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Editorial Office

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

Correspondence regarding editorial matters, articles, reviews and news items should be addressed to the Editor, SLDJ, Dr. Upul B. Dissanayake, Dept. of Oral Pathology , Faculty of Dental Sciences, University of Peradeniya, Sri Lanka.
Tele: 0812 385821/ 0812 387500/ 0812 2392068
0777 393318

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

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GUEST EDITORIAL

Is altruism out of place in dentistry?

As health professionals dental surgeons interact with patients all the time. Is this interaction different from that between (medical) doctors and their patients? The answer is perhaps yes and no. There are some situational differences between these interactions, but in essence the philosophical basis of the doctor-patient relationships in both medical and dental professions must be the same. However, there is a widespread notion that the relationship between dentists and their patients, at least in private practice, is dominated more by pecuniary considerations than altruistic ones. Evidence for this came in a British study not long ago, when medical and dental students were compared on their declared motives for choosing their respective professions. Sadly, a majority of dental students declared financial motives as what made them choose the dental profession whereas the majority of medical students stated that the opportunity to serve humanity attracted them to the medical profession. But is dentistry not concerned with serving humanity? Do not altruistic motives determine the way dentists relate to their patients? Nowadays a great deal of emphasis is being laid in dental curriculum development on the need to produce 'caring dentists'. How can dentists be 'caring' if all what they 'care' is money and nothing else? I believe it is time that the dental profession looked at itself introspectively on this important issue on which the esteem for the profession in the minds of the public will depend. In short, the profession needs a paradigm shift from monetary concerns to altruistic considerations.

The British Medical Journal (BMJ) carried out a study in 2002 asking its wide readership of doctors, allied professionals, academics and even patients on the theme: What's a good doctor and how do we make them? The respondents identified a staggering figure of nearly 70 good qualities a doctor must have. Among them, compassion, understanding, empathy, honesty, competence, commitment, humanity and self-sacrificing were the predictable qualities most people expected from a doctor. There were also the less predictable qualities of courage, creativity, a sense of justice, respect, optimism and grace cited by the respondents as qualities in a good doctor. It is evident that the society expects the doctor to demonstrate lofty ideals not expected from any other profession. A pertinent comment in this regard came from one of the respondents: "Good doctors, unlike good engineers, good accountants, or good firemen, are not just better than average at their job. They are special in some other way too. Extra dedicated, extra humane, or extra selfless." Is there any one out there in our profession who thinks dentists may be exempted from any of the stated qualities particularly the predictable ones? I believe the public is entitled to expect the same qualities from their dentists as much as they expect of their doctors.

The question of "how do we make them?" as in the above-mentioned study naturally falls on the teachers in the dental school. Are our teachers addressing these issues sufficiently? The teachers must also lead by example in establishing good communication with patients. The standard of communication between doctors and patients, let alone between dentists and their patients, is in a lamentable state in Sri Lanka. It is time that medical teachers addressed this issue too. Could our students emulate their teachers as 'role models'?.
I wish to conclude this little note with the following quotation from William Osler:

"Medicine arose out of the primal sympathy of man with man, out of a desire to help those in sorrow, pain, or sickness, nothing more nothing less".

Dr M A M Sitheeque

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Sri Lanka Dental Journal 2006; 36(02)60-67

Microbial biofilms and the clinical significance

S. Rajapakse

Introduction

Our recognition of bacteria as unicellular life forms is entrenched in the pure culture paradigm. In the study of bacteriology, information about many bacterial activities has been obtained using liquid cultures/suspensions of bacteria. The knowledge gained from works of Koch and Pasteur has shown that this mode of bacterial growth has been very useful in understanding the role of bacteria in disease pathogenesis and it has also been instrumental in revealing various aspects of microbial physiology. However, bacteria rarely exist in nature as pure culture planktonic growth. Studies on enumeration of bacteria in a number of natural, industrial and medical ecosystems using quantitative recovery methods have established that the majority of microbes persist attached to surfaces within a given aquatic system-biofilms- and not as free floating organisms.[1] Significance of biofilms has gradually emerged since its first description in 1936 and the first recognition of their ubiquity.[2,3]

Definition and characteristics

Biofilms are defined as matrix-enclosed bacterial populations adherent to each other and/or to surfaces or interfaces. This definition includes microbial aggregates and floccules which form at air-water interfaces and suspensions and also adherent populations within pore spaces of porous media.[1]

The matrix that holds the biofilm together consists of primarily exopolysaccharide material (EPS), proteins and DNA secreted by the cells.[4,5] Non-cellular materials such as mineral crystals, corrosion particles, and clay or silt particles may also be found in the biofilm matrix, depending on the environment in which the biofilm has developed. Based on extensive analytical research on biofilms in a number of aquatic systems, it has been shown that biofilm populations predominate in virtually all nutrient-sufficient aquatic systems independent of system geometry and of the type of ecosystem involved, and that these populations have a very significant metabolic activity. Extensive quantitative analysis of different aquatic systems enables to predict the extent of biofilm formation in a particular aquatic system based on the principles[1] given below,

- Metabolically active bacteria show a remarkable avidity for adhesion to surfaces, and this tendency is especially pronounced in wild type cells in natural environments.

- The extent of biofilm accretion on surfaces in any aquatic system is controlled by the amount of nutrients available for cell replication and for exopolysaccharide production.

Sunethra Rajapakse (Correspondance) B.D.S(SL), M.Phil(SL), PhD(Aus) Senior Lecturer, Department of Oral Medicine and Periodontology, Faculty of Dental Sciences, University of Peradeniya. E-mail: Sunethra.Rajapakse@newcastle.ac.uk

- In extremely oligotrophic environments, organic nutrients tend to associate with available surfaces, and to trigger local biofilm development, but bacteria generally do not adhere to surfaces in very nutrient-deficient ecosystems.

Microbial composition of the biofilm can be derived from either a single species or a community derived from a multiple microbial species.

The above basic principles on biofilm formation reveal that the oral environment where the soft and hard tissue surfaces are continually bathed in nutrient rich medium favours optimal bacterial growth and biofilm formation. The nutrient rich medium/saliva is constantly being replenished by host derived nutrients allowing unimpeded growth of microbial biofilms on the oral surfaces. Also when bacteria are present biofilm formation is favoured on metal and plastic surface of medical devices which are bathed in body fluids, such as contact lenses, and artificial heart valves, indwelling catheters, artificial joint replacements, endotracheal tubes and voice prosthesis with resultant infections caused by these biofilms. In order to manage biofilm infections it is necessary to have an understanding of biofilm formation, the structure and how biofilms are maintained successfully in their natural habitat.

Contrary to the earlier understanding of biofilms as a homogenous distribution of cells in a uniform exopolysaccharide matrix, it is now clear that biofilm has significant variability and heterogenecity. Using new microscopic and molecular technologies, researchers have shown that biofilms are not simply organisms containing slime layers but highly structured, properly coordinated, functional communities.[6] Also biofilms consist of a variable distribution of cells, cellular aggregates, their extra cellular polymers, and void spaces or water channels which may or may not be continuous with the bulk liquid phase. These fluid channels are used for transport of substrate, waste products and signal molecules used for communication between microbes.[2] Biofilm formation is a step-wise process and there is emerging evidence that expression of genes required at each and every stage of biofilm development is well-regulated.[7,8] Biofilm development starts with the adhesion of planktonic microorganisms to a surface. It has been found that following adhesion to a surface, bacteria undergo a phenotypic change with expression or derepression of a large number of genes and also derepression of exopolysaccharide (EPS) synthesis. As a result, biofilm bacteria differ phenotypically from their planktonic (free-living) counterparts. Once adhered, the attached bacteria undergo cell division in order to form microcolonies embedded in EPS matrix followed by growth and maturation of the biofilm. Adherence of bacteria from the same species or from different species leads to form a complex biofilm structure with metabolic and functional cooperation between them. Microcolony in a biofilm determines its maximum size and suppresses its cell division to control the number of cells present at a given site by quorum sensing.[9] All these events and the reaction of biofilm to external stimuli are coordinated through various signal transduction molecules. Reaction to external stimuli leads to expression of vast set of genes so that most suitable phenotype emerges. Therefore, with the growth of the biofilm, changes occur in its micro environment (nutrient availability, osmolarity, pH etc.) with resultant changes in phenotype of its constituent bacteria as well as establishment of different species of bacteria. For example the enzyme

urease in *Streptococcus salivarius* was found to be differentially regulated during biofilm growth.[10] It has also been shown that *Streptococcus mutans* glycosyltransferase and fructosyltransferase genes are differentially expressed in mature biofilms compared with initial biofilms.[11] Likewise there is differential expression of proteins observed between biofilm and planktonic cells. Further these differences in protein expression are observed with respect to the same species between different stages of biofilm development.[12] All these phenotypic changes enable microorganisms to thrive in a particular environment. Once matured and established, the biofilm sheds its constituent bacteria from time to time. These bacteria would colonize new habitats with new biofilm formation which would help in the continued existence of the species.

Ecological advantages of biofilm life

When considering the ubiquitous nature of biofilms it seems that this form of growth is advantageous to the microbes in many ways.

- Biofilm environment confers protection for its constituent bacteria from protozoan grazing. It also enhances biofilm resistance to environmental stresses such as UV radiation, pH shift, osmotic shock and dessication.[13] Apart from facilitation of initial attachment of bacteria to surfaces, exopolysaccharide (EPS) produced by biofilm bacteria is a major factor which is responsible for those protective functions. In addition, EPS physically prevent access of certain antimicrobial substances into the biofilm thereby protecting the biofilm against antimicrobial agents. Restriction of the diffusion of compounds from the immediate environment into the biofilm by the ion exchanging ability of the EPS helps in achieving most of those functions.[14] This may explain the low efficacy of antimicrobial agents used in biofilm-associated infections.[15] The slower growth rate of bacteria in a biofilm may also account for low susceptibility to antimicrobial agents. These factors explain the failure of antimicrobial therapy/chemical plaque control measures in establishing long lasting health in patients affected by biofilm related diseases such as periodontal disease and caries. It has been reported that the concentration of antimicrobial agents which kills planktonic bacteria might have to be increased up to 10-1000 fold to have the same efficacy on microbes in a biofilm.[16,17,18] The reliability of sampling techniques which are directed to obtain planktonic bacteria as well as the determination of minimum inhibitory concentrations of antimicrobial agents using phenotypically different planktonic bacteria may also be responsible for reduced efficacy of antimicrobial substances in biofilm related infections.

- The existence of highly permeable water channels throughout the biofilm provides an effective means of nutrient uptake and removal of metabolic end products including toxic substances.[1] Growing in a biofilm enhances the metabolic cooperation between biofilm bacteria so that each member is benefited by being a part of a large community.

- Horizontal gene transfer is important for the evolution and genetic diversity of natural microbial communities. The prevalence of plasmids in bacteria from diverse habitats and the dwelling of biofilm bacteria in close proximity to each other in their natural setting, make conjugation a likely mechanism by which biofilm bacteria exchange genetic material within and between populations. Acquisition of a gene which confers resistance to tetracycline by the oral microbe *Streptococcus* growing in a biofilm from a strain of *Bacillus subtilis* which harbours the conjugative transposon has been shown in an in vitro study.[19] This indicates gene transfer between non-oral bacteria and oral commensals and the possible mechanism by which biofilm bacteria acquire resistance to antibacterial substances.

