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Editorial Board

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Continuing Professional Development (CPD) and Professionalism

Keeping skills and knowledge up to date throughout the career is of utmost importance for perfect professionalism. In accordance to keep abreast with future advanced dentistry, we all must intentionally and continuously focus on updating our skills and knowledge.

Myanmar Dental Association is pleased to partner with FDI (Fédération dentaire international) World Dental Federation, local as well as international Medical and Dental Universities, Non Government Organizations (NGOs) and Medical Research Centers to provide continuing professional development as part of Myanmar Dental Council, Continuing Professional Development (CPD) Committee. Through the Committee, we promote our dental profession by providing high level continuous dental education, clinical excellence, as well as dental and oral research relevant to the profession.

CPD can support dentists and dental care professionals in maintaining and updating their skills, knowledge and behavior throughout their working life and more. CPD for dental professionals is defined in law as lectures, seminars, courses, individual study and other related activities. Attending local and international conferences can be included in CPD record if they can be reasonably expected to advance professional development relevant to practice.

In many developing countries, undertaking CPD record is a compulsory part of registration with the General Dental Council. Dental professionals are highly recommended to do the minimum verifiable CPD requirements such as medical emergencies, disinfection and decontamination and radiography and radiation protection. We also need to keep up to date by doing CPD record in legal and ethical issues, complaint handling, early detection of oral cancer etc. in contribution to patients’ safety.

As a registered dental professional, we must develop, maintain, and work within our professional knowledge and skills and make sure our personal behavior assures patients’ confidence by adapting the CPD record. Then only can we provide the best possible treatment and care to patients. Myanmar Dental Association desires to join forces with other Medical and Dental Societies which will be to the benefit of all members and ultimately, is committed to serving our local community and the advancement of dentistry.

Professor Dr. Swe Swe Win
Vice President, Myanmar Dental Association,
Chairman, Academic committee

References: www.gdc-uk.org
Alpha tricalcium phosphate biomaterials for potential clinical application in bone regeneration

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Abstract
Regeneration of oral and craniofacial tissues is one of the significant developments in treatment modalities. It combines innovative application of basic science, clinical science and engineering technology. Synthetic bone substitutes have been developed in a fast pace and research is ongoing to develop more efficient biomaterials with promising ability to regenerate lost bone structure. Tricalcium phosphate biomaterials are among them. In this review, recent innovations to develop more effective biomaterials are discussed.

Introduction
Oral and craniofacial defects may be uncomfortable to the patient and affect functions such as mastication and articulation such that structure, function, aesthetics and pain must all be managed effectively resulting in treatment challenges that are often more complex than in other parts of the body. In daily clinical practice, the dental surgeons frequently encounter considerable challenge when facing with periapical, periodontal and peri-implant bone defects as well as reduced alveolar bone volume for prosthetic rehabilitations. Recently, innovative application of basic science, clinical science and engineering technology altogether renders the regeneration of lost bone structure. The techniques to repair orofacial defects apply accepted therapies for restoring tissue structure and/or function elsewhere in the body, and include synthetic materials (alloplasts), patient’s own tissue (autografts), tissue from other person of the same species (allografts) and animal tissues (xenografts). All these have specific advantages and disadvantages. Although autogenous bone is considered as the gold standard, it has significant limitations, including donor site morbidity, inadequate amount, and inappropriate form. These limitations have prompted increasing research in alternative bone grafts such as allografts and xenografts. Though they are attractive sources, there are several problems encountered in using them, including the risk of disease transmission, immunogenicity, loss of biologic and mechanical properties secondary to its processing, increased cost, and non-availability world-wide due to financial and religious concerns. Consequently, significant efforts are being made to develop ideal synthetic bone graft substitutes. Bone grafts and their substitutes can be divided according to their properties of osteoconduction, osteoinduction, and osteogenesis (Table 1).
Term | Definition | Examples
--- | --- | ---
Osteogenic | Contains Osteoprogenitor cells or stimulates (committed) osteoprogenitor cell proliferation | Bone marrow, bone graft
Osteoconductive | 3-D scaffold on which committed osteoprogenitor cells produce bone; supports ingrowth of capillaries and cells from the host bed (fracture ends); guides repair in the location where normal healing will occur if left untreated | Demineralized bone matrix, bone graft, calcium phosphate ceramics
Osteoinductive | Bone morphogenesis: phenotypic conversion of stem cells from a non-osseous environment to chondrocytes and osteoblasts; allows repair in a location that would not normally heal if left untreated; able to induce bone formation at an ectopic (extra-skeletal, non-osseous) site | Bone graft, dentine matrix, demineralized bone matrix, bone morphogenetic proteins (BMPs)

Table 1. Fracture healing and bone morphogenesis terminology (Glowacki, 1992; Kenley, 1993; Winn, 1998; Burg, 2000)

**Tissue engineering principles**

New therapeutic strategy termed tissue engineering has been developed (Langer, 1993). Tissue engineering is one of the biomedical technologies developed to assist the regeneration of body tissues to treat large size defects that are not possible to self-repair. Three basic component; scaffolds, cell sources and signals (the tissue engineering triad) are necessary to optimize development of a single tissue, hybrid organ or interface.

*In vivo* tissue regeneration, one of the tissue engineering strategies, applies scaffolds and signal molecules to stimulate local host cells for tissue regeneration. In bone tissue engineering, bone morphogenetic protein-2 (BMP-2) has shown its beneficial effects on bone regeneration (Yasko, 1992; Bostrom, 1998; Schimandle, 1995; Sandhu, 1995). However, there are some problems to be solved such as short shelf life, inefficient delivery to target cells and high price.

**Calcium phosphate biomaterials as osteoconductive scaffold**

One of the first studies reported using calcium phosphate for bone repair was performed by Albee and Morrison in 1920. In 1970s, calcium phosphates were synthesized, characterized and used to a large extent. The earliest application of calcium phosphate salts was in the form of powders (Ferraro, 1979). Synthetic calcium phosphates are salts of orthophosphoric acid (H₃PO₄), and thus can form compounds that contain H₂PO₄⁻, HPO₄²⁻ or PO₄³⁻. The calcium phosphate salts constitute a wide group of compounds (Elliot, 1994). Table 2 summarizes the chemical name, the formula, the abbreviation, and the calcium to phosphorus ratio (Ca/P) of some calcium phosphate compounds. Calcium phosphate salts vary by their composition and their crystal structures, leading to specific physicochemical properties.
<table>
<thead>
<tr>
<th>Name</th>
<th>Formula</th>
<th>Abbreviation</th>
<th>Ca/P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dicalcium phosphate anhydrate or monetite</td>
<td>CaHPO₄</td>
<td>DCPA</td>
<td>1.00</td>
</tr>
<tr>
<td>Dicalcium phosphate dihydrate or brushite</td>
<td>CaHPO₄·2H₂O</td>
<td>DCPD</td>
<td>1.00</td>
</tr>
<tr>
<td>Octacalcium phosphate</td>
<td>Ca₈(PO₄)₄(HPO₄)₂·5H₂O</td>
<td>OCP</td>
<td>1.33</td>
</tr>
<tr>
<td>Tricalcium phosphate</td>
<td>Ca₃(PO₄)₂</td>
<td>TCP</td>
<td>1.50</td>
</tr>
<tr>
<td>Hydroxyapatite</td>
<td>Ca₁₀(PO₄)₆(OH)₂</td>
<td>HA</td>
<td>1.67</td>
</tr>
</tbody>
</table>

Table 2. Main biologically relevant calcium phosphate salts (From Barrèr et al., International Journal of Nanomedicine, 2006: 1(3) 317–332)

Calcium phosphate biomaterials such as hydroxyapatite and tricalcium phosphate are commonly used biomaterials because they are highly biocompatible, osteoconductive and gradually resorbed and replaced with bone. Two major distinct phases of anhydrous tricalcium phosphate (TCP) crystals exist: α-TCP and β-TCP phases. The α-TCP crystallizes in the monoclinic space group, and β-TCP crystallizes in the rhombohedral space group. Despite their similar chemical composition, their different crystallographic features confer different resorption features: α-TCP is more soluble than β-TCP, and it is obtained after heating the β-TCP to more than 1170°C. Clinically, α-TCP is a major reagent in the composition of cements as they hydrolyze into apatitic structures, but it is also sold under the form of powder, blocks, or granules, like β-TCP. Hydroxyapatite is the most stable since it crystallizes in hexagonal space group.

