developed. Moreover, she had received repeated injections of local anaesthetic to counteract the ineffective analgesia. Under these circumstances, it is conceivable that oral microbial flora or possibly some more virulent microbes had entered the parotid gland in sufficient quantity to produce an acute intraglandular abscess. This explains the swelling, fever and severe pain that developed three days after the extraction. It is also understandable that the repeated injections through the medial pterygoid muscle may have caused muscle damage and an organizing haematoma in the pterygomandibular space, causing restricted mouth opening.

A search of the literature on the medline failed to find any report of a parotid abscess developing as a complication of an IANB. Thus it would appear that this is the first reported case of a parotid abscess arising as a complication of an IANB.

Apart from a faulty IANB technique, patient's condition might have been caused by the needle being contaminated with microbes and this could have been prevented by adherence to aseptic techniques. Alternatively, a poor state of oral hygiene at the time of the IANB might have contributed to this outcome. For this reason, it is a good practice to instruct the patient to rinse the mouth with 0.2% Chlorhexidine for at least one minute duration before any invasive procedure such as the IANB.

Although the IANB technique of the dental surgeon who had performed the extraction might have been faulty, it is also possible that anatomical features of the patient's jaw might have contributed to this event as stated in the section under surgical anatomy above. A possible explanation could be that the patient's mandibular foramen was located in a relatively anterior position and the needle was thus directed in a posterior direction towards the posterior end of

the ramus thus leading to its entry into the capsule of the parotid gland.

Accidental injection of local anaesthetic solution into the inferior alveolar artery or vein which may precipitate the ocular and other complications described earlier can be avoided by using an aspirating technique before depositing the local anaesthetic.

Conclusion

It is important for dental surgeons to be aware of complications that may arise from local anaesthetic injections in general and from inferior alveolar block injection in particular. Dental surgeons must adhere to stringent aseptic technique while administering the IANB. It is also advisable to carry out oral prophylaxis before any extraction or at least the employment of an antiseptic mouthwash before IANB or other invasive procedures. They also must take precaution against entry of the local anaesthetic into inferior alveolar artery or vein by aspirating to ensure that the tip of the needle has not entered a blood vessel.

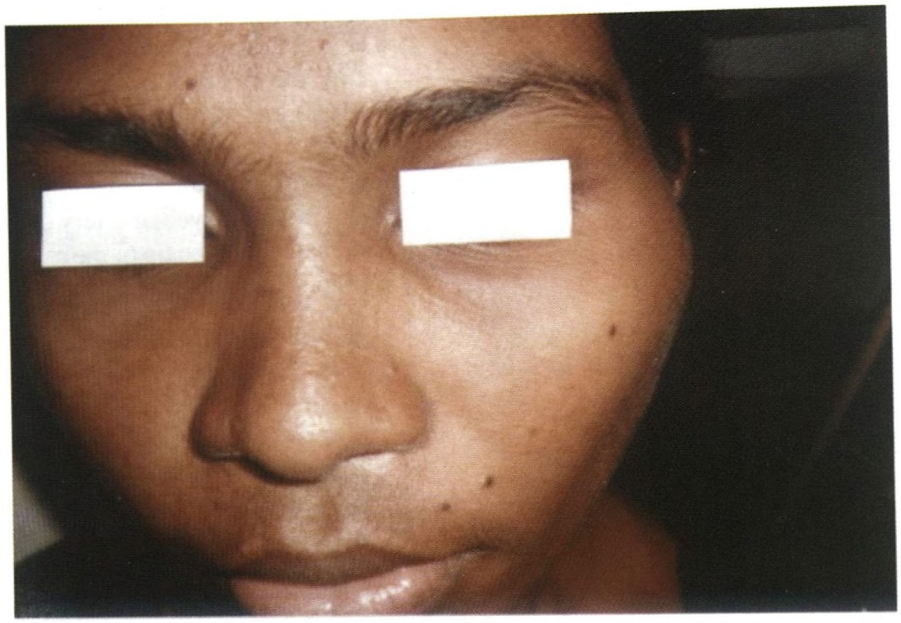

Figure 1: Frontal appearance of patient's face with arrows pointing to the left pre-auricular swelling