Clinical significance of biofilms

Once established, the biofilm's resistance to natural surfactants, phagocytosis, and antibiotic therapy allows it to remain as a continuing nidus of living bacteria even long after all planktonic bacteria are killed by these host defense factors and antibacterial agents. Therefore, clinicians have to face the following inherent problems associated with biofilm infections;

a) Symptoms typically recur even after repeated treatments with antibiotics,
b) biofilm infections are rarely resolved by host immune system (biofilm bacteria release antigens and stimulate the production of antibodies, yet they are resistant to defense mechanisms),
c) Continuous stimulation of immune response may even cause damage to the surrounding tissue. Periodontal diseases provide a classic example for this and therein clinicians encounter all of the above problems.

Therefore, novel strategies should be developed with better understanding of the biofilm formation and its dynamics. Common oral diseases; caries and periodontal diseases are primarily biofilm-associated polymicrobial infections. However, these diseases are multifactorial and as such oral hygiene practices, diet, and patient's tissue (hard and soft) resistance play an equally significant role. Traditionally management of these diseases in a population is directed towards primary prevention together with therapeutic intervention. Primary prevention strategies used at present include oral health education in order to achieve optimum control of dental plaque (oral biofilm), usage of supplementary fluorides to strengthen the tooth surface and application of sealants to eliminate ecological niches on tooth surfaces. Treatment of both these diseases is restricted to biofilm control using mechanical/chemical means, and restoration of lost tissue using variety of artificial material. Despite the advances in clinical dentistry including material sciences, scientists so far have failed in developing a material which properly merges with the tooth substance. As a result, tooth-filling material interface is left with microscopic voids making it susceptible to bacterial colonization with subsequent recurrence of disease. Further all these treatment methods in the management of caries and periodontal diseases are costly as well as time and labour intensive. With the recent advances in biofilm research scientists have been able to unfold answers to why biofilm infections are recalcitrant to current prophylactic and treatment methods. Although factors which were not known

previously with regard to physical and chemical properties and population dynamics of biofilms have now been revealed, our knowledge on biofilm dynamics is far from complete.

Various strategies have been considered with respect to the prevention and treatment of biofilm infections: this includes:
Targeting various biological activities occuring in biofilm formation without disturbing the balance of the normal flora. This could potentially be achieved by,

- Interference with signal transduction systems, Signal molecules coordinate expression of genes in order to transform planktonic bacteria to suit the biofilm life. Targeting signal molecules in developing countermeasures against biofilms would affect the formation, maintenance and survival of biofilm bacteria.

- Altering characteristics of the surface on which the biofilms are formed eg; tooth surface or the salivary pellicle to impede bacterial colonization; manipulating protein film on the enamel to reduce bacterial adhesion

- Replacement therapy where potentially pathogenic microorganisms are replaced by genetically modified organisms that are less virulent. However, the replacement organism, should not cause disease itself and it must colonize persistently and replace the pathogen effectively. The organism must have a high degree of genetic stability too. Supercolonizing strain of *Streptococcus mutans* is one such genetically modified organism which lacks lactate dehydrogenase and therefore does not form lactate.[20] It also produces mutacin which enables it to replace wild type strains of *Streptococcus mutans* effectively. Further animal experiments involving lactate dehydrogenase-deficient ureolytic *Streptococcus mutans* have shown promising results in caries prevention.[21] The rationale behind the replacement therapy is based on the assumption that the disease is caused by a single organism. However, some of these diseases are polymicrobial, eg: caries and periodontal diseases. There is also a possibility that the genetically modified organism may further undergo genetic transformation and become an opportunistic pathogenic strain.

- Immunization is considered another strategy for the prevention of dental caries and periodontal disease. In both caries and periodontal disease, active immunization and passive immunization have shown promising results.[22,23,24] However, clinically applicable vaccinations for human beings with respect to these diseases are not yet developed. Further, it has to be kept in mind that these vaccinations are directed against epitopes of single bacterial species whereas both caries and periodontal diseases are considered as ecologically driven polymicrobial diseases.[25] Moreover, lasting results from immunization is questionable since these microbes are biofilm-associated, with an ability to undergo genetic transformation which would alter the antigenicity.

- Furthermore, signal molecules used by biofilm bacteria are also likely targets of future preventive and therapeutic interventions of biofilm infections. This line of approach interferes with the population dynamics of interdependent microbes and leads to a collapse of the biofilm system. However, one should remember that the constituent microbial species may have more than one method in achieving the same objective during their existence with respect to the formation, maintenance and the survival of the biofilm.

- In biofilms, aforementioned fluid channels are kept open by surfactant molecules secreted by the members of the biofilm.[26] and this would prevent clogging of fluid channels so that the micro-environment around bacterial colonies is maintained. Targeting these surfactant molecules or their respective genes is another approach which is under scrutiny in order to achieve disruption of biofilms. Also this kind of approach would enable us to minimize the problem of development of bacterial resistance which is of importance in therapy.

- Phage therapy may also be another possible approach which is being considered for management of biofilm related problems. Biofilm control in this instance is thought to be achieved by bacteria being infected by a specific bacteriophage with subsequent lysis of the bacteria.[27] This lysis could lead to disruption of the biofilm architecture with resultant imbalance generated in the system. However, this mode of approach may be more appropriate to face with problems created by biofilms in the environmental and industrial aquatic systems such as marine environment.

In conclusion, advances in microbiology have enabled us to successfully counteract acute microbial infections affecting mankind. However, there are numerous challenges encountered by clinicians as well as microbiologists in the management of biofilm-associated infections such as device or implant related infections, periodontal diseases and caries.[28] Understanding of microbial life from the biofilm perspective has just begun. From the available information we know that these biofilm bacteria are organized in to three-dimensional structures with primitive circulatory systems (fluid channels) and they are functioning as well-protected communities with a high level of cooperation and communication between them. This kind of organization is not merely a result of their extensive genetic repertoire but the genotype's remarkable capability to respond phenotypically to the environmental stimuli. Hence, in achieving the objective of overcoming challenges associated with biofilm-related infections, it is necessary to understand the complexity of the intercellular interactions and communications within a biofilm which make biofilms a highly successful mode of bacterial growth. Such an understanding would be vital in determining specific molecules to be targeted in the development of future therapeutic or prophylactic interventions.

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Speech considerations in dentistry

J.U. Weerasinghe

Speech is a basic physiologic function involving lungs, pharynx, oral and nasal cavities. It is a mode of communication of human. Since generation of speech sounds mainly occurs at oro-pharyngeal region tissue abnormalities in this area can affect speech. The structural and functional tissue abnormalities in the oro-pharyngeal region may arise from a disorder or as a consequence of treatment procedures. The present article attempts to discuss the normal speech mechanism and common speech problems that may occur due to changes in the oral environment.

Production of human speech is described acoustically as a source-filter model.[1] The excitation source is the glottal wave arising from vibrating vocal cords with the air stream from the lungs. The acoustic filter is the vocal tract, which modifies the flow and direction of the air stream to produce different sounds. The overall shape of the vocal tract may differ in each sound. Main stages of human speech comprised of phonation, articulation, and resonance and these events are subjected to neurological control. Phonation is the production of basic vowel tone in larynx. During articulation new sounds are created and then modified with the help of active (lips, tongue, soft palate and pharynx) and passive (teeth, alveolar ridge, hard palate and uvula) articulators. Resonance which occur within the oral-pharyngeal-nasal cavities and paranasal sinuses, gives a characteristic quality to the sound. Neurological control determines the arrangement of sounds to form a pattern of communication.

The velopharynx directs the passage of air column into nasopharynx during speech. Velopharyngeal port closure is achieved by normal apposition of the soft palate (velum) together with the posterior and lateral pharyngeal walls.[2] Dysfunction of the velopharynx due to any structural, neurological, physiological or mechanical causes result in improper closure of the velopharyngeal port.

With regard to the oral cavity environment, articulation and resonance are the two major areas where the dental professionals should focus their attention. It is interesting to observe the important role played by the oral cavity in the production of speech sounds. Speech sounds are classified into vowels and consonants. Vowels (*a,e,i,o,u)* are produced with minimum interruption to the airflow. The shape of the mouth and the pharynx and the position of the tongue

Jayantha Weerasinghe (Correspondence) Senior Lecturer in Oral and Maxillofacial Surgery, Faculty of Dental Sciences, University of Peradeniya, Sri Lanka. Tel: 081223974002, E mail : juw@pdn.ac.lk

determine each vowel. Parameters that determine vowels are the height of the tongue, backness of the tongue and state of lip rounding.

Consonants involve some obstruction to the airflow along the vocal tract. Parameters that determine consonants include occurrence of vocal cord vibration, place of the articulation (obstruction to the airflow) and the manner of articulation of the airflow (degree of obstruction).

Based on the place of articulation consonant sounds can be classified as follows:

Bilabial- both lips are close together (*p. b*),
Labio-dental- lower lip against upper teeth (*fa*-n),
Dental- tip of the tongue against upper teeth (*thi*-n),
Alveolar- tip of the tongue against upper alveolus (*so*-ng),
Palato-alveolar- front of the tongue against anterior palate (*shi*-p),
Glottal- back of the tongue against soft palate (*ho*-tel).

Based on the manner of articulation consonants can be classified as follows:

Plosives- there is momentarily complete closure and rapid release of articulators (*p.b*),
Fricatives- allow escape of air through a narrow orifice (*f, v*)
Affricates- plosives released with frication (*chur*-ch).
Nasals- lowering the velum causes the air to passes through the nasal cavity (*m, n*)
Approximants- articulators approach each other but not close enough (*j, w*).
Laterals- air stream escapes around the sides of the tongue (*l*)
Trills- series of rapid closures (*r*)

Altered structure and function of articulators and the oral environment bring about changes in articulation and resonance thereby affecting the speech outcome. Cleft lip and palate abnormality is a congenital condition that affects speech due to the structural and functional changes in lips, alveolus, dentition and hard palate affecting articulation of speech sounds. Furthermore velopharyngeal dysfunction due to soft palate defect brings about hypernasality, which is defined as perception of excessive amount of nasal resonance accompanying normal non-nasalized sounds.[3] Various investigation modalities are being applied for the evaluation of hypernasal speech in children undergoing cleft surgery.[4] These investigations are necessary to evaluate the success of treatment.

Minor modifications in speech sounds may be observed in other developmental conditions such as tongue-tie, malocclusions including Class III status. In these instances place and manner of articulation are affected. Inflammatory conditions that affect paranasal sinuses and conditions that produce swellings in the oro-pharyngeal region may alter the shape and available volume of vocal tract, results in affecting the resonance.