Histological and histomorphometrical comparative study of the degradation and osteoconductive characteristics of alpha and beta-tricalcium phosphate in block grafts was carried and reported that α-TCP porous blocks showed higher initial solubility in vivo than β-TCP blocks degradation of α-TCP seemed to be aligned with bone formation (Yamada et al., 2007). Kihara et al. (2006) studied biodegradation process of α-TCP particles and new bone formation in a rabbit cranial defect model and found that α-TCP particles are osteoconductive and space-maintaining. We conducted a preclinical study to assess whether α-TCP can be applicable in vertical bone augmentation (Nyan et al., 2012). We found that alpha tricalcium phosphate prevents soft tissue migration into the space and gradually resorbed and replaced with new bone.

**Strategies to promote effective bone regeneration**

If pharmacological compounds can upregulate the expression of intrinsic growth factors to stimulate bone growth, the strategy to combine such compounds with an osteoconductive bone substitute as drug carrier scaffold would be more cost-effective for bone regeneration. Ideal drug carrier scaffold should have the ability to deliver the drug at appropriate time & proper dose; the presence of a substratum that will enhance cell recruitment and attachment and potentiate chemotaxis; the presence of a void space to allow for cell migration and to promote angiogenesis and the ability to biodegrade without generating an immune or inflammatory response and without producing toxic waste products (Lieberman et al, 1999).

**Alpha TCP with statins**

Topically applied simvastatin, a cholesterol-lowering drug, has been shown to stimulate BMP-2 mRNA expression in osteoblasts and promote bone growth (Mundy et al. 1999; Sugiyama et al. 2000; Garrett et al. 2001; Ohnaka et al. 2001; Thylin et al. 2003). A preclinical study was carried out by using alpha tricalcium...
phosphate (αTCP) as drug carrier and scaffold which is highly biocompatible, osteoconductive and degradable biomaterial (Nyan et al., 2008). Various doses of simvastatin were combined with αTCP and grafted in rat calvarial defects. The results indicated that 0.1 mg simvastatin is the optimal dose for maximum bone regeneration of the defects in this study model. To clarify the mechanisms underlying the stimulation of bone regeneration by this combination material, another study was performed to analyze the cellular and molecular changes in bone defects at early time points after the material application (Nyan et al., 2009). The results suggested that simvastatin combined with alpha tricalcium phosphate induced bone regeneration in rat calvarial defects by augmenting osteogenic cell proliferation, migration, recruitment and differentiation in the early phase of bone healing which were associated with increased expression of BMP-2 and TGF-b1. Alpha TCP could release simvastatin sufficiently at the early time, provide spaces into which osteogenic cells migrated and recruited. Moreover, its gradual dissolution allowed a smooth exchange with newly formed bone and we for the first time reported that 0.1mg simvastatin+14mg α-TCP is the best combination for optimal bone regeneration. Simvastatin is a widely prescribed cholesterol lowering drug and has been proven for its safety. It is readily available and less expensive compared with recombinant growth factors. Moreover, it is chemically stable. The results of this preclinical study suggest that the combination of simvastatin with α-TCP would be applicable clinically as an effective, simple and safe biomaterial for reconstructive treatments.

We recently evaluated the osteoconductivity of three different bone substitute materials: α-tricalcium phosphate (α-TCP), β-tricalcium phosphate (β-TCP), and hydroxyapatite (HA), combined with or without simvastatin (Rojbani et al., 2011). In α-TCP group, the amount of newly formed bone was significantly more than both HA and control groups but not significantly yet more than β-TCP group. Degradation of α-TCP was prominent and β-TCP showed slower rate while HA showed the least degradation. Combining the materials with Simvastatin led to increasing in the amount of newly formed bone. These results confirmed that α-TCP, β-TCP, and HA are osteoconductive materials acting as space maintainer for bone formation and that combining these materials with simvastatin stimulates bone regeneration and it also affects degradability of α-TCP and β-TCP. Conclusively, α-TCP has the advantage of higher rate of degradation allowing the more bone formation and combining α-TCP with simvastatin enhances this property.

**Alpha TCP with green tea extract EGCG**

Epigallocatechin-3-gallate (EGCG), the most abundant and biologically active catechin in green tea, has anti-inflammatory and anticancer properties, the ability to reduce serum lipid and blood pressure and to modulate immune response (Hegarty et al., 2000; Wu et al., 2002; Rahman et al., 2006; Tosetti et al., 2009). Additionally, EGCG was found to induce apoptotic cell death of osteoclast-like multinucleated cells and ameliorated experimentally induced arthritis in mice (Nakagawa et al., 2002; Lin et al., 2009; Lee et al., 2010). Such pharmacological effects of catechins may be useful for prophylaxis or treatment of inflammatory bone disease (Morinobu et al., 2008). Recent in vitro studies show that EGCG increases bone mineral nodules in cell lines. However, there has been no study that investigates the effect of the bone regenerative capacity of EGCG in vivo. Our recent work aimed to investigate effects of the combination of epigallocatechin-3-gallate (EGCG) and α-tricalcium phosphate (α-TCP) on bone regenerative capacity (Rodriguez et al., 2011). The combination of α-TCP particles and 0.2 mg EGCG stimulates maximum bone regeneration and this combination would be potentially effective as bone graft material.
Conclusion

The reported pharmacological approach in tissue engineering using alpha tricalcium phosphate biomaterials would be considered very promising and cost-effective in maxillofacial bone regeneration in periapical, periodontal and peri-implant bone defects as well as reduced alveolar bone volume for prosthetic rehabilitations.

References


Nyan M., Miyahara T., Noritake K., Hao J., Rodriguez R., Kuroda S., Kasugai S. Molecular and tissue responses in the healing of rat


Proliferating Cell Nuclear Antigen and Ki-67 Expression in Solid/Multicystic Ameloblastoma and Uncystic Ameloblastoma

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¹ Department of Oral Medicine, University of Dental Medicine, Yangon
² Department of Oral Medicine, University of Dental Medicine, Mandalay

Abstract

Ameloblastoma is slow growing, locally invasive, epithelial odontogenic tumour of the jaws with high recurrence rate and quite common in Myanmar population. The proliferative activity of ameloblastoma is important to predict its biologic behaviour. The purpose of the study was to determine the proliferative activity of ameloblastoma in both SMA (Solid/multicystic ameloblastoma) and UA (Uncystic ameloblastoma). Forty paraffin embedded tissue specimens from both SMA and UA were immunohistochemically performed by using monoclonal anti-PCNA (1:1000) and monoclonal mouse anti-human Ki-67 antigen (1:100). Immunoreactivity was evaluated by mean labeling index (LI). The highest mean LI of PCNA was presented in plexiform type of SMA (75.50±4.10%) and in luminal type of UA (73.35±21.89%). PCNA expression was significantly higher in peripheral ameloblast like cells in both SMA and UA (p=0.000). Mean LI of PCNA in SMA (74.63±5.55%) was significant higher than UA (67.30±14.25%) (p=0.034). The highest mean LI of Ki-67 was presented in granular type of SMA (14.59±5.51%) and in luminal type of UA (11.24±4.42%). Ki-67 expression could not exhibit remarkable differences between peripheral and central area of SMA (p=0.674)and also between basal and luminal portion of UA (p=0.946). No significant difference of mean LI of Ki-67 was found between SMA and UA (p=0.289). When compare the mean LI of PCNA and Ki-67 between SMA and UA, PCNA was significantly higher than Ki-67 (p= 0.000). Based upon the results, SMA demonstrated more aggressive biologic behaviour than UA and PCNA was found to be the reliable immunohistochemical marker than Ki-67 for evaluating cell proliferation in ameloblastic tumours.

INTRODUCTION

Odontogenic tumours are lesions derived from epithelial, ectomesenchymal, and/or mesenchymal elements that are or have been part of the tooth-forming apparatus. These tumours are found exclusively within the jawbones or in the soft mucosal tissue overlying tooth-bearing areas (Philipsen et al., 2005).