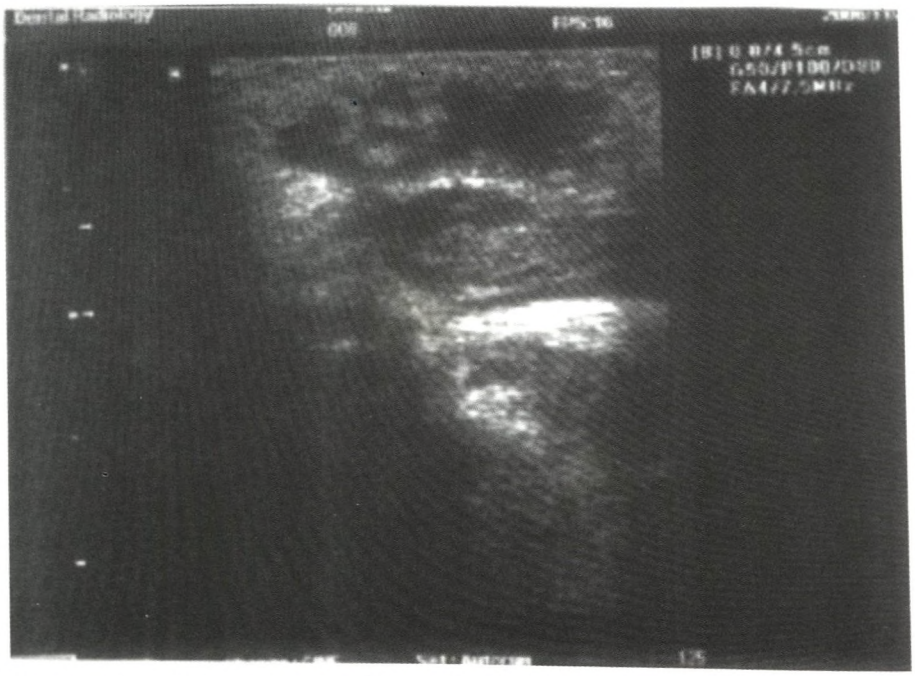

Figure 2: Ultrasonogram of the patient's left parotid gland showing Intra-glandular hypoechoic area indicating the presence of fluid.

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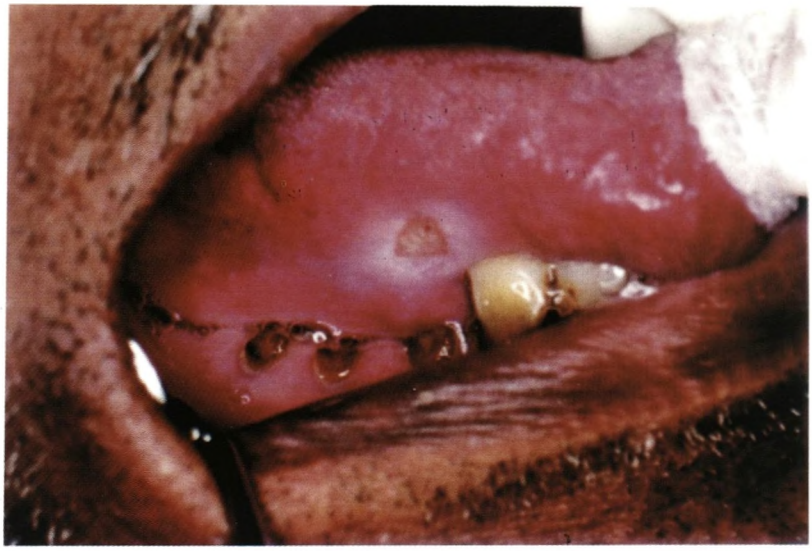
Sri Lanka Dental Journal 2007; 37(01) 55

Self Assessment / Oral Diagnosis (SAOD)

A 71 year old male presented with a non-healing ulcer on the right ventral surface of the tongue. The ulcer has been there for several months. He experienced pain at the site of the ulcer when he wakes up from sleep. He chews betel with all ingredients and 10 quids per day for more than 20 years and takes a quarter bottle of alcohol regularly once a week. He has never smoked. Examination revealed an ulcer measuring 0.5 X 0.5 cm with a diffuse keratotic halo as shown in the photograph.