In the dental surgeon's point of view "speech" is one of the important factors that should be taken into consideration when delivering routine dental care for their patients. Extraction of upper and lower anterior teeth may cause difficulties in the production of certain fricative sounds. During prosthodontic treatments, maintenances of adequate space is necessary for the movements of the tongue in speech and swallowing. Equal attention on speech is needed in delivering restorative, orthodontic and periodontal treatments too.

Special attention for the speech outcome is being focused during major oral surgical procedures.

In cleft surgery, appropriate palate repair procedures that bring about anatomical repositioning of soft palate musculature are adopted for a better speech outcome. Postoperative speech therapy is successfully utilized in cleft patients who have a functioning velopharyngeal mechanism. In cleft orthognathic surgery the amount of advancement of maxilla by Le Fort osteotomy could interfere with the already existing impaired velopharyngeal mechanism by moving the soft palate further forward. This could be compromised with a bimaxillary procedure in which a mandible pushback also is performed. Maxillary ostectomy procedures which may change the volume of sinuses can affect the resonance. In order to restore the oro-pharyngeal mechanism in maxillectomy procedures, prosthetic rehabilitation is essential. Resection of large amount of oro-pharyngeal tissue and reconstruction of defects with pedicle or microvascular free flaps during surgical treatment of oral cancers also can affect the speech. Bulky flaps inside oro-pharyngeal region can affect resonance. As such, forearm free flaps are considered best in reconstruction of oral mucosal defects, being less bulky and pliable with minimal interference of speech. Surgeons must take care of leaving at least minimum required amount of tongue tissue behind, in glossectomies when ever possible, to maintain the articulation in speech. In conclusion, adequate knowledge on speech process and speech related problems associated with structural and functional disturbances of the oro-pharyngeal region, will be beneficial for dental professionals in delivering dental care for their patients with the aim of restoring articulation and preserving oral resonance.

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Review Article

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Pathological resorption of dental hard tissues

E. A. P. D. Amaratunga

Resorption of dental hard tissues is considered pathological when it occurs in situations other than physiological shedding of deciduous teeth. Resorption of enamel, dentine and cementum of permanent teeth is always pathological and often a result of trauma causing inflammation of the dental pulp, periodontal tissues or both. Root resorption could be one of the serious dental complications leading to loss of teeth. Different authors have proposed various classifications for resorption of dental hard tissues; some are confusing while the others are incomplete. Classification broadly based on aetiology of resorption as given below, would be more meaningful clinically, as the selection of appropriate treatment is largely related to the stimulation that initiate the resorption.

Classification of pathological resorption of dental hard tissues:

1. External root resorption
    - 1.1 Resorption associated with traumatic injury
        - a. Surface resorption
        - b. Inflammatory resorption
        - c. Replacement resorption
    - 1.2 Resorption associated with pulp necrosis and apical pathology
    - 1.3 Resorption associated with pressure in the periodontal ligament
2. Internal root resorption
3. Idiopathic root resorption

Before discussing the types of root resorption in detail, it would be appropriate to consider the general mechanism of root resorption and more importantly, the protective mechanisms involved in preventing such resorption. Resorption of teeth can be initiated by an inflammatory condition, mechanical stimulation or by pressure in the periodontal ligament as a result of neoplastic process or unerupted teeth.[1] Although there are certain differences, root resorption is somewhat similar to that of resorption of bone. A cell that is primarily responsible for the resorption of bone is the osteoclast.[2] However, macrophages,[3] monocytes[4] and osteocytes[2] have also been reported to play a role. Osteoclasts are large multinucleated cells derived from monocyte, macrophage, histiocyte series.[5] Dentine resorbing cells or dentinoclasts are comparatively smaller than osteoclasts and contain fewer nuclei.[6,7]

Pathological resorption of dental hard tissues requires two main steps. Firstly, there should be an injury to the root surface and secondly, persistent irritation by which the stimulation for resorption will be continued.[8,9]Initial injury damages the nonmineralized tissues covering the root surface, initiating an inflammation. In case of external root resorption, the periodontal ligament and precementum would be subjected to the initial

Dr. E.A.P.D. Amaratunga (Correspondence)

BDS (SL), MS (Col), MSc (Lond), FDSRCS (Eng), FFDRCS (Ire), Senior Lecturer, Consultant Oral Pathologist, Department of Oral Pathology, Faculty of Dental Sciences, University of Peradeniya, Sri Lanka, E-mail : prasada@pdn.ac.lk

injury. The odontoblast layer and predentin are the nonmineralized tissues that would be subjected to the initial injury in internal root resorption. Severity of the injury could vary and it determines the type of root resorption. Different types of root resorption in relation to the severity of the injury have been discussed in the latter part of the article. Injury may be of mechanical in origin such as dental trauma, surgical trauma and pressure from a neoplasm/an impacted tooth. Chemical irritation as a result of a treatment procedure such as bleaching of teeth using 30% hydrogen peroxide could also cause an initial injury to the tooth surface.[10] Multinucleated odontoclasts will appear in such a damaged site following injury and initiate the resorption process.

Persistent stimulation of the resorbing cell is necessary for the resorption process to progress continuously after the initial injury. Without such stimulation after an initial injury, the resorption process will cease and repair with cementum like material will take place over a period of 2-3 weeks. In case of an extensive damage however, bone cells can migrate and attach to the damaged root surface before cementoblasts and start the production of bone resulting in ankylosis of the tooth. Common stimulating factors responsible for continuous stimulation of odontoclasts are infections and pressure in the periodontal ligament. There are situations where cervical root resorption could occur without an identifiable cause or injury and thus referred to as "idiopathic" root resorption.

Is there a mechanism to protect the external root surface from resorption?

Composition of the cementum is very much similar to that of bone. Further, the cementum is physically located closer to bone being separated from each other only by the thin periodontal ligament. However, cementum is more resistant to resorption compared to bone.[11,12] As such, there should be physiological mechanisms involved in inhibiting cementum resorption. It has been shown experimentally that the periodontal ligament is capable of producing a low molecular weight proteolytic activity inhibitor referred to as "anti-invasion factor". This secretory product plays an important role in protecting the periodontal ligament and the root surface being invaded by the resorbing cells.[12,13,14] Confirming the protective power of the periodontal ligament, it has been shown that in vitro incubation of bone and bone cells together with periodontal tissue harvested from newly extracted teeth, obstructed the bone attachment property and resorption capacity of respective cells. However, a similar experiment only without periodontal tissue incubation indicated no change in the resorption properties of the osteoclasts. These observations suggest that the periodontal ligament plays an important role in protecting and maintaining the integrity of the tooth root.

The second line of defence against resorption lies within the cementum itself. This barrier with anti-resorption properties is the hyaline layer of Hopwell-Smith[15] or "intermediate cementum".[16] This is a hypercalcified[17] layer of cementum placed adjacent to the root dentine which shows much resistance to resorption and, more

importantly, seals off the peripheral ends of the dentinal tubules and prevents continuous irritation of the periodontal ligament from the noxious agents in the necrotic pulp via the dentinal tubules.[18,19] As already indicated before, stimulation of resorbing cells through such irritation is necessary for the progressive resorption of roots.

Resorption of teeth may begin either from the external surface of the root or from the internal surface of the pulpal wall of the root. The general terms, external resorption and internal resorption are used respectively to distinguish the two types.

1. External root resorption

External resorption is mostly associated with dental trauma, pulp necrosis or excessive pressure in the periodontal ligament. External resorption without an identifiable aetiology has been discussed under idiopathic resorption in this article.

1.1 External resorption associated with traumatic injury

a. Surface resorption

Surface resorption is a transient process caused by minor injuries to the periodontal ligament and cementum. This is a sub clinical process and, patients usually do not experience discomfort. Radiological examination usually shows no changes, as the resorption defect is very small. Following injury, cellular scavenging of tissue debris and microbes will take place as a part of the general inflammatory response to tissue injury. In addition, the clastic cells such as osteoclasts and fibroclasts will be deployed and resorption will occur as a part of the scavenging process. In the absence of continuous irritation and stimulation of resorbing cells, the resorption process will not continue. New periodontal ligament and cementum begin to appear as early as one week after the injury.[19,20,21] Surface resorption requires no treatment.

b. Inflammatory resorption

When the traumatic injury is more severe and the resultant inflammatory reaction is more intense, the activity of the resorbing cells will progress over a longer period of time. This would lead to destruction of the intermediate cementum layer which normally seals the outer end of the dentinal tubules. Destruction of intermediate cementum would open up a communication channel between the pulpal tissues and the injury site on the outer surface of the tooth. As such, noxious substances from the necrotic and infected pulp can readily escape through the exposed dentinal tubules to the periodontal ligament and the alveolar bone and provide necessary stimulation for the progression of the resorbing process. Resorbing cells will penetrate into the dentine and resorption process will spread inside the root. However, the resorbing process will not reach the pulp, at least until late stages due to circumpulpal dentine which is generally spared from destruction.[22] Without an intervention, the resorption process would penetrate into the pulp and will continue to destroy the root and the alveolar bone leading to loss of the tooth.

Inflammatory root resorption can be effectively arrested by early removal of the necrotic and infected pulp tissue followed by a comprehensive endodontic treatment. [23,24,25] The endodontic therapy arrests the resorption process which occurs on the external surface of the root by eliminating the continuous stimulation of the resorbing cells by the noxious agents in the necrotic pulp coming through damaged dentinal tubules.

Use of calcium hydroxide (CH) as an intra-canal dressing prior to obturation has shown some beneficial effects on healing of the resorption defect.[23,24,26] Its antibacterial activity and low solubility generate a long-term effect in the root canal by removing the resorbing cell stimulation factor from the root canal. CH has an alkaline pH which increases the pH of dentine up to a level of 8.0-10.0 and thereby inhibits the activity of osteoclastic acid hydrolases in the periodontal tissues and stimulates the activity of alkaline phosphatases.[27] In contrast, it has been experimentally shown that the pH of the dentine has not been significantly changed even if the roots of teeth with exposed dentinal tubules are embedded in a medium containing CH for a period of 10 days.[28,29] However, the recommendations on the duration of CH treatment prior to obturation with gutta-percha vary considerably. Some studies[24,30] recommend 1 or 2 weeks of CH dressings prior to obturation whereas another study[31] recommends 6-24 months of CH dressings. CH treatment with long duration has been shown to be beneficial if there has been a delay in pulp removal.[24]

c. Replacement resorption

Luxation injuries and avulsion of the teeth can cause necrosis of the periodontal ligament and loss of viable cells that are required for healing with cementum formation. Extent of the damage to the cells of the periodontal ligament depends on the severity of trauma. In case of avulsion, the extent of the damage would also depend on the duration of the tooth out of the socket and drying time due to inappropriate storage. If only less than 20% of the root surface is involved, resorption may be transient and repair with viable cells from the adjacent healthy periodontal ligament is possible.[32,33] If the damage is more than 20% of the root surface, bone may come into direct contact with the root surface before the regeneration of the periodontal ligament.[26] When bone and osteoclasts are in direct contact with dentine, resorption would continue without any further stimulation. Although the exact mechanism is not clearly known, it has been suggested that cementoblasts on the root surface are replaced by osteoblastic type cells with a capability of responding to the signals of the factors involved in physiological bone remodelling.[11] It can also be hypothesized that the root becomes a part of the skeletal system in the absence of the complete periodontal wrapping around it, and subjected to go through the normal remodelling process of the bone. As the resorbed cementum and dentine is replaced by alveolar bone, this type of resorption is called replacement resorption.