Ameloblastoma is the most frequently encountered odontogenic neoplasm of the jaws with a strong predilection for the posterior region of the mandible. Accounts for approximately half (48.9%) of the odontogenic tumours with female to male and maxilla to mandible ratios of 1:1.7 and 1:8 respectively (Laxmidevi et al., 2010).
The two most common subtypes are SMA and UA.

These two common types have different recurrence rates after surgical treatment. Moreover, the UA differs in biologic behaviour from the SMA, with a considerably lower rate of recurrence. Even within the UA group, difference in biological behaviour and prognosis apparently exists, with the intramural lesion, which is characterized by intramural proliferation of neoplastic tissue, being more aggressive than their simple unicystic form or the intraluminal unicystic ameloblastoma. Based on these differences, it has been suggested that solid and multicystic ameloblastomas and intramural unicystic ameloblastomas should be treated by resection, whereas the simple and intraluminal unicystic forms of this tumour can be enucleated (Meer et al., 2003).

On the other hand, the assessment of cell proliferation activity in tumours has become a common tool used by histopathologists to provide useful information for assessing diagnosis, used as indicator of aggressiveness and prognostic information. Proliferation markers refers to specific proteins or other factors whose presence in actively growing and dividing cells serves as an indicator for such cells. Today, the most common method for determining proliferative activity is the use of immunohistochemical techniques, which are increasingly being applied in routine pathology (Bologna-Molina et al., 2013).

Currently, new markers are being added to evaluate cell proliferation. However, PCNA is still used as a first-choice marker of cell proliferation. Furthermore, many investigations of tumour cell proliferative activity have used PCNA and Ki-67 to evaluate cell proliferation in oral tumours (Salehinejad et al., 2011).

In Myanmar, although ameloblastoma is common odontogenic tumour there were limited studies and the studies were mainly based on clinical interest and not reflecting the biological behaviour. Therefore, the purpose of this study is to predicts its biologic behaviour, as suggestion for more aggressive treatment when recurrence occur, as well as for regular follow up and prognostic value by using both monoclonal anti-proliferating cell nuclear antigen (PCNA) antibody and monoclonal anti Ki-67 antibody on SMA and UA ameloblastoma.

**Proliferating Cell Nuclear Antigen (PCNA)**

Proliferating cell nuclear antigen (PCNA) is a 36 KD protein identified as an auxiliary protein of DNA polynuosense delta. Its distribution in the cell cycle, increasing through G1, peaking at the G1/S-phase interface, decreasing through G2, and reaching low levels which are virtually undetectable by immunocytochemical methods in M-phase and quiescent cells, makes it a useful marker for proliferating cells(Figure 1) (Coltrera and Gown et al., 1991).

The half-life of PCNA exceeds 20 hours and PCNA expression may be used as a marker of cell proliferation because cells remain a longer time in the G1/S phase when proliferating. Furthermore, this protein has an essential role in nucleic acid metabolism as a component of the DNA replication and repair mechanism (Oliveria et al., 2007).

**Ki-67**

The Ki-67 antigen (Ki-67) is a classic marker of cellular proliferation that has been widely applied in the diagnostic, research. Over 10 years later, immunostaining with antibodies to the Ki-67 antigen is well established as a quick and efficient method for evaluating growth fractions of various tumour types because of its distinctive reaction patterns that exclusively involve proliferating cells. The large number of citations evidences the extensive use of Ki-67 as a marker of proliferation in pathology, which is increasing year on year (Ross and Hall, 1995).

The Ki-67 antigen is preferentially expressed during the late G1, S, G2 and M phase of the cell cycle, whereas resting, non-cycling cells (G0 phase) lack Ki-67 expression. Because of its
absence in quiescent cells (G0 phase), this protein developed into a widely used tumour marker in the fields of research and pathology. The Ki-67 protein has been shown to have an extremely short half-life of around 20 minutes throughout the cycle, with major catabolism occurring at the end of mitosis, indicating that few cells that have left the cycle will contain the antigen. The standard antibody for the detection of Ki-67 is MIB-1. The fraction of MIB-1-positive tumour cells (the MIB-1/Ki-67 labeling index) is often correlated with the clinical course of cancer; Ki-67 is of prognostic value for many types of malignant tumours (Thmann et al., 2004).

Figure 1. Theoretical cell cycle distributions for immuno-cytochemical staining of the three proliferation markers. The thickness of the line for PCNA staining refers to the peak staining observed in cells. (adapted from Coltrera and Gown et al., 1991)

This study was aimed study the proliferative activity of ameloblastoma by using proliferating cell nuclear antigen (PCNA) and Ki-67 in Myanmar patients

**MATERIALS AND METHODS**

**Materials**

**Study Population**

(40) Cases of histopathologically proven ameloblastoma

**Primary Antibodies**

1. Anti-proliferating cell nuclear antigen (PCNA)
   
   Code No-DAKO M 0879
   
   1:1000 dilutions (DAKO, USA)

2. Monoclonal mouse anti-human Ki-67 antigen, Clone MIB-1
   
   Code M 7240
   
   Lot-00004924
   
   1:100 dilutions (DAKO, USA)

**Secondary Antibody**

Dako REALTM EnVisionTM/HRP, Rabbit/Mouse (ENV)

Dextran coupled with peroxidase molecules and goat secondary antibody molecules against rabbit and mouse immunoglobulins.

**Methods**

**Type and place of Study**

Hospital and Laboratory based analytical study was conducted at:

1. Department of Oral Medicine, University of Dental Medicine, Yangon
2. Department of Oral and Maxillofacial Surgery, University of Dental Medicine, Yangon
3. Department of Oral Medicine, University of Dental Medicine, Mandalay
4. Department of Plastic and Maxillofacial Surgery, Yangon General Hospital, Yangon
5. Research Laboratory of School of Postgraduate Studies and Research of International Medical University, Kuala Lumpur, Malaysia

**Selection of Cases**
Histologically proven biopsy specimen of SMA and UA ameloblastoma cases were selected starting from January 2010 to December 2012. Previously treated and recurrent cases of SMA and UA ameloblastoma, cases of SMA and UA ameloblastoma if the patient did not agree to involved in this study, cases of desmoplastic ameloblastoma and cases of peripheral ameloblastoma were excluded.

RESULTS AND DISCUSSION

Table 1. Distribution of PCNA labeling index in different Types of SMA

<table>
<thead>
<tr>
<th>Type of SMA</th>
<th>Number of cases</th>
<th>PCNA labeling index</th>
<th>Range %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean% LI±SD</td>
<td></td>
</tr>
<tr>
<td>Follicular</td>
<td>6</td>
<td>74.73±5.61</td>
<td>65.50-80.89</td>
</tr>
<tr>
<td>Plexiform</td>
<td>7</td>
<td>75.50±4.10</td>
<td>67.96-79.94</td>
</tr>
<tr>
<td>Mixed</td>
<td>5</td>
<td>75.35±8.10</td>
<td>65.81-83.52</td>
</tr>
<tr>
<td>Granular</td>
<td>2</td>
<td>69.48±2.09</td>
<td>68-70.96</td>
</tr>
</tbody>
</table>

The highest mean labeling index in plexiform type and the lowest labeling index in granular type of SMA

Figure 2. Plexiform type of SMA (A) H&E staining × 200 (B) Immunohistochemical expression of PCNA × 200

Figure 3. Granular type of SMA (A) H&E staining × 200 (B) Immunohistochemical expression of PCNA × 200

Expression of Proliferating Cell Nuclear Antigen (PCNA) in SMA and UA
In the present study, the plexiform type of ameloblastoma presented the strongest PCNA labeling index, while the granular type of SMA showed the weakest labeling index. Table 1, Figure 2(B), 3(B)

The results were compatible with the studies of Hirayama et al. (2004). Maya et al., (2012). Proliferative activities between the different histologic types of the SMA were not statistically significant. These findings were consistent with Broboza et al. (2005), Salehinejad et al. (2011), Bologna-Molina et al. (2013), who did not find the significant differences of proliferative activity among the difference histological types of SMA.