1. Which of the following additional investigations are needed to arrive at the diagnosis?
   a. examination of the lymph nodes
   b. ultrasonography
   c. swab and culture
   d. full blood count
   e. erythrocyte sedimentation rate

2. What is the most likely diagnosis?
   a. homogenous leukoplakia
   b. non-homogenous leukoplakia
   c. traumatic ulcer
   d. squamous cell carcinoma
   e. candidal infection

3. Which of the following is the most appropriate management option?
   a. extract retained roots adjacent to the ulcer and observe the progress of the lesion
   b. incisional biopsy for routine histopathology
   c. apply topical steroid and observe for a period of two weeks
   d. excise the lesion and primary closure
   e. application of an antifungal agent topically

Answers

1. a
2. c
3. a

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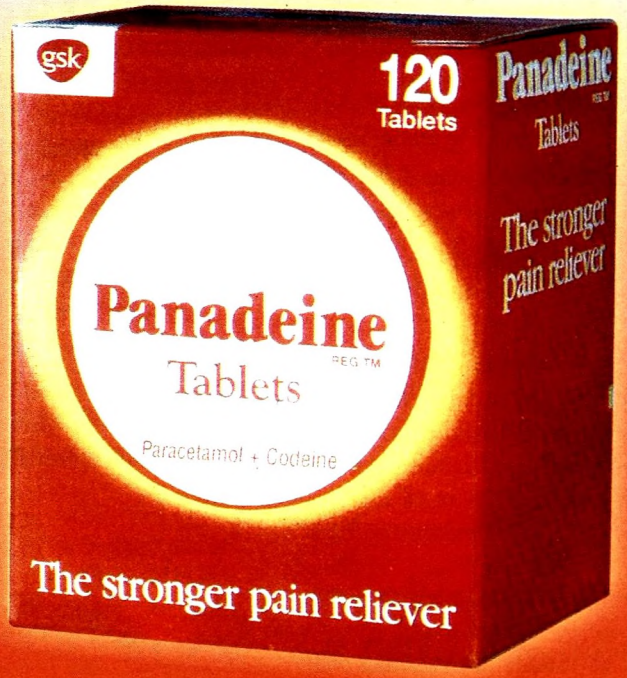

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2.Reviews - Reviews are detailed surveys of published research pertinent to dentistry and associated sciences. They should be critical in nature and should not normally exceed 3000 words and 30 references.

3. Research articles - Articles resulting from research work belong to this group. Results from routine clinical examinations or laboratory investigations will not be considered under this category. Subjects may vary from clinical trials to basic science research, historical analysis to dental economics. They should not exceed 3000 words and 30 references. A reasonable number of tables and illustrations will be accepted.

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5. Case reports - Reports such as of rare diseases or conditions. Modifications to accepted treatment procedures, new management methods etc. may be included in this category.

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7. Miscellaneous topics - Subjects unlimited and the format are free. These may also include details of scientific meetings, conferences, annual sessions, examinations, news and views, visits and obituaries.

8. Proceedings of annual sessions - Abstracts from annual sessions of the SLDA and other colleges will be published under this category.

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Tables - The tables should be numbered in the order" of appearance in Arabic numerals, Each table should have a brief explanatory title. Each table; should be typed on a separate sheet, with due regard to the proportion of the printed column/page.

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Journals

Standard journal article

Bartlett IG, O'Keefe P. The bacteriology of the perimandibular space infections. JOral Surg 1979; 37: 407409.

Corporate (collective) author

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Chapter in book

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

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International statistical classification of diseases and related health problems, 10th revision, vol 1. Geneva: World Health Organisation, 1992; 550--564

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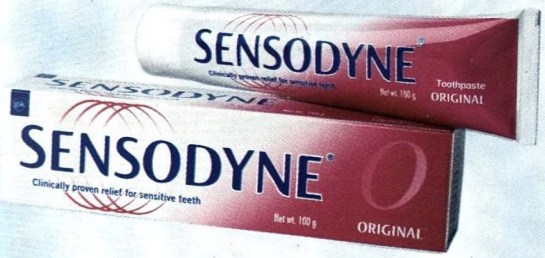

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