Fusion of root dentine directly with bone is called ankylosis. Clinically, an ankylotic tooth lacks physiological tooth movement of healthy teeth and produces a special metallic percussion sound. As the adjacent unaffected teeth continue to erupt, ankylosed teeth will show submergence or infraocclusion of the crown compared to the rest of the dentition. Periodontal spaces and the resorbed dentine defect of the ankylotic tooth is completely replaced by bone. As such, there is no radiolucecy around the root of an ankylotic tooth.

There is no effective treatment for replacement resorption. However ankylotic tooth will remain in function for several years. There seems to be a maturation or age factor that influences the speed of the resorption and eventual loss of tooth. In younger patients, a replanted tooth with necrotic periodontal ligament would survive for a period of 3-7 years whereas, in older patients for decades or for the life time.[34]

1.2 Resorption associated with pulp necrosis and apical pathology

Infections and necrosis of the dental pulp will eventually lead to periapical periodontitis. Inflammatory cells in the periapical region will release chemical mediators such as osteoclast activation factor, macrophage chemotactic factor and prostaglandins that stimulate the resorbing process. Almost all the teeth with peri-radicular inflammation would undergo some degree of apical external root resorption. It has been shown that the presence of bacteria in the necrotic pulp is a significant factor that would enhance the resorbing process.[35,36]

Removal of the necrotic pulp tissues, irrigation with antibacterial agents and effective endodontic treatment will arrest the resorption process and lead to cemental and osseous repair.

1.3 Resorption associated with pressure in the periodontal ligament

Orthodontic forces and pressure in the periodontium caused by unerupted teeth or space occupying lesions such as cysts and neoplastic lesions could stimulate resorbing cells and initiate resorption.

Continuous and excessive orthodontic forces will result in continuous resorption in the apical third of the tooth. Teeth are generally asymptomatic and the pulp remains vital unless the orthodontic forces are exceedingly high and interfere with the apical blood supply. Removal of excessive forces will result in cessation of the resorbing process.

Pressure induced external root resorption occurs in association with impacted teeth exerting pressure on roots of adjacent teeth or as a result of space occupying pathological lesions. During eruption of the permanent teeth, pressure resorption of maxillary lateral incisors by the canines and mandibular second molar by the third molar are not uncommon. This type of resorption is usually arrested when the stimulus is discontinued.[1,26] Slow growing space occupying lesions such as cysts, ameloblastomas and giant cell granulomas cause more root resorption than rapidly growing lesions. Rapidly growing tumours are more destructive to the bone than the roots of teeth.[1,8] This type of resorption is generally asymptomatic and the involved tooth remains vital unless the impacted tooth or the pathological lesion is located closer to the apical foramen interfering with the apical blood supply. Radiologically the resorption defect is always detectable in relation to the stimulus eg: impacted tooth or a tumour.

2. Internal root resorption

Progression of root resorption starting from the inner surface of the wall of the root canal is called internal root resorption. A layer of odontoblasts and predentine lines the pulpal side of the dentine. It seems that this odontoblast-predentine layer has a protective function of the root dentine from resorption by acting as a barrier.[26,37] Chronic pulpal inflammation and bacterial toxins destroy the odontoblast-predentine barrier exposing the raw dentine wall of the pulp canal, which is now susceptible for the action of resorbing cells. Odontoblasts have no resorbing ability. Resorbing cells probably migrate to the pulp through the apical foramen.[1,37]

Internal root resorption is generally asymptomatic. When it occurs in the cervical or coronal areas, a pinkish hue may appear because of the granulation tissues visible through the progressively thinning wall with enamel and dentine.[38] Radiologically, internal resorption shows

a uniform round to oval shaped radiolucent enlargement.

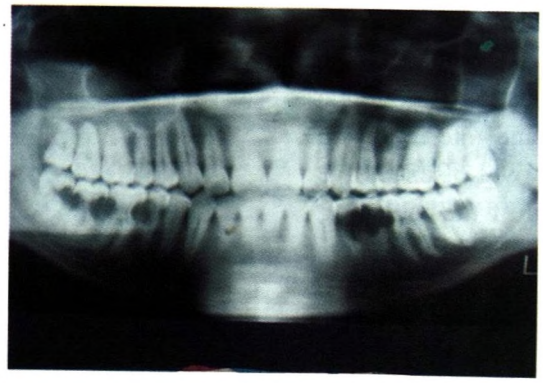
As already indicated before, resorbing cells responsible for internal resorption are of pulpal origin or move into the pulp via the apical foramen. As such when internal resorption is clinically detected, early pulpectomy is essential to arrest the resorbing process. Delay in doing so would lead to progression of resorption towards the external surface eventually producing an external communication with the periodontal ligament space drastically reducing the success of the treatment outcome.[38]

3. Idiopathic root resorption

Idiopathic root resorption is a form of progressive inflammatory external resorption but lacks an identifiable cause or stimulation. As it affects the cervical area of the tooth around the epithelial attachment, it is also called cervical resorption.

Some of the patients with idiopathic root resorption present with a history of previous orthodontic treatments, trauma, bleaching, periodontal treatments or dento alveolar surgery. In the majority of the cases however, the aetiology is uncertain, hence it is termed idiopathic resorption. Idiopathic resorption occurs in teeth with an apparently healthy pulp. As such, the resorption process is neither stimulated nor sustained by the necrotic pulp.[39] As shown in figure 1 multiple teeth involvement is not uncommon.

Idiopathic resorption probably starts from a small area of damage to the cervical cementum. Resorbing cells penetrate through and spread the resorption defect into the cementum and dentine. The source of the continuous stimulation for the resorbing cells is still far form clear. It is believed that the bacteria present in the gingival sulcus colonise the affected root surface and provide the necessary stimulation for the cells to continue the process.[26] Spreading resorption defect causes destruction to dentine sparing a layer of dentine around the dental pulp. There is no predictable treatment available at present for idiopathic root resorption.



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Research Article 1

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Host response at the invasive front of oral squamous cell carcinoma (OSCC): histopathological and immunohistochemical evaluation

U. B. Dissanayake, D.M. Dissanayake, A.K. Suraweera, E.A.P.D. Amaratunga.

Abstract:

Objective: Objective of the present study was to evaluate the association if any, of histopathological grade of the tumour cell population with that of sub-tumoral immune response of the host at the confronting invasive front of the oral squamous cell carcinomas (OSCCs) using histopathological and immunohistochemical methods.

Material and methods: Series of 75 formalin fixed paraffin embedded oral squamous cell carcinomas (OSCCs) were used for this study. The histopathological parameters of tumour cell population, namely degree of keratinisation, cellular pleomorphism, mitotic count and sub-tumoral lympho-plasmocytic cell filtrate (LPCI) were assessed using scale in Bryne's grading system. Scores for three parameters of the tumour cell population (keratinisation, cellular pleomorphism and mitoses) were summed into a total score. T cell fraction in the sub-tumoral cell infiltrate was stained immunohistochemically using monoclonal antibody CD45RO-UCHL1. In case of estimating the T cell index over thousand cells from each chosen area were counted and the immuno positive and negative cells were recorded separately. T cell index for each tumour was estimated as the percentage of immuno positive cells out of total infiltrate. Association of sub-tumoral LPCI verses score for the individual histopathological parameter/ total score of the tumour cell population was statistically evaluated. Correlation between T cell index versus score for the individual histopathological parameter/total score of the parameters of the tumour cell population was also studied separately.

Results: Significantly higher LPCIs were observed in tumours with high keratin scores at their invasive fronts (χ^2=10.15,p=0.01). No association was found between other parameters.

Upul B. Dissanayake (Correspondance) B.D.S, M,Phil, Ph D. Senior Lecturer, Head, Department, Department of Oral Pathology, Faculty of Dental Sciences,University of Peradeniya, Peradeniya. E-mail: upulbd@pdn.ac.lk

D.M. Dissanayake M.B.B.S.(SL), D. Path (SL), MD (SL), Ph.D (UK) Consultant Haematologist, Senior Lecturer, Department of Pathology, Faculty of Medicine, University of Peradeniya, Sri Lanka. E-mail: dhammikamdisa@yahoo.co.uk

A.K. Suraweera Department of Oral Pathology, Technical Officer, Faculty of Dental Sciences

E.A.P.D. Amaratunga BDS (SL), MS (Col), MSc (Lond), FDSRCS (Eng), FFDRCS (Ire), Senior Lecturer, Consultant Oral Pathologist, Department of Oral Pathology, Faculty of Dental Sciences, University of Peradeniya, Sri Lanka, E-mail : prasada@pdn.ac.lk

Conclusion: The relationship between the degree of keratinisation and grade of LPCI has not been established before. This finding harmonizes with the known fact that highly keratinised tumours show heavy sub-tumoral immune cell filtrate and better prognosis. However, elucidation of significantly higher LPCI may be due to emergence of keratin in unrelated foreign sites like sub-epithelial areas where keratinisation does not normally occur rather than true immune response against invading tumour. As such, further insights into the finding is needed to elaborate the specificity of the relationship.

Key words: Tumour immunity,
Host response

Introduction:

Most of the solid tumours are found to contain large numbers of infiltrated lymphocytes suggesting that cellular immune system recognizes the malignant neoplasm as foreign and the need to attack it.[1,2] Strong sub-tumoral lympho-plasmocytic cell infiltrate against the invasive front of a tumour is suggestive of better prognosis and low grade of malignancy in certain tumours.[3,4] Patients with immune deficiencies and those who are on immune suppressive therapy are at an increased risk of developing malignancies.[5,6,7] Clinical observations further indicated that cancer is relatively rare in children and young adults but appears more frequently with advancing age when the efficiency of the immune system declines. Tatake *et al,* (1989) experimentally demonstrated the lysis of target tumour cell lines derived from head and neck and oral cancer by lymphokine activated killer cells generated from peripheral blood lymphocytes of patients with oral cancer.[8] The study further highlighted that the tumour infiltrating immune cells can be the potential source for lymphokines activated killer cells. Alterations in T lymphocyte subpopulations in the peripheral blood samples of patients with head and neck squamous cell carcinomas have also been demonstrated.[9] However, though the host could mount an immune response against altered antigens expressed by various tumours, it is an obvious fact that most of the patients with malignant tumours still die of their disease. This implies that the role played by the immune response against tumours is complicated and less certain.

It has been shown that the histo-morphological characteristics at the deeper-most invasive front of OSCCs are of utmost importance in anticipating behaviour, planning treatments and predicting the prognosis of the tumour.[10,11,12,13,14] Potentially important interaction between tumour cell population and immune cell response at the deep invasive front has also been demonstrated.[2,4,15]

We hypothesise, that the degree of peri-tumoral lympho-plasmocytic cell infiltration at the invasive front, has a decisive implication on the malignancy grade of the tumour cell population and prognostic outcome of the tumour. As T cells play a major role in both cell mediated immune mechanism and development of immunity against tumours the T cell density in the sub tumoural area may remark the intensity of the cell mediated immune reaction mounting against the invading tumour.[2,3,14,16,17,18] Degree of keratinisation, grade of cellular

pleomorphism and the number of mitosis in the tumour cell population collectively substantiate the virulence of the tumour cell population. Sub-tumoral lymphoplasmocytic cell infiltrate at the confronting invasive front is supposedly considered to be the morphologic substrate of the immune response of the host against the tumour.