Table 2. PCNA expression in peripheral cell layer and central portion of SMA

<table>
<thead>
<tr>
<th>Cytological pattern</th>
<th>Number of cases</th>
<th>PCNA labeling index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral cell</td>
<td>20</td>
<td>82.978±5.59</td>
</tr>
<tr>
<td>Central cell</td>
<td>20</td>
<td>67.41±6.50</td>
</tr>
</tbody>
</table>

PCNA expression is significantly increased in peripheral cells layer of SMA
Paired sample t test (p=0.000)

Figure 4. Mixed type of SMA showing follicular as well as anastomosing strands
(A) H&E staining × 200 (B) Immunohistochemical expression of PCNA × 200

The peripheral epithelial cells of the nests of the SMA showed strong PCNA labeling index than that of central cells. (Table 2, Figure 4.B) These results were also in accordance with the studies of Miyake et al. (2004), Ahmed and El-Azab (2008), Salehinejad et al. (2011) and Maya et al. (2012), they verified the higher PCNA labeling index were seen in the basal and suprabasal layers with the stellate reticulum area showing less number of positive cells. This result may be interpreted that the cellular proliferation and consequently the ameloblastoma growth are concentrated in the peripheral areas composed by ameloblast like cells.

Table 3. Distribution of PCNA labeling index in different types of UA
The table below shows the PCNA labeling index for different types of unicystic ameloblastoma (UA).

<table>
<thead>
<tr>
<th>Type of UA</th>
<th>Number of cases</th>
<th>PCNA labeling index</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean% LI±SD</td>
<td>Range %</td>
</tr>
<tr>
<td>Luminal</td>
<td>3</td>
<td>73.35±21.89</td>
<td>52.91-96.45</td>
</tr>
<tr>
<td>Luminal &amp; intraluminal</td>
<td>2</td>
<td>70.99±13.36</td>
<td>61.54-80.44</td>
</tr>
<tr>
<td>Luminal, intraluminal &amp; intramural</td>
<td>3</td>
<td>57.21±14.58</td>
<td>47.94-74.02</td>
</tr>
<tr>
<td>Luminal &amp; intramural</td>
<td>12</td>
<td>67.69±12.97</td>
<td>46.20-80.71</td>
</tr>
</tbody>
</table>

The highest mean labeling index in luminal unicystic ameloblastoma and lowest labeling index in luminal, intraluminal & intramural type of UA.

Figure 5. Luminal type of UA (A) H&E staining × 200 (B) Immunohistochemical expression of PCNA × 200

As for Unicystic Ameloblastoma (UA), differences in mean labeling indices for PCNA was observed among the various subtypes. The highest mean labeling index was detected in luminal unicystic ameloblastoma proper and the lowest in combination of luminal, intraluminal and intramural type (Table 3, Figure 5, B).

Proliferative activities between the different histologic types of the UA were not statistically significant. This finding is similar to the findings of Bologna-Molina et al. (2013) who showed that the luminal unicystic ameloblastoma had higher PCNA labeling index than the intraluminal unicystic ameloblastomas and mural unicystic ameloblastoma but there were no statistically difference among them.

Kim and Yook (1994) found that the area of plexiform intraluminal growth showed the highest proliferating activity among the unicystic variants. Meer et al. (2003) reported that the highest mean labeling index in the intraluminal and intramural unicystic ameloblastoma types within the unicystic group. However, because the number of cases of each subtype was limited, we would be reluctant to draw any definite conclusions from current data.
Table 4. PCNA labeling index in basal cell layer and luminal portion of UA

<table>
<thead>
<tr>
<th>Cytological pattern</th>
<th>Number of cases</th>
<th>PCNA labeling index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean% LI±SD</td>
</tr>
<tr>
<td>Basal cell</td>
<td>20</td>
<td>72.56±14.78</td>
</tr>
<tr>
<td>Luminal cell</td>
<td>20</td>
<td>62.20±15.03</td>
</tr>
</tbody>
</table>

PCNA expression is significantly increased in basal cells layer of UA
Paired sample t test (p=0.000)

Figure 6. Comparison of PCNA labeling index between SMA and UA

The mean PCNA labeling index of basal cells were significantly higher proliferating index than that of luminal cells (Table 4). This result may be interpreted that the cellular proliferation and consequently the ameloblastoma growth in UA are concentrated in the basal cells layer.

Table 5. PCNA labeling index in SMA and UA

<table>
<thead>
<tr>
<th>Type of ameloblastoma</th>
<th>Number of cases</th>
<th>PCNA labeling index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean% LI±SD</td>
</tr>
<tr>
<td>SMA</td>
<td>20</td>
<td>74.63±5.55</td>
</tr>
<tr>
<td>UA</td>
<td>20</td>
<td>67.30±14.25</td>
</tr>
</tbody>
</table>

Higher PCNA labeling indices in SMA compared to the UA
Independent sample t test (p = 0.034)
In this study, labeling index of PCNA ranged from 65.50% to 83.52% in SMA and from 46.20% to 96.45% in UA (Table 5). When the mean labeling indices of the solid multicystic ameloblastoma (mean = 74.63 %) and unicystic groups (mean = 67.30 %) were compared, a statistically significant difference was obtained (p=0.034), with the higher PCNA labeling indices in the solid and multicystic ameloblastomas compared to the UA respectively. (Figure 6, Figure 7.B and Figure 8.B)

Our results were similar to results of Li et al. (1995) who showed that the solid ameloblastomas exhibited higher mean PCNA labeling index than all areas of unicystic ameloblastomas. It was also supported by results of Sandra et al. (2001), unicystic ameloblastoma showed lower values than plexiform and follicular ameloblastomas. Again, Maya et al. (2012) reported that the cystic type showed a low positive PCNA labeling index. By comparing SMA and UA, higher PCNA positivity may indicate higher cellular proliferation rate, which would explain the more aggressive nature of the SMA compared to the UA.

Table 6. Distribution of Ki-67 Labeling indexin different types of SMA
<table>
<thead>
<tr>
<th>Type of SMA</th>
<th>Number of cases</th>
<th>Ki-67 labeling index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean% Li±SD</td>
</tr>
<tr>
<td>Follicular</td>
<td>6</td>
<td>12.74±6.50</td>
</tr>
<tr>
<td>Plexiform</td>
<td>7</td>
<td>10.81±6.61</td>
</tr>
<tr>
<td>Mixed</td>
<td>5</td>
<td>11.22±6.12</td>
</tr>
<tr>
<td>Granular</td>
<td>2</td>
<td>14.59±5.51</td>
</tr>
</tbody>
</table>

Mean labeling indices of granular type was highest and lowest in plexiform type of SMA.

Figure 9. Granular type of SMA (A) H&E staining × 200 (B) Immunohistochemical expression of Ki-67 × 200

Figure 10. Plexiform type of SMA (A) H&E staining × 200 (B) Immunohistochemical expression of Ki-67 × 200

Expression of Ki-67 Antigen in SMA and UA

Among the different types of SMA, mean labeling index of follicular type was higher than plexiform and mixed type in present study (Table 6). Proliferative activities between the different histologic types of the SMA were not statistically significant. The granular type of ameloblastoma presented the strongest Ki-67 labeling index while the plexiform type of SMA showed the weakest labeling index. (Figure 9. B, Figure 13. B)

Sandra et al. (2001) revealed that Ki-67 labeling indices of follicular ameloblastoma were higher than
that of plexiform ameloblastoma as suggested by previous researchers (Nakamura et al., 1994 and Li et al., 1995). The findings of Hirayama et al. (2004); and Piattelli et al. (1998) are not similar to above studies, as well as the present study. In the study of Koizumi et al. (2004); no statistically significant difference was shown in Ki-67 labeling index between follicular and plexiform patterns of ameloblastoma.