Aim of the present study was to evaluate the association if any, of histopathological grade of the tumour cell population with that of sub-tumoral immune response of the host at the confronting invasive front assessed by histopathology and immunihistochemistry in oral squamous cell carcinomas.

Material and methods

Sample

Seventy five formalin-fixed paraffin-embedded wax blocks of 75 primary oral squamous cell carcinoma which had deep invasive front were selected from the archives of the Department of Oral Pathology Faculty of Dental Sciences for the study. The mean age of the patients was 61.96±12.32 and there were 55 males and 20 female.

Histopathological grading

Six micron sections were cut from each biopsy and stained with haematoxylin and eosin for histopathological assessment. Only the most anaplastic fields in the most invasive areas of the tumours were used for scoring. Tumour cell population was assessed using degree of keratinisation, nuclear pleomorphism and number of mitotic figures per high power field. Each of the morphological parameters was scored on a point scale running from 1- 4.[10,11,12] Total score of tumour cell population for each tumour was calculated by summing the three scores into a total. Host response was assessed histopathologically on the basis of the degree of lymphoplasmocytic cell infiltration (LPCI) in the sub-tumoral area opposite to the deep invasive front selected for scoring. Same point scale used in Bryne's classification for assessment of immune cell response of the host was used for grading of the infiltrate in the present study.[10,11,12] In analysing the results grades I and II in the point scale were considered as low and grades III and IV were considered as high, separately for each parameter.

Immunohistochemical staining

Consecutive section from each tumour was mounted on sialine coated, slides for immuno staining of the T cell fraction of the lymphoplasmocytic cell infiltration. Phosphate buffered saline (PBS) was used as the diluent for washing and rinsing steps throughout the immunohistochemistry protocol except for the purposes of antigen retrieval.

Antibody

The antibody used was **CD45RO-UCHL1** (DAKO, Cat No; 0505). CD45RO-UCHL1 is a human specific mouse monoclonal antibody belonging to the IgG2b class, raised against CD45 transmembrane protein molecule of the cell membrane. Antibody labels most thymocytes, subpopulation of resting T cells within both CD4 and CD8 subsets, and mature, and activated T cells. B cells and NK cells are consistently negative.

Positive and negative controls

One section from each tumour block was used as the internal negative control by omitting the primary antibody and by incubating with the diluent buffer (PBS). A section from a reactive lymph node acted as a positive control. One positive control was included for each immunohistochemical run.

Staining procedure

Sections were dewaxed, and rehydrated in a series of alcohol and incubated in 0.5% hydrogen peroxide (H_2O_2) in 70 % methanol in PBS for twenty minutes to eliminate endogenous peroxidase activity. The antigen retrieval was done following microwave (Toshiba ER665ET, 650W) method in citrate buffer (pH 6.0). The slides were then bench cooled, washed with PBS and incubated with normal goat serum (1:30 dilution) in PBS for 20 minutes to block non specific immune reactions. Incubation with the primary antibody was done for a period of one hour (T cell antibody; dil 1:50, DAKO, Cat No; 0505). Once primary incubation was completed the slides were rinsed in PBS and incubated with biotinylated secondary antibody (Daco-Duet) (dil 1:100 in PBS) for twenty minutes. Following a further PBS wash, the sections were treated with biotin-streptavidin complex (Dako-Duet) (dil 1:100) for a further twenty minutes. Visualization (chromagen) was obtained with 0.05% diaminobezidine tetra hydrochloride (DAB) (Sigma Cat No: 5637) in 0.1% hydrogen perioxide in PBS for 10 minutes. The sections were rinsed in tap water, and counter stained with Harries' haematoxylin. After dehydration in a graded series of ethanol (70%, 90% and 100%) and section were cleared with xyline and mounted in DePex.

Assessment of immunocytochemistry

Sections stained with T cell antibody were examined under light microscopy. Immuno positive T cells were clearly identified by their brown membrane staining. Ten samples, which did not show brown membrane staining, were excluded from the sample. Total of 65 tumours, positive for the antibody were assessed quantitatively. Cell counts were made at X400 magnification using 10 x 10 square eyepiece graticule (Graticule Ltd.) on a conventional light microscope. Cells were counted in the same subtumoral area used for histopathological grading. Over thousand cells from each chosen area were counted and the immuno positive and negative cells were recorded separately. T cell index for each tumour was estimated as the percentage of immuno positive cells out of total infiltrate. Reproducibility was determined by counting and recounting replicates of the same fields until consistency was reached (data not shown) One observer performed all the counts to eliminate the inter-observer variability.

Statistical method

Chi square method was used to assess any significant difference between parameters. A significance level was accepted at the 0.05 level. The relationship between parameters were investigated using pearson correlation coefficient (r).

Results

The statistical analysis showed that oral squamous cell carcinomas with high keratin scores at the deep invasive fronts showed significantly higher lympho-plasmocytic cell infiltrate (χ^2=10.15,p=0.01) (Table 1). However LPCI was not significantly different either in the tumours between high and low grades of pleomorphism (χ^2=1.94,p=0.16) or between tumours with high and low mitotic counts (χ^2=2.88,p=0.09) (Table 1). Pooled data for keratinisation, pleomorphism and mitosis when dichotomised as high and low,

also showed no significant difference in the LPCI between two groups of tumours (χ^2=0.64,p=0.42) (Table 1).

Table 2 shows that the T cell index was not significantly different between tumours with high and low grades of pleomorphism (χ^2=4.35,p=0.11), between tumours with high and low mitotic counts (χ^2=3.28,p=0.19) or between tumours with high keratin score and low keratin score (χ^2=2.82,p=0.24). When data was pooled for keratinisation pleomorphism and mitosis as total score again no significant difference was found in the T cell index, between high and low grades (χ^2=.30, p=0.6).

Table 3 shows values for the correlation coefficient (r) between parameters studied in the present cohort. No correlation was found between the T cell index and the individual histopathological parameters of the tumour cell population namely Keratinization (r=0.087) pleomorphism (r=0.169), and mitotic count (r=0.226) (Table 3).

Figure 1 gives a scattergram to show the correlation between the T cell index and the total score of the tumour cell population of the individual tumours. The distribution of the scatter gram shows that there is no correlation between two parameters.

Table 1. The association between the parameters of the tumour cell population and sub-tumoral lymphoplasmocytic cell infiltration (n=75)

Parameter	Grade	LPCI		Significance (Chi², p vaule)
		Grade I & II	Grade III & IV	
Keratinisation	Grade I & II	7 (9%)	4 (15%)	χ^2=10.15, p=0.01
	Grade III & IV	49 (65%)	15 (%)	
Pleomorphism	Grade I & II	28 (36%)	6 (8%)	χ^2=1.94, p=0.16
	Grade III & IV	28 (36%)	13 (16%)	
Mitosis	Grade I & II	24 (32%)	4 (5%)	χ^2=2.88, p=0.09
	Grade III & IV	32 (42%)	15 (20%)	
Total (Keratinisation	Grade I & II	10 (15.3%)	20 (30.7%)	χ^2= 0.64, p=0.42
Mitosis, Pleomorphism)	Grade III & IV	14 (21.5%)	21 (31.7%)	

Table 2. The association between parameters of the tumour cell population and immuno stained T cell fraction (T cell Index) of the sub-tumoral lymphoplasmocytic cell infiltrate (n=65)

Parameter	Grade	T cell index	T cell index	Chi², p vaule
Keratinisation	**Grade I & II**	7 (10.9%)	3 (4.6%)	χ^2=2.82, p=0.24
	Grade III &IV	23 (35.3%)	32 (49.1%)	
Pleomorphism	**Grade I & II**	17 (26.3%)	11 (17%)	χ^2=4.35, p=0.11
	Grade III &IV	13 (20%)	24 (36.9%)	
Mitosis	**Grade I & II**	8 (12.3%)	17 (26%)	χ^2=3.28, p=0.19
	Grade III & IV	22 (38.8%)	18 (27.8%)	
Total Score	**Grade I & II**	14 (21.5%)	21 (31.7%)	χ^2=0.30, p=57
	Grade I & II	7 (10.9%)	3 (4.6%)	

Table 3. Correlation between T cell Index and parameters of the tumour cell population (n=65)

Parameter	TCI (T Cell Index)
Keratinisation	r = 0.087
Pleomorphism	r= 0.169
Mitosis	r= 0.226

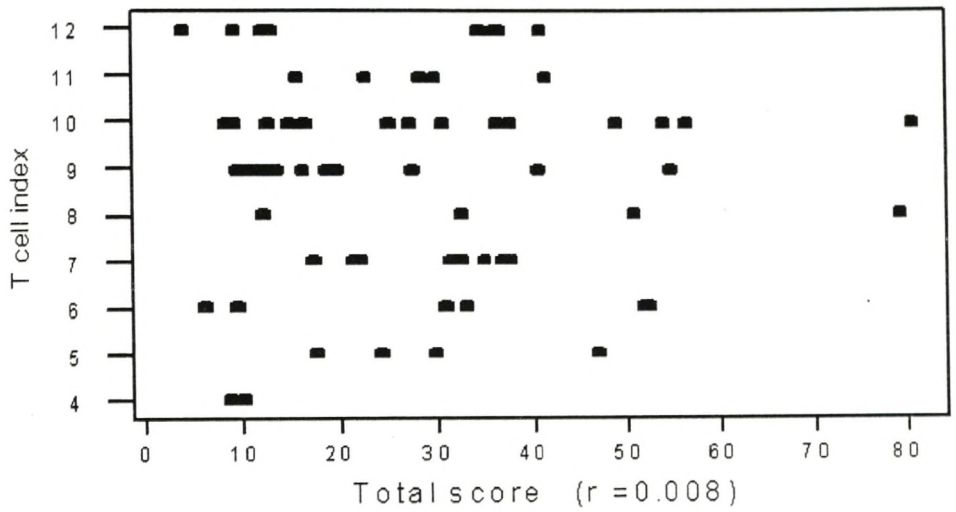

Figure 1. Correlation between T cell Index and total score of the parameters (Keratinisation, pleomorphism, mitosis) of the tumour cell population (n=65)

Discussion.

Literature reveals that the cell mediated immune mechanism has a bigger role in mounting immune response against tumours.[1,2,17] With the aim of evaluating the local T cell response against invading OSCC, parameters of tumour cell population namely degree of keratinisation, cellular pleomorphism and number of mitoses versus host factor which directly symbolized the lympho-plasmocytic cell infiltration at the sub tumoral area, were considered in the present study. We omitted the pattern of invasion from our panel of parameters as it is thought to be responsible for representing both tumour cell population and host response.[18] However, Slootweg *et al*, (1994) demonstrated a correlation between pattern of invasion and the peri-tumoral plasmo-lymphocytic cell infiltrate using Aneroth's histopathological scale.[14]

Significantly higher lympho-plasmocytic cell infiltrates were observed in tumours with high keratin scores at their invasive fronts in our study. The relationship between the degree of keratinisation and grade of lympho-plasmocytic cell infiltrate has not been established before. This finding harmonizes with the known fact that highly keratinised tumours show better prognosis.[19] But, emergence of keratin in unrelated foreign sites like sub-epithelial areas where keratinisation does not normally occur may have been the reason for the elicitation of significant higher levels of lympho-plasmocytic cell infiltrate rather than true immune response against invading tumour. However, further insight into the finding is needed to elaborate the specificity of the relationship between keratinisation score and illusion of higher immune response at the invasive front. Other two parameters of tumour cell population namely mitotic count and degree of pleomorphism showed no relationship with peri-tumoral lympho-plasmocytic cell infiltrate in terms of histopathological grading. To our knowledge, a direct histological evaluation between tumour cell

population and peri-tumoral immune cell infiltrate in OSCCs has not been reported before for comparison.

Based on the our hypothesis, the monoclonal antibody CD45RO-UCHL1 was used and it immuno labelled the majority of the T cell population in the infiltrate including thymocytes, subpopulation of resting T cells within both CD4 and CD8 subsets, and mature, and activated T cells. However, results showed that no association was found between T cell index (proportion of immuno positive T cells in the peri-tumoral infiltrate) and keratinisation, cellular pleomorphism or mitotic count assessed by Bryne's histopathological scale. There was no association found between the T cell index and the total histopathological score of the cancer cell population too. Studying the peri-tumoral cell infiltration in SCCs and premalignant lesions obtained from different sites of the body Perez *et al,* (1999) also highlight that there was no correlation between nature of the immune cell infiltrate and the histological grade of oral squamous cell carcinomas.[4] Analysing 146 cutaneous melanomas Brocker *et al* (1988) reported a reduction in the amounts of T lymphocytes during the tumour progression.[15] In contrast to this Hiratsuka *et al,* (1984) demonstrated an association between immune labelled T cell infiltrate and the local invasion in OSCCs.[2] Further Sevennevig *et al,* (1984) correlated the lympho-plasmocytic cell infiltrated with that of prognosis of the colorectal carcinomas.[20]

It has been demonstrated that the expression of MHC antigens is compulsory to present tumour cell antigen to the T lymphocytes for a proper host response to be developed.[21] Confirming this finding, Sadanaga *et al,* (1994) showed that local immune response may prevent tumour invasion, only when HLA-DR antigen is expressed by the tumour cells.[22] However, most of the OSCCs show down regulation of MHC antigens in their tumour cells, resulting in failure of development of immune response against invading tumours. As such studying T cell index together with the expression of MCH antigens emerges as areas for future studies.

In conclusion, it may be said that there is no association found between grade of tumour cell population and the lymphoplasmocytic cell infiltrate assessed both by histopathological and immunohistochemical methods at the invasive front areas of oral squamous cell carcinomas apart from keratinisation. However, further studies analysing the different sub sets of T cells fraction together with the expression of MHC class 1 and II antigens would be valuable in clarifying the mechanisms by which host immune system interact with cancer cell population.

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Research Article 2

Sri Lanka Dental Journal 2006; 36(02)90-97

Correlation of Matrix Metalloproteinase -9 (MMP-9) expression with clinico-pathological parameters of oral squamous cell carcinomas (OSCCs).

P.R Jayasooriya, A.K Suraweera, N. Bandoh, Y. Harabuchi and E.A.P.D Amaratunga

Abstract

Objectives: The purpose of the present study was to evaluate the expression of MMP-9 and to correlate these findings with clinico-pathological features of oral squamous cell carcinomas (OSCCs) from Sri-Lanka.

Material and methods Tissue specimens from 30 patients with primary OSCC were used in the study. Expression of MMP-9 was evaluated in tumour cells and stromal cells within the tumour and stromal cells and epithelium in adjacent normal tissues using immunohistochemistry. MMP-9 expression was categorized into four grades depending on the number of positive cells and then the correlation between these four grades and clinico-pathological features were analyzed statistically using Chi-square test at 5% level of significance.

Results The results revealed, 28 (93%) of OSCCs with MMP-9 expression in tumour cells. With reference to stromal cells within the tumour, 27 (90%) OSCCs showed MMP-9 positivity. However, in the normal tissues adjacent to OSCC, MMP-9 expression was seen in only 4 (13%) samples. As such MMP-9 expression was significantly increased in malignant tissue compared to adjacent normal tissues ($p<0.05$). No correlation was observed between MMP-9 expression and clinico-pathological features such as age, site, size and histopathological grade of the tumour. In addition, no statistically significant differences were observed in MMP-9 expression levels in patients with and without lymph node metastasis ($p>0.05$).

P.R. Jayasooriya (Correspondance) BDS (SL), PhD (Japan), Senior Lecturer, Dpartment of Oral Pathology, Faculty of Dental Sciences, University of Peradeniya, Peradeniya. E-mail: primalij@yahoo.com

A.K. Suraweera Technical officer, Department of Oral Pathology, Faculty of Dental Sciences, University of Peradeniya, Peradeniya. E-mail:anuraaks@yahoo.com

N. Bandoh MBBS (Japan), PhD (Japan) Senior Lecturer, Department of Oto-larygology, Head and Neck Surgery, Asahikawa Medical College, Japan. E-mail: bando@asahikawa-med.ac.jp

Y. Harabuchi MBBS (Japan), PhD (Japan) Head, Department of Oto-larygology, Head and Neck Surgery, Asahikawa Medical College, Japan. E-mail: hyasu@asahikawa-med.ac.jp

E.AP.D. Amaratunge BDS(SL), MS (Col), MSc (London), FDSRCS (England), FFDRCS (Ire) Department of Oral Pathology, Faculty of Dental Sciences, University of Peradeniya, Peradeniya. E-mail: prasada@pdn.ac.lk

Conclusion: In conclusion, relatively high number of OSCCs in the present study was found to express MMP-9 when compared to other studies. In addition, it is not possible to predict lymph node metastasis depending on MMP-9 expression in patients with OSCCs.

Key words: Matrix metalloproteinases, Oral squamous cell carcinoma, tumour invasion

Introduction

Squamous cell carcinomas (SCC) account for approximately 97% of all malignant tumours of the oral cavity.[1] Although, treatment guidelines have been developed for OSCCs according to TNM staging, histopathological diagnosis and the site of the lesion, patients' survival rate still remains poor. The poor prognosis has been attributed mainly to extensive local invasion and frequent spread to lymph nodes.[2, 3] As such an understanding of molecular and cellular mechanisms involved in tumour invasion would be useful to predict metastasis and recurrences and thus provide better management for patients with OSCC. One such factor, namely matrix metalloproteinases (MMP) has gained much attention in the recent years as degradation of extracellular matrix is essential for malignant tumour invasion and angiogenesis.

Matrix metalloprotenases are a family of Zinc dependent proteinases capable of degrading all matrix components. At present twenty one members of the human MMP gene family are known and are classified into four groups depending on the substrate specificity. They are the collagenases (MMP1, 8 and13), gelatinases (MMP2 and 9), stromelysins (MMP3, 10 and 12) and membrane type MMPs (MT-MMP).[4,5] Out of the four groups, gelatinase A (MMP-2) and gelatinase B (MMP-9) breakdown the components of the basement membrane are crucial in invasion of malignant tumours. Even though, there are numerous reports on the expression of MMPs and its relationship with the clinical stage, lymph node metastasis and histopathological grade of head and neck SCCs, these results are controversial.[6,7,8,9,10] In addition, studies on MMPs expression in OSCCs from South East Asia where the molecular aetiopathogenesis differs from that of OSCCs from other parts of the world are limited.[2] Therefore, the aim of the study was to evaluate and correlate the MMP-9 expression with clinico-pathological features such as age, site, size, histopathological grade and lymph node metastasis of OSCCs from Sri-Lanka.

Material and methods

The study was based on thirty cases of OSCCs randomly selected from archives of the Department of Oral Pathology, Faculty of Dental Sciences, University of Peradeniya. Relevant clinical data of the patients was obtained from their clinical records. Initial, histopathological diagnosis was made using incisional biopsies, and the tissue for the study was obtained from the excisional biopsies performed at the time of surgery.

Immuno histochemical staining

Four ìm thick sections were taken from formalin fixed paraffin embedded specimens and placed on sialane coated slides. The specimens were deparafinized in xylene and rehydrated in a series of alcohol. Then, the sections were incubated in 3% hydrogen peroxide for 30 minutes to block the endogenous peroxidase activity. The slides were placed in 10 mM citric acid buffer at pH 6.0 and subjected to antigen retrieval for 10 minutes at 750W in a micro-wave oven. Mouse monoclonal antibody against MMP-9 (56-2A4;

FUJI Chemical Industry, Takaoka, Japan: 5ìg/ml) was used as the primary antibody. The primary antibody was incubated overnight at 4°C and followed by incubation with secondary antibody for 30 minutes. Visualization was obtained by immersing the slides in freshly prepared 0.02% diaminobenzidine solution for 10 minutes. The sections were counter stained with Mayer's haematoxylin, dehydrated in graded alcohol and cleared with xyline and mounted.[6]

Assessment of immuno staining

The results of the immunohistochemical staining for MMP-9 was evaluated semi-quantitatively on the basis of a four point scale as follows; (-) negative staining, (+) low expression (< 10% positive cells), (++) moderate expression (10-50% positive cells), and (+++) diffuse expression (>50% positive cells).[11] For the statistical analysis, patients were divided into two groups: those with no or low expression and those with moderate or diffuse MMP-9 expression. Correlation between the grade of MMP-9 expression and clinico-pathological features were analyzed using Chi-square test. (Level of significance =0.05)

Results

MMP-9 expression was mainly observed in the cytoplasm of tumour cells. The surface epithelium over the tumour also showed strong MMP-9 positivity (Fig 1). In addition to MMP-9 expression in tumour cells, it was also detected in the stromal fibroblasts of tumour induced stroma, endothelial cells and inflammatory cells. Therefore, it was decided to evaluate the MMP-9 expression (depending on the number of positively stained cells) in tumour cells and stromal cells separately. The results are given in Table 1. Accordingly MMP-9 expression was detected in 28 (93%) OSCC specimens. Out of the 28 immuno-positive tumours, 18 (60%) specimens showed moderate to diffuse expression of MMP-9 with reference to tumour cells (Fig 2). In stromal cells within the tumour, 90% (27/30) of the OSCCs showed MMP-9 positivity. As such, there was no statistically significant difference in MMP-9 expression between tumour cells and stromal cells within the tumour (Chi-square test: $p > 0.05$). None of the OSCCs were MMP-9 negative in both tumour and stromal cells.