Table 7. Ki-67 labeling index in peripheral cell layer and central portion of SMA

| Cytological pattern | Number of cases | Ki-67 labeling index |  |
|--------------------|-----------------|----------------------|  |
|                    |                 | Mean% LI±SD          | Range % |
| Peripheral cell    | 20              | 12.12±6.88           | 3.53-24.16 |
| Central cell       | 20              | 11.79±5.54           | 2.18-23.62 |

Higher expression of Ki-67 expression observed in peripheral ameloblast like cells than central area of SMA

Paired sample t test (p = 0.674)

Figure 11. Follicular type of SMA (A) H&E staining × 200 (B) Immunohistochemical expression of Ki-67 × 200

In this study, there was higher expression of Ki-67 expression in peripheral ameloblast like cells than in stellate reticulum like cells located in central area of SMA but no significant difference was found. (Table 7, Figure 11.B) Similarly, Bologna-Molina et al. (2009) and Gomes et al. (2010) showed that cellular proliferation index of Ki-67 positive nuclei are mainly located in peripheral ameloblast like cells in the follicular as well as in the plexiform areas of the solid ameloblastoma. Otero et al. (2012) also showed that there was significantly higher expression of Ki-67 in peripheral ameloblast like cells than in stellate reticulum like cells. Stellate reticulum like cells in ameloblastoma and in stellate reticulum cells in developing teeth tend to be negative to this marker.

Thus, it was suggested that the growth of ameloblastomas is produced by peripheral expression of the follicles (Ong’uti et al., 1997; Gomes et al., 2010). In this study, although Ki-67 staining was higher in the basal cells than the central stellate reticulum-like cells, due to the paucity of these neoplasms, no significant differences were found.
Table 8. Distribution of Ki-67 labeling index in different types of UA

<table>
<thead>
<tr>
<th>Type of UA</th>
<th>Number of cases</th>
<th>Ki-67 labeling index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean% LI±SD</td>
</tr>
<tr>
<td>Luminal &amp; intraluminal</td>
<td>2</td>
<td>7.00±2.45</td>
</tr>
<tr>
<td>Luminal, intraluminal &amp; intramual</td>
<td>3</td>
<td>10.17±1.55</td>
</tr>
<tr>
<td>Luminal &amp; intramual</td>
<td>12</td>
<td>10.34±4.79</td>
</tr>
</tbody>
</table>

The highest value was found in luminal type and lowest in luminal & intraluminal type of UA.

![A](image1.png)  ![B](image2.png)

Figure 12. Luminal type of UA (A) H&E staining × 200 (B) Immunohistochemical expression of Ki-67 × 200

When the various subtypes of UA were considered, the highest value was found in the luminal type of UA (11.24 %). Intramural type is the second highest labeling indices. The lowest indices were found in luminal and intraluminal type. (Table 8, Figure 12.B)

Proliferative activities between the different histologic types of the UA were not statistically significant. This finding was also similar to Bologna-Molina et al. (2008) and could be explained by the fact that luminal type of UA contains less stellate reticulum like cells, as compared to the other subtypes of UA and SMA.

Table 9. Ki-67 labeling index in basal cell layer and luminal portion of UA

<table>
<thead>
<tr>
<th>Cytological pattern</th>
<th>Number of cases</th>
<th>Ki-67 labeling index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean% LI±SD</td>
</tr>
<tr>
<td>Basal cell</td>
<td>20</td>
<td>10.26±5.58</td>
</tr>
<tr>
<td>Luminal cell</td>
<td>20</td>
<td>10.35±4.40</td>
</tr>
</tbody>
</table>

Mean labeling index of the immunostaining in basal cell layer and that of luminal area in UA Paired t test (p = 0.946).

No significant difference were found in the distribution of Ki-67 immuno expression between basal cells and luminal cells of UA (Table 9)
Table 10. Ki-67 labeling index in SMA and UA

<table>
<thead>
<tr>
<th>Type of ameloblastoma</th>
<th>Number of cases</th>
<th>Ki-67 labeling index</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean % Li±SD</td>
<td>Range %</td>
</tr>
<tr>
<td>SMA</td>
<td>20</td>
<td>11.87±5.99</td>
<td>3.27-22.96</td>
</tr>
<tr>
<td>UA</td>
<td>20</td>
<td>10.12±4.16</td>
<td>4.84-22.12</td>
</tr>
</tbody>
</table>

Higher Ki-67 expression observed in SMA than UA

Independent sample t test (p = 0.289).

Figure 13. Comparison of Ki-67 labeling index between SMA and UA

In the present study, Higher Ki-67 expression observed in SMA than UA but not significant. (Table 10, Figure 13) Bologna-Molina et al. (2008, 2013) and Nafarzadeh et al. (2013) studied on Ki-67 expression in ameloblastoma, and demonstrated that higher Ki-67 expression observed in SMA than UA but there is no significant difference. The possible reason might be the difference in the morphology of the tumours, with the solid lesions providing large follicles or plexiform sheets for analysis, whereas only a thin lining is available in the unicystic cases.

Meer et al. (2003) stated that the expression of Ki-67 markers has been studied in ameloblastoma and the expression level of this marker was significantly higher in UA compared with SMA. This may have resulted in the inclusion of greater numbers of basal and parabasal cells in the unicystic group, thus resulting in higher mean labeling indices. The reason for the variable results regarding differences in mean labeling indices of Ki-67 between unicystic and solid lesions is still unclear. A possible explanation might be the difference in methodology, especially the counting protocol. The published reports were frequently failed to provide sufficient detail or explanation.
Table 11. Comparison of expression of PCNA and Ki-67 in SMA and UA

<table>
<thead>
<tr>
<th>Histological type</th>
<th>PCNA (%) Median (IQR)</th>
<th>Ki-67 (%) Median (IQR)</th>
<th>z statistics</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMA</td>
<td>75.36 (9.84)</td>
<td>11.03 (10.32)</td>
<td>3.92</td>
<td>0.0001</td>
</tr>
<tr>
<td>UA</td>
<td>70.92 (26.80)</td>
<td>9.40 (5.24)</td>
<td>3.92</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

PCNA expression is significantly higher than Ki67 expression
Wilcoxon signed-rank test (p= 0.0001)

Comparison of the Value of PCNA and Ki-67 as Markers of Cell Proliferation in Ameloblastoma

Median labeling index of PCNA is significantly higher than Ki-67 in both SMA and UA (Table 11). The results were in line with the published study by Bologna-Molina et al. (2013) as the percentages were higher for PCNA in all types of ameloblastomas.

This higher PCNA positivity in the nuclei of the ameloblastic tumour cells can be explained by the following factors:

PCNA is an essential molecule for the synthesis of DNA. It expressed in non-proliferating cells undergoing DNA repair. This protein has half-life of at least 20 hours within the tissues. This finding could indicate that nuclei can continue to express PCNA even after completing the cell cycle. The lower the dilution of antibody, the higher is the percentage of PCNA expressed in the nucleus. (Bologna-Molina et al., 2013). Therefore, all of the points mentioned above could explain the increased positivity of PCNA compared with other markers of cellular proliferation, such as Ki-67. Ki-67 has significantly lower cellular proliferation index than PCNA in the nuclei of the ameloblastic tumour cells.

This difference can be explained by the following factors:

The expression of the human Ki-67 protein is strictly associated with cell proliferation. The fact that the Ki-67 protein is present during all active phases of the cell cycle (G1, S, G2, and mitosis) but absent from resting cells (G0) makes
it an specific cell proliferation marker for determining the growth fraction of a given cell population (Schalzen and Gerdes., 1996). In addition, the Ki-67 protein has been shown to have an extremely short half-life of around 20 minutes throughout the cycle, with major catabolism occurring at the end of mitosis, indicating that few cells that have left the cycle will contain the antigen. (Ross and Hall., 1995)

Since the procedures in immuno-histochemical studies affect the results, incomparable results of the present and the previous studies are probably due to different experimental conditions such as appropriate pH for citrate buffer, duration of slice exposure to hydrogen peroxide solution, hydrogen peroxide concentration and the type of antibody used.

CONCLUSION AND RECOMMENDATION

Present study indicated that the PCNA labeling indices were significantly higher in the peripheral cells of tumour nests and strands than in the central cells of the SMA as well as higher in basal cells than luminal portion of UA. Therefore, the cellular proliferation and consequently the ameloblastoma growth are concentrated in the peripheral areas composed by ameloblast like cells.