MMP-9 expression was also evaluated in tissues adjacent to the tumour and only the stromal cells were found to express MMP-9 in this area. The normal epithelium adjacent to the tumour did not express MMP-9. Accordingly, in the stromal tissues adjacent to the tumour, MMP-9 expression was seen in only 4 (13%) samples (Table 1). As such MMP-9 expression was significantly higher in malignant areas compared to the adjacent normal tissues.

No significant correlation was found between MMP-9 expression and clinical parameters or histopathological parameters (Table 2). Although tumours with MMP-9 over expression showed more frequent lymph node metastasis compared to the tumours without MMP-9 expression, the difference was not statistically significant ($p > 0.05$).

Discussion

Tumour invasion and metastasis is an extremely complex multi step process. Activities of extracellular matrix degrading enzymes are compulsory for the purpose of tumour cell dissemination. Basement membrane is the first barrier of epithelial tumour cell invasion. The ability of MMP-9 to initiate basement membrane destruction and further, collagen and non-collagen components degradation suggest its importance in tumour invasion. MMP-9 expression is detected in malignant keratinocytes located at the tumour-stroma interface.[12] In addition, MMP-9 has been

shown to be a key factor in determining the invasive phenotype of at least some sub populations of OSCC tumour cells.[13] MMP-9 occurs in different forms in biological samples such as latent enzyme, active enzyme or complexed with inhibitors. Assays such as immunohistochemistry, ELISA and zymography are used to detect MMPs. Both ELISA and zymorgraphy are used to differentiate active and latent forms of the enzyme. However, disturbance of the spatial relationship as a result of physical destruction of the tissue architecture in preparation is one of the disadvantages of these techniques.

MMP-9 is overexpressed in tumour cells as well as in stromal cells. It has also been experimentally shown that MMPs produced by stromal cells make a significant contribution to squamous cell carcinogenesis in a transgenic mouse model.[14] As such, we employed immunihistochemistry as the method of choice for the present study as it can be used to evaluate MMP-9 expression in both tumour cells and stromal cell seperately. In addition none of the previous studies to the best of our knowledge has evaluated MMP-9 expression in tumour cells and stromal cell separately. According to the results of the present study, relatively high number of OSCCs expressed MMP-9. The percentage of OSCCs that expressed MMP-9 in the present study is similar to that of the percentage reported by Jordan *et al,* (2004).[5] In contrast, Katayama *et al,* (2004) demonstrated a low percentage of OSCCs with MMP-9 in their study.[6] Frachi *et al,* (2002) also indicated a small percentage of OSCCs with MMP-9 over-expression.[11] The reason for this difference may be attributed to the clinical stage of the disease. Accordingly, higher proportion of OSCCs in these two studies belonged to clinical stage 1 and 2 compared to the present study where all the OSCCs were either stage 3 or 4. Metalloproteinases are often over-expressed by the stromal cells adjacent to the invasive tumour fronts. Among non-malignant cells, fibroblasts, endothelial cells and inflammatory cells synthesize different MMPs.[16] Results of the present study show both stromal and tumour cells to express MMP-9 in varying intensities. In addition, stromal cells showed MMP-9 over-expression with or without MMP-9 over-expression in the tumour cell population.

No correlation was found between MMP-9 expression and clinico-pathological parameters in the present study. Similar results were reported by Yorioka *et al,* (2002)[17] and Patel *et al,* (2005).[2] However, significantly higher MMP-9 scores have been reported in patients with lymph node involvement and distant metastasis.[6] The reason for this difference may also be attributed to clinical stage and size of the lesion, as all the samples in Katayama's study were T1 and T2 lesions compared to T3 and T 4 lesions in the present study. In addition, aetiopathological differences may have also contributed to this difference.

Several studies have attempted to delineate the type of the MMPs that are necessary for the growth and spread of OSCC. Accordingly, combined actions of several MMPs are considered essential for efficient degradation of all complex components in the extra cellular

matrix. As we have only evaluated one antibody (MMP-9) the present study has limitation in addressing this issue.

In conclusion relatively high number of OSCCs in the present study was found to express MMP-9 when compared to other studies. Usage of MMP-9 over-expression as a predictive marker of lymph node metastasis may not be feasible especially on OSCCs of advanced clinical stage.

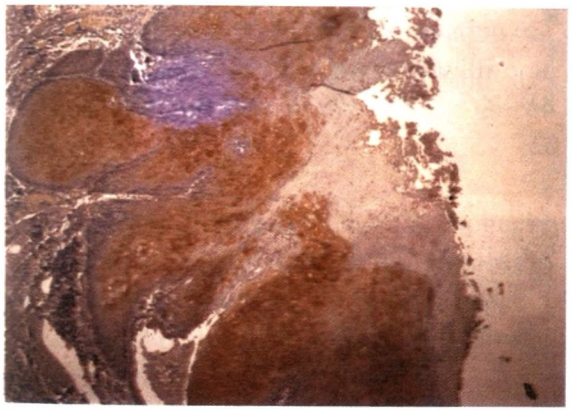

Figure 1. Photomicrograph shows MMP-9 expression in the surface epithelium over the tumour (x10).

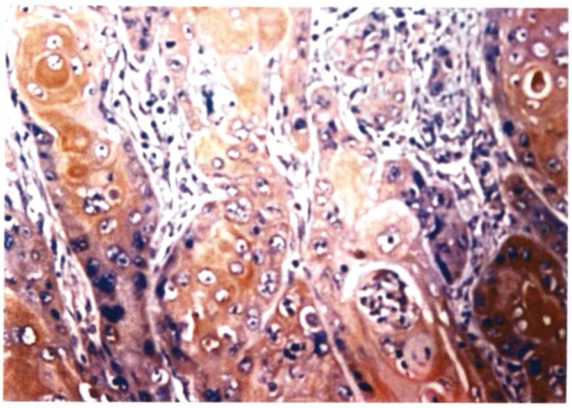

Figure 2. Photomicrograph shows MMP-9 expression in SCC tumour cells: note: +++, diffuse expression of MMP-9 within tumour cells and stromal cells (x10).

Table 1. The Number and percentage of OSCCs with different grades of MMP-9 expression (n= 30).

Type of tissue	MMP-9 negative tumours	Tumours with MMJ-9 expression (percentage positivity)		
		<10% of cells	10-50% of cells	>50% of cells
Tumour cells within the lesion	2 (6.6%)	10 (33.3%)	13 (43.3%)	5 (16.6%)
Stromal cells within the lesion	3 (10%)	7 (23.3%)	14 (46.6%)	6 (20%)
Stromal cells in normal adjacent	26 (86.6%)	3 (10%)	1 (3.3%)	0 (0%)

Table 2. Correlation between MMP-9 expression (in tumour cells) and clinico-pathological parameters of OSCCs.

Parameter	Number	MMP-9 expression (percentage positivity)		(Chi-square)
		(-)negative (<10% cells positive)	11-100% cells positive	
Age: Below 50 yrs:	10	4	6	p>0.05
Above 51 yrs:	20	8	12	
Gender: Male:	24	10	14	p>0.05
Female:	06	02	04	
Size: TI-T2:	02	00	02	p>0.05
T3-T4:	28	12	16	
Site: Tongue:	06	03	03	
FOM:	03	02	01	p>0.05
BM	17	06	11	
RMA	04	01	03	
Histopathology:				
WDSCC	14	06	08	p>0.05
MDSCC	15	05	10	
PDSCC	01	01	00	
Lymph-node metastasis				
Present	16	05	11	p>0.05
Absent	14	07	07	

FOM: Floor of the mouth, BM: Buccal mucosa, RMA: Retromolar area, WDSSC: Well differentiated squamous cell carcinoma, MDSCC: Moderately differentiated squamous cell carcinoma, PDSCC: Poorly differentiated squamous cell carcinoma

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Case Report

Sri Lanka Dental Journal 2006; 36(02) 98-101

Malignant Peripheral Nerve Sheath Tumour (MPNST) of zygomatic region presenting as a painful swelling: A case report

R.D. Jayasinghe, A.M. Attyagala, E.A.P.D. Amaratunga

A case of Malignant Peripheral Nerve Sheath Tumour (MPNST) located in the zygomatic region of a 36 year old female is presented. Clinical history was short and the main complaint was pain in the zygomatic region of three weeks duration.Ultrasound scanning and Fine Needl Afpiration Biopsy (FNAB) were inconclusive. As such, an exploration biopsy was carried out under general anesthesia. Debulking surgery was performed prior to sending the patient for oncological management. This case highlights the importance of immunohistochemical staining in the diagnosis of MPNST as the Haematoxylin and Eosin (H and E) histopathological findings alone could be confusing.

Key words- Malignant Peripheral Nerve sheath tumours, Neurofibromatosis, Zygomatic region

Introduction

Malignant Peripheral Nerve Sheath Tumour (MPNST) also known as malignant schwannoma is a rare tumour which has a significant association with neurofibromatosis (von Recklinghausen's disease). Association with neurofibromatosis type 1 has been shown in 60-80% of MPNSTs and more than 60% of tumours are derived from neurofibromas. Only a minority of MPNSTs are sporadic and arise de novo.[1] The classical histological features of MPNST include spindle shaped cells with hyperchromatic nuclei and high mitotic activity. In addition to the more common conventional type of MPNST, three other histopathological types have been described. These include Epithelioid (eMPNST), Glandular and Malignant triton tumour.[1] The epithelioid variant accounts for 5-10% of MPNST and has no association with neurofibromatosis.[1]

Confirmation of histopathological diagnosis of MPNST requires demonstration of neural origin of the tumour and with routine H and E sections alone, it may be confused with other spindle cell lesions.

Dr Ruwan Duminda Jayasinghe (Correspondence) BDS (SL) MS(Col) Lecturer, Department of Oral Medicine and Periodontology, Faculty of Dental Sciences University of Peradeniya, E-mail : ruweanduminda@yahoo.com

Dr. A.M.Attyagala BDS (Sri Lanka), MS Col, FDSRCS (Eng). Senior lecturer and Head,Department of Oral and Maxillofacial Surgery ,Faculty of Dental Sciences, University of Peradeniya E-mail : manjuatty@yahoo.com

Dr. E.A.P.D. Amaratunga BDS (SL), MS (Col), MSc (Lond), FDSRCS (Eng), FFDRCS (Ire), Senior Lecturer, Consultant Oral Pathologist, Department of Oral Pathology, Faculty of Dental Sciences, University of Peradeniya, Sri Lanka, E-mail : prasada@pdn.ac.lk

Case Report

A 36 year old woman presented to the oral and maxillofacial clinic, Faculty of Dental Sciences, Peradeniya with the complaint of pain in the left zygomatic region, of three weeks duration. Severity of the pain was gradually increasing with significant tenderness over the left zygomatic region. Past medical history was not significant. In the clinical examination a tender, diffuse, firm swelling was found over the left side zygomatic region. Head and neck, general and neurological examination was normal.