PCNA labeling indices was significantly higher in the SMA compared to the UA. Higher PCNA positivity indicate higher cellular proliferation rate, which would explain the more aggressive nature of the SMA. Therefore, adequate surgical management should need to consider for SMA.

When compared between PCNA and Ki-67 labeling indices, PCNA labeling indices was significantly higher in both SMA and UA than Ki-67. Moreover, no significant difference in Ki-67 expression was found in peripheral and central portion of SMA and basal cell layer and luminal portion of UA. Therefore, PCNA was found to be more liable immunohistochemical marker for evaluating cell proliferation in ameloblastic tumours.

Because of limited numbers of cases of each subtype of both SMA and UA, proliferative activity of PCNA and Ki-67 were shown to be not statistically significant. Therefore, further studies with large sample sizes with equal subtype are required to draw definite conclusion on the proliferative activity not only among the different types of benign cases but also malignant ameloblastomas.

Further tumour behaviour studies on ameloblastoma using various types of markers such as MMPs,TIMP-2, Heparanase and CD147 for ECM degradation, expression of CD138, Cadherins for cell adhesion, WNT5A and Podoplanin for cell migration, Cyclin E, P21, p27 for cell proliferation and molecular marker involved in bone invasion including interleukin -1, interleukin-6, and tumor necrosis factor alpha (TNF-α) are warranted.

REFERENCE


Evaluation of Serum Copper and Zinc Levels in Betel Quid Associated Oral Submucous Fibrosis and Oral Squamous Cell Carcinoma Patients

Su Mon Than*, Sein Shwe**, Zaw Moe Thein**, Swe Swe Win**
**Department of Oral Medicine, University of Dental Medicine, Yangon
*Department of Oral Medicine, University of Dental Medicine, Mandalay

ABSTRACT

Oral submucous fibrosis (OSMF) is a well-recognized oral premalignancy and oral squamous cell carcinoma (OSCC) is one of the most common malignancy which are related to betel quid chewing habit. Trace elements, copper and zinc were found to be associated with the etiopathogenesis of oral premalignancies and oral carcinogenesis. Measurements of copper and zinc levels in the serum of OSMF and OSCC patients may be helpful in understanding the pathogenesis and rendering effective treatment. The purpose of the study is to evaluate serum copper and zinc in OSMF and OSCC patients with betel quid chewing habit. A cross sectional descriptive study was conducted on clinically diagnosed 30 OSMF patients and histologically proven 30 OSCC patients. The serum concentration of copper and zinc were measured by atomic absorption spectrometry. Serum copper levels were significantly increased (P = 0.000) and serum zinc levels were significantly decreased (P = <0.05) in both OSMF and OSCC patients. Although there was no significantly difference between increase serum copper level in both OSMF and OSCC cases, serum zinc level was significantly decrease in OSMF cases in compare to OSCC cases (P = < 0.05). These findings indicate that trace elements copper and zinc have a role to play in pathogenesis as well as management of OSMF and OSCC.

INTRODUCTION

Oral submucous fibrosis (OSMF) is a chronic disease and a well-recognized potentially malignant condition of the oral cavity characterized by inflammation and a progressive fibrosis of the lamina propria and deeper connective tissues (Sudarshan et al., 2012). Oral squamous cell carcinoma (OSCC) accounts for approximately 3% of all malignancies and more than 90% of oral cancers, and affects mostly adult males, predominantly alcohol and tobacco users, between the sixth and seventh decades of life (Marocchio et al., 2010).

Both OSMF& OSCC is believed to have multifactorial causes, among these chewing of areca nut alone or areca nut including betel quid is the major etiological factor (Marocchio et al., 2010). Areca nut has shown to have high copper content compared to commonly eaten nuts, plays as an initiating factor in stimulating fibrinogenesis by upregulation of lysyl oxidase activity. It is a copper dependent enzyme and play a role in cross-linking of collagen (Punnya et al., 2011).

The role of copper and zinc in human cancer etiology are much less studied. The potential
role of iron, copper, and zinc in cancer etiology is supported by several plausible mechanisms. As transition metals, copper can generate the reactive oxygen species (ROS) including hydroxyl radicals. These reactive oxygen species can attack DNA and cause DNA mutation, thus contributing to the pathological process of cancer (Wu et al., 2004). Zinc is an essential to the function of several transcription factors, proteins that recognize certain DNA sequences and regulate gene transcription. Zinc protects against free radical injury and may affect immune response (Wu et al., 2004). In contrast, zinc may play an anti-carcinogenic role by stabilizing the structure of DNA, RNA, and ribosome. Serum zinc levels are decreased in OSMF patients which can acts as indicator for malignant transformation (Sudarshan et al., 2012).

Trace elements have been extensively studied in recent years to access whether they have any modifying effect in the etiology of cancer. Therefore, this study may provide the exact role of copper and zinc in pathogenesis as well as management of betel quid associated OSMF and OSCC patients.

Aim

To evaluate serum copper and zinc levels in betel quid associated oral submucous fibrosis and oral squamous cell carcinoma patients

Objectives

1. To evaluate serum copper and zinc levels in oral submucous fibrosis patients with betel quid chewing habit
2. To evaluate serum copper and zinc levels in oral squamous cell carcinoma patients with betel quid chewing habit
3. To compare the serum copper and zinc levels in oral submucous fibrosis and oral squamous cell carcinoma patients with betel quid chewing habit

MATERIALS AND METHODS

Types of Study

Cross-Sectional Descriptive study

Place of Study

This study was conducted in

(1) Department of Oral Medicine, University of Dental Medicine, Yangon
(2) Department of Oral and Maxillofacial Surgery, University of Dental Medicine, Yangon
(3) Department of Plastic, Oral and Maxillofacial Surgery, Yangon General Hospital
(4) Department of Clinical Pathology, National Health Laboratory, Yangon

Study Population

30 Oral submucous fibrosis (OSMF) patients and 30 Oral squamous cell carcinoma (OSCC) patients

Study Procedure

Patients were selected according to the selection criteria. After obtaining the consent, patients entitled for this study were interviewed and examined according to proforma. Emphasis was laid on recording any oral habit of chewing betel quid containing processed and raw areca nut. 5ml of venous blood was drawn from median cubital vein under aseptic condition. The blood was allowed to clot for 1 hr and sent to NHL within 2 hrs after taking the blood. In NHL, the blood tube was centrifuged at 1000 rpm for 15 mins to obtain serum. After obtaining serum samples, 1.5 ml of serum was mixed with 1.5 ml of deionized water for copper determination (1:1) and 0.5 ml of serum was mixed with 2.5 ml of deionized water for zinc determination (1:5).

The serum samples were be centrifuged again at 1000 rpm for 15 mins to mix serum and deionized water. For determination of serum copper and zinc level, copper and zinc were
used by performing the standard conditions. Copper standard was prepared by diluting copper stock standard solution with 10% glycerol and zinc standard was prepared by diluting the stock standard solution with 5% glycerol. The Atomic Absorption Spectrometry machine absorbed standard solution first and then absorbed serum sample. Then estimation of copper and zinc were carried out by atomic absorption spectrometry.