Ultrasound scanning of the lesion revealed a 20x 9 mm sized lesion with well defined margins. Posterior enhancement/hyper-echoic shadow was noted. As these features were inconclusive and might be suggestive of an organized abscess, fine needle aspiration biopsy was performed. Results of FNAB were inconclusive too. As such an exploration biopsy was carried out under general anesthesia. Pre-auricular incision was made and opened into the zygomatic region. An irregular, extensive tumour was noted deeper to the parotid gland. Facial nerve branches were not involved. During the surgery, the lesion gave an impression of a connective tissue malignancy. Due to the extensive tumour infiltration, only debulking surgery was performed and the resected tissue specimen was sent for histopathology. The patient was referred to the Oncological unit, General Hospital, Kandy for further management and she underwent a course of local radiotherapy and chemotherapy.

Pathological findings

H and E sections taken from the specimen showed a proliferating mass of spindle cells containing large granular nuclei. In places, cells were arranged in a rolled manner where as in other areas, tumour cells were arranged to form a solid mass. Proliferative activity was high.

Immunohistochemical staining for S-100 and smooth muscle actin was performed. Tumour cells were weakly positive for S-100 and showed no positivity for smooth muscle actin. Histopathological features were suggestive of a spindle cell sarcoma and the immunohistochemical staining favored the diagnosis of MPNST.

Discussion

Sarcomas in head and neck region are relatively uncommon. MPNST is a very rare tumour with an incidence of only 0.001% in the general population and is mostly associated with neurofibromatosis (von Recklinghausen's disease).[2,3] MPNST is even rarer in the head and neck region and only few cases have been reported in the western medical literature. Only 20% of MPNSTs occur in the head and neck region. MPNSTs that are not associated with neurofibromatosis, classically develop in large nerves of the trunk or extremities.[2,3] These tumours show a very poor prognosis with a high risk of local spread and distant metastasis.

Wide local excision is the treatment of choice in the management of head and neck sarcomas. Adjunct radiotherapy is recommended for high grade and larger tumours whereas adjunct chemotherapy is recommended for the tumours with high risk of distant metastasis. Watanabe *et al,* (2001) identified the Ki67 labeling index as a marker of prognosis.[3] They further highlighted

that the MPNSTs with the Ki67 labeling index of more than 25%, showed poor prognosis.[3] Size and the site (location) and the proliferative activity of the tumour have been shown to have some value in predicting the prognosis of MPNSTs. Accordingly, larger tumours located at deep sites with high proliferative activity would have a poor prognosis in terms of histopathology too.[3] In most of the head and neck MPNSTs time duration between the initial clinical diagnosis and recurrence or metastasis was shorter regardless of the treatment. MPNST of the present patient had a deeply located, infiltrative tumour with a high proliferative activity as such a close follow up is necessary in order to identify the possible recurrence or metastasis. A recent review of 120 MPNSTs reported over a period of 71years at the Mayo clinic demonstrated, 5 year survival rate of 47%-66% after complete resection of localized disease. Ducatman *et al*, (1986) studied MPNST patients with and without neurofibromatosis and reported 5 year survival rates of 16% and 53% respectively.[4]

Diagnosis of MPNST is a challenge. The MPNST resembles a fibro sarcoma in its general organization but the tumour cells (spindle cells) show wavy or comma shaped outlines and nuclear contour of schwann cells. Cellular and nuclear pleomorphism is quite marked and the mitotic activity is usually high. Nuclear pallisading is an important histopathological feature but it may not be present in 50% of cases and when present, is found only in scattered areas. Rarely, MPNSTs may contain epithelioid rhabdoid and pleomorphic cells usually with varying amount of spindle cells. Differential diagnosis of MPNST includes malignant melanoma, clear cell sarcoma, epithelioid sarcoma, angiosarcoma and synovial sarcoma. S-100, EMA and CD34 are the basic markers of peripheral nerve sheath and used for the determination of the type of differentiation within the MPNST. Immunostaining with epithelial, endothelial and melanotic markers are necessary to exclude the other conditions such as carcinoma, angiosarcoma and malignant melanoma. S-100 was weakly positive and smooth muscle actin was negative in our case confirming the neural origin.

Although the S-100 protein is regularly and diffusely positive in benign nerve sheath tumours, its positivity is around 30% in high grade malignant nerve sheath tumours. Most MPNSTs express diffuse positivity for S-100 protein but some cases are completely negative. NSE, which is another neural tissue marker, is most often positive in e-MPNST but its specificity is limited.

Identification of specific genetic patterns with electron microscopy or molecular analysis would be helpful in cases where results of immunostains are inconclusive. Critical genetic changes in tumourgenesis of MPNST are the alterations in NF1, P16 genes and p53and retinoblastoma (RB) pathways.

The histology and immunostaining of the presented case was consistent with the diagnosis of MPNST. The patient presented here had no clinical features of neurofibromatosis, so it was a tumour arising de novo with no detected precursor lesion.

According to the clinical symptoms and tumour extension, MPNST of the present case may have derived from one of the branches of the trigeminal nerve (Maxillary division). The tumour in this case was inoperable because of the diffuse neoplastic infiltration which made identification of the exact nerve of its origin impossible. This caused some diagnostic difficulty as the connection between tumour and the nerve trunk of origin is an important observation in the diagnosis of MPNST.

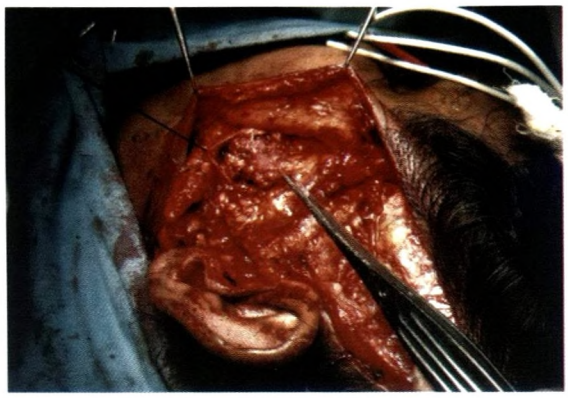

Figure 1. Intra operative view showing tumour mass

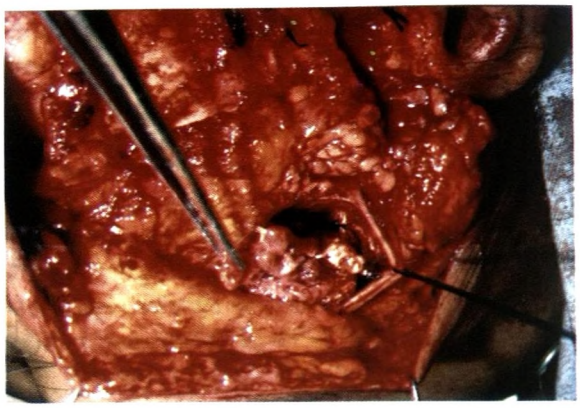

Figure 2. Intra operative view showing tumour mass and the facial nerve branch

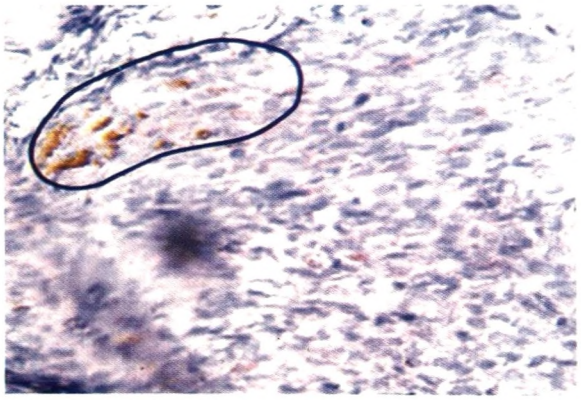

Figure 3. S-100 positive tuomur cells in some areas (x10)

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Self Assessment / Oral Diagnosis (SAOD)

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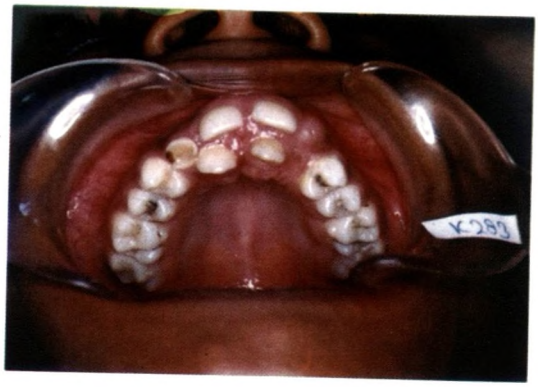

Based on the photograph state whether the statement is true or false

1. Upper left permanent lateral incisor is not erupted.
2. Upper right permanent canine is not erupted.
3. Upper right deciduous canine is present
4. Bulge of the upper left permanent canine is not seen.
5. The possible age of the patient is 10-12 years.
6. The problem is spacing.
7. The investigation for upper write permanent canine is not important.
8. Simple removable appliance can align the upper lateral incisors.
9. Multiple tooth movements are not required to correct the malocclusion.
10. Inversion of fully pre adjusted edge wize brackets at the beginning of the treatment is not helpful.

Prepared by

Dr. K.A. Kalyanaratne,
BDS(SL), MS(Col), MORTH, RCS(UK)
Division of orthodontics
Faculty of Dental Sciences
University of Peradeniya Sri Lanka

Answers

1. F,	6. F,	2. T,	7. F
3. T,	8. F	4. F,	9. F
5. T,	10. F		

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The Sri Lanka Dental Journal publishes the following categories of articles which have relevance to Dentistry and allied sciences.

1. Leading articles - One article per issue. It may be solicited by the Editor. Authors are welcome to submit leading articles on current topics of interest. One's expertise or commentaries on general practice etc. They should be approximately 1500 words in length. References should be 20 or less.

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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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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.

No authror given

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

SENSODYNE

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Dentine hypersensitivity is a common condition characterized by short, sharp tooth pain.

Up to 40% of adults have dentine hypersensitivity but many don't seek help. Dentine hypersensitivity is easily treated, so it's important for you to recognize the symptoms so you can consult your dentist immediately.

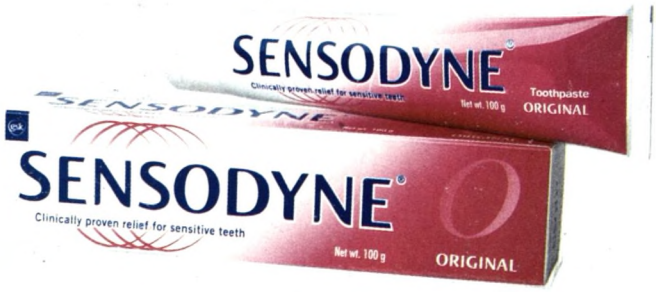
What causes dentine hypersensitivity?
Your teeth become hypersensitive when dentine - the inner, hard tissue of teeth - becomes uncovered, exposing the tooth's sensitive surface.

Dentine can be exposed by gum recession or enamel loss caused by:

* *Harsh tooth brushing*
* *Excessive flossing*
* *Intake of acidic food and drink*
* *Frequent vomiting*
* *Gum disease*
* *Previous dental work or*
* *The use of dental products with abrasive ingredients*

What usually triggers the pain?

* *Exposure to cold or hot food, liquids or air (including drying up of teeth)*
* *Exposure to sweet or sour food and drinks*
* *Tooth brushing*



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