![Figure 1. Serum samples of the patients](image1.jpg)

![Figure 2. Determination of serum copper and zinc by Atomic Absorption Spectrophotometry](image2.jpg)

**RESULTS AND DISCUSSION**

**RESULTS**

Table 1. Distribution of OSMF patients in relation to serum copper level

<table>
<thead>
<tr>
<th>Serum copper level</th>
<th>OSMF (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frequency</td>
</tr>
<tr>
<td>Increase (&gt;1.4 ppm)</td>
<td>17</td>
</tr>
<tr>
<td>Upper limit (1.0-1.4 ppm)</td>
<td>11</td>
</tr>
<tr>
<td>Normal (0.7-1.0 ppm)</td>
<td>2</td>
</tr>
<tr>
<td>Student’s t test</td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td></td>
</tr>
</tbody>
</table>
### Table 2. Distribution of OSMF patients in relation to serum zinc level

<table>
<thead>
<tr>
<th>Serum zinc level</th>
<th>OSMF (n=30)</th>
<th>Frequency</th>
<th>Percent</th>
<th>Mean Serum Level (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decrease (&lt;0.5 ppm)</td>
<td></td>
<td>5</td>
<td>16.7</td>
<td>0.2355±0.4637</td>
</tr>
<tr>
<td>Lower limit (0.5-1.0 ppm)</td>
<td></td>
<td>18</td>
<td>60.0</td>
<td>0.8125±0.5220</td>
</tr>
<tr>
<td>Normal (&gt;1.0 ppm)</td>
<td></td>
<td>7</td>
<td>23.3</td>
<td>1.2512±0.5828</td>
</tr>
<tr>
<td><strong>Student’s t test</strong></td>
<td></td>
<td></td>
<td></td>
<td>2.2403</td>
</tr>
<tr>
<td><strong>P value</strong></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

### Table 3. Distribution of OSCC patients in relation to serum copper level

<table>
<thead>
<tr>
<th>Serum copper level</th>
<th>OSCC(n=30)</th>
<th>Frequency</th>
<th>Percent</th>
<th>Mean Serum Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increase (&gt;1.4 ppm)</td>
<td></td>
<td>16</td>
<td>53.3</td>
<td>1.766±1.0452</td>
</tr>
<tr>
<td>Upper limit (1.0-1.4 ppm)</td>
<td></td>
<td>9</td>
<td>30.0</td>
<td>1.4167±0.8191</td>
</tr>
<tr>
<td>Normal (0.7-1.0 ppm)</td>
<td></td>
<td>5</td>
<td>16.7</td>
<td>0.8904±0.0476</td>
</tr>
<tr>
<td><strong>Student’s t test</strong></td>
<td></td>
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<td>6.1406</td>
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<td><strong>P value</strong></td>
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<td>0.000</td>
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</tbody>
</table>

### Table 4. Distribution of OSCC patients in relation to serum zinc level

<table>
<thead>
<tr>
<th>Serum zinc level</th>
<th>OSCC(n=30)</th>
<th>Frequency</th>
<th>Percent</th>
<th>Mean Serum Level (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decrease (&lt;0.5 ppm)</td>
<td></td>
<td>1</td>
<td>3.3</td>
<td>0.2334±0</td>
</tr>
<tr>
<td>Lower limit (0.5-1.0 ppm)</td>
<td></td>
<td>13</td>
<td>43.3</td>
<td>0.7340±0.2920</td>
</tr>
<tr>
<td>Normal (&gt;1.0 ppm)</td>
<td></td>
<td>16</td>
<td>53.3</td>
<td>1.497±0.9511</td>
</tr>
<tr>
<td><strong>Student’s t test</strong></td>
<td></td>
<td></td>
<td></td>
<td>2.12</td>
</tr>
<tr>
<td><strong>P value</strong></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>
DISCUSSION

(1) Evaluation of Serum Copper and Zinc Levels in Oral Submucous Fibrosis Patients with Betel Quid Chewing Habit

In this study, serum copper level was measured in clinically diagnosed 30 OSMF patients. Among them, serum copper level was increased (>1.4 ppm) in 17 patients (56.7%), upper limit (1.0-1.4 ppm) in 11 patients (36.7%) and normal (0.7-1.0 ppm) in 2 patients (6.7%) and serum copper level was highly significantly increased in oral submucous fibrosis patients (P value=0.000) (Table 1).

Most of the areca nuts from the consumed betel quid were already processed and had higher copper content and it might be the possible reason of significantly increased serum copper level in OSMF patients.
Out of 30 patients, 5 patients (16.7%) were decrease serum zinc level (<0.5 ppm), 18 patients (60.0%) were lower limit (0.5-1.0 ppm) and 7 patients were within normal limit (>1.0 ppm). Although 5 patients were decrease in serum zinc level, overall serum zinc level was significantly decreased in OSMF patients (P value= <0.05) (Table 2).

In this study, decrease serum zinc level might be due to increase serum copper level or other causes of immune system or might be the indicator for malignant transformation (Varghese et al., 1987). Shetty et al. (2013) explained that lower serum zinc level in OSMF patients might be due to the consumption of zinc in counter reacting to oxidants which were generated due to tobacco or high copper of areca quid metabolism.

(2) Evaluation of Serum Copper and Zinc Levels in Oral Squamous Cell Carcinoma Patients with Betel Quid Chewing Habit

Serum copper level was measured in histologically proven 30 OSCC patients. Among them, 16 patients (53.3%) were increased (>1.4 ppm), 9 patients (30.0%) were upper limit (1.0-1.4 ppm) and 5 patients (16.7%) were normal (0.7-1.0 ppm) and serum copper level was highly significantly increased among OSCC patients (P value= 0.000) (Table 3).

Increased in serum copper level in OSCC was due to not only increased copper content of processed areca nut but also more likely to get long time exposure with carcinogens from the betel quid.

As for the serum zinc level, out of 30 OSCC patients, 1 patients (3.3%) was decrease serum zinc level (<0.5 ppm), 13 patients (43.3%) were lower limit (0.5-1.0 ppm) and 16 patients (53.3%) was normal serum zinc level (> 1.0 ppm) and serum zinc level was significantly decreased in OSCC patients (P value= <0.05) (Table 4).

Serum copper level was decrease in OSCC patients due to increase serum copper level or their dietary deficiency of zinc or use of zinc in tumour development. Dar et al. (2008) stated that tumour development is associated with an imbalance in trace element metabolism and low levels of zinc may decrease the overall antioxidant defenses. Varghese et al. (1987) also reported that a decreased level of serum zinc level was associated with the carcinogenesis may be due to increased utilization of zinc by tumour tissues.

(3) Comparison of Serum Copper and Zinc Levels in Oral Submucous Fibrosis and Oral Squamous Cell Carcinoma Patients with Betel Quid Chewing Habit

In this study, mean serum copper level of OSMF was 1.39± 0.247 ppm and mean serum copper level of OSCC was 1.33± 0.295 ppm. Even though, there is no significantly difference between serum copper level of OSMF and OSCC patients (P=0.353) (Figure 3), the results showed elevated level of serum copper in OSMF compared with OSCC patients.

Regarding the mean serum zinc levels, OSMF was 0.825± 0.363 ppm and OSCC was 1.25±0.687 ppm and significant difference was found between mean serum zinc level of OSMF and OSCC patients (P= < 0.05) (Figure 4). More decreased level of mean serum zinc was found in OSMF compared with OSCC patients.

Balasubramanian and Chitra (2012) concluded that high levels of zinc were found in OSCC patients than OSMF patients, the similar results were found in this study.
CONCLUSION

After statistical analysis, serum copper level was significantly increased and serum zinc level was significantly decreased in both OSMF and OSCC patients. Mean serum copper level was more increase and mean serum zinc level was more decrease in OSMF than OSCC patients. The role of copper cannot be segregated from that of zinc because of the well-elucidated biochemical relatedness. Zinc bears an inverse relationship with copper and has been implicated in the modulation of mucosal metallothionein, thereby interfering with the absorption of copper. The present study highlighted the role of copper and zinc in etiopathogenesis, malignant transformation as well as modification in treatment modalities for both OSMF and OSCC. Likewise, long term follow up is mandatory for OSMF patients with decreased serum zinc level to observe malignant transformation.

REFERENCES


Early Detection In Oral Potentially Malignant Disorders (OPMDs) With Oral Cancer Screening

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Abstract
This study sought to measure the baseline awareness of oral cancer in Myanmar and aimed to increase not only public awareness and knowledge of oral cancer but also to reduce oral cancer deaths and morbidity by early detection with oral screening with toluidine blue staining as well as oral brush biopsy. Methods: Baseline awareness was evaluated by self-administered questionnaires to individuals and suspicious oral lesions were assessed by oral cancer screening with toluidine blue staining along with oral brush biopsy. Results: A total of 542 subjects participated in the survey, about 349 subjects have tobacco habits including smoking and smokeless. Among the tobacco habitues, 25 of respondents were suffered from oral potentially malignant disorders (OPMD) and 14 out of 25 were male and 11 were female. All of them were prepared to seek proper treatment and follow up. Conclusion: Oral cancer screening for early detection and tobacco use cessation program are utmost important for control and prevention of oral cancer thereby reduction in mortality.

INTRODUCTION
The incidence of oral cancer is increasing in both developed and developing countries (Warnakulasuriya 2009). In Southeast Asia, oral squamous cell carcinomas (OSCCs) account for 40% of all cancers compared with approximately 2-4% in developed countries (Mehrotra et al., 2006). Oral cancer is the 6th most common cancer globally and represents a group of conditions with a range of sites and a varied etiology. In Myanmar, oral cancer ranks 6th in male, 10th in females and contribute 3.5 % of whole cancer (Htun- Naing- Oo et al., 2011).

A significant proportion of oral cancers develop from premalignant lesions such as leukoplakia, erythroplakia and oral submucous fibrosis etc. WHO has identified prevention and early detection as major targets in the battle to control the oral cancer burden worldwide (Peterson, 2008). The most logical approach to decreasing morbidity and mortality associated with OSCCs is to increase detection of suspicious oral potentially malignant disorders (OPMDs) and early detection of OSCCs.

Targeted screening of high-risk individuals might be more effective than mass screening in facilitating early detection of oral cancers. Visual detection alone is not adequate to differentiate precancers and early oral cancers from benign lesions regardless of the expertise of the clinician. Adjunctive techniques that increase the ability to differentiate between benign abnormalities and dysplastic or malignant changes have been suggested during the last decade. These include the use of toluidine blue, oral brush biopsy, chemiluminescence and tissue autofluorescence (Fedele, 2009).

Toluidine blue (tolonium chloride) has been used in vital tissue staining to aid in detection of
mucosal abnormalities of cervix and oral cavity for more than forty years (Patton et al., 2008). Oral brush biopsy is a cytological method that utilizes a brush to obtain a complete trans-epithelial biopsy cell specimen. The use of combination of toluidine blue and oral brush biopsy has been attempted for early detection of oral cancer. The combination was found to be highly sensitive and moderately specific for oral cancers (Gupta et al., 2007).

Materials and Methods

422 households were selected from Ward 133 and 120 households were selected from Sit-Pin village according to sample size determination done by Department of Medical Research (Lower Myanmar). Oral examination team was comprised into 5 groups in which 13 dental surgeons including teaching staffs and post graduate candidates from Department of Oral Medicine, Yangon and 8 dental house surgeons. Suspicious visible oral mucosal lesions from respondents with oral habits (i.e betel quid chewing, smoking, and alcohol drinking) were stained by toluidine blue (TB). Oral brush biopsy was done on TB positive lesions along with cytologic examination.

Materials for toluidine blue staining were needed 1% aqueous toluidine blue, 1% acetic acid and sterile cotton applicators. As for oral brush biopsy, interdental brushes, 95% ethyl alcohol and glass slides were used.

Results and Discussion

542 respondents were collected from Ward 133 and Sit-Pin village in East Dagon Township. In this project, 59.23% were female and 40.77 % were male. Most of respondents were females because males were manual workers and most of them were absent at the time of oral screening.

Out of 542 persons, only betel quid chewers were 284, though 394 were associated with oral habits including chewing, drinking and smoking. Among 284 betel quid chewers, 240 (85%) were added with tobacco such as 92, 100, signal, etc.
Out of 4, one respondent had attended to follow up for only one time (Figure-4). In spite of providing not only health education but also needful medications, there was recognized lack of willingness for regular treatment. It might be due to difficulties in communication, negligence and lack of awareness of oral cancer as well as socioeconomic burden.

**Conclusion & Recommendation**

Betel quid chewing was found to be a common habit in both men and women in the population. All persons of oral dysplastic smear were found to be associated with betel quid usage. Regular opportunistic screening by oral examination of betel quid chewers could achieve the improvement outcome to reduce the risk of oral cancer.

The high mortality rate in cancer is commonly attributed to the negligence in detection of the disease at an early treatable stage. As the key role of dental surgeons, all should participate in early detection or screening and refer early to reduce the occurrence of malignant transformation. The ultimate goals are to increase awareness of oral cancer, to reduce both mortality and morbidity, and to improve patient’s quality of life.

The association of betel quid use with the increase of precancerous conditions and oral cancerous lesions highlights the importance of early detection, oral health education as well as to put great effort on not only tobacco cessation but also betel quid cessation program.

**References**

Comparative Study of the Anesthetic Efficacy of Articaine and Lignocaine in Mandibular First Molars

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Abstract

Pain control is an important part of dentistry and particularly of tooth extractions and surgical procedures. The achievement of successful local anesthesia is a continual challenge in dentistry. Not all conventional inferior alveolar nerve blocks (IANB) result in successful anesthesia. The purpose of this randomized controlled trial was to compare the effectiveness of 4% articaine with 1:100,000 epinephrine mandibular infiltration with that of 2% lignocaine with 1:100,000 epinephrine conventional IANB in mandibular posterior teeth. Using independent study design, a total of 64 patients, 18-59 yrs old, requiring tooth extractions of mandibular first molars randomly received either 1.8ml of articaine buccal and lingual infiltration or 1.8ml of lignocaine IANB. Anesthesia was determined using Heft – Parker Visual Analog Scale (VAS) and Wong – Baker Faces Pain Scale (FPS). Anesthetic efficacy was considered successful if the patients felt no pain or mild pain (VAS < 54mm and FPS of 0-4) during extraction. According to VAS, 84.4% of articaine infiltration and 87.5% of lignocaine IANB were successful (p=0.719) and the success rate determined by FPS were 90.6% and 84.4% respectively (p=0.453). There were no statistically significant differences (p>0.05). The onset of subjective lip numbness and the injection discomfort of articaine and lignocaine groups were not significantly different (p>0.05). In conclusion, infiltration anesthesia with 4% articaine for mandibular first molars can be a useful alternative for clinicians because it has a similar success rate compared with lignocaine IANB.

Introduction

In dentistry, the ability to provide a patient with clinically adequate pain control was a concern of dentists throughout the world. For most surgical procedures, dentists are able to manage operative pain & discomfort by using intraoral administration of local anesthetics. Thus, local anesthesia and its administration techniques are integral parts of management of painful dental procedures.

Although local anesthesia for the extraction of mandibular posterior teeth has principally been obtained through the administration of inferior alveolar nerve block (IANB), not all IANB injections result in successful pulpal anesthesia (Claffey et.al., 2004). Clinical studies have found failure of 44% to 81% with the IANB (Nusstein et.al., 1998). Anesthetic failure after conventional IANB may be caused by several factors such as technical errors, presence of limitation of mouth opening and collateral innervations (Pogrel et. al., 1997 ; Yonchak et.al., 2001).Therefore, many studies have developed considerable interest in inducing mandibular pulpal anesthesia by means of administering buccal infiltration (BI) injection of anesthetic solution adjacent to molars.

The efficacy of infiltration anesthesia for
mandibular molars has been considered inadequate because the mandible has dense, thick cortical bone at that region. Haas et al. (1990) reported that BI of lignocaine or prilocaine solution was not very effective for pulpal anesthesia in adult mandibular posterior teeth. Recently, articaine hydrochloride has been claimed to be efficacious for anesthesia of mandibular pulp and lingual soft tissue by BI, as well as palatal soft tissue anesthesia by means of maxillary labial infiltration because of its superior diffusion through bony tissue (Haas et al., 1990). The superiority of 4% articaine with epinephrine over 2% lignocaine with epinephrine after mandibular infiltration was confirmed by the work of Kanaa et al. (2006). Robertson and colleagues (2007) also found that 4% articaine has 75%-87% anesthetic success in mandibular molars when administered via BI. These results show that BI can be as effective as IANB in anesthetizing the pulp of mandibular molars by using articaine. However, neither study compared the effectiveness of the two methods directly in the extractions of mandibular molars.

The purpose of this study was to compare the efficacy of articaine infiltration anesthesia to the efficacy of conventional lignocaine IANB of the same dose in the extractions of mandibular first molars.

**Material and Methods**

This study was approved by the Ethical Board of the University of Dental Medicine, Yangon and written informed consent was obtained from each subject. Sixty-four patients (28 men and 36 women) between 18 and 59 years of age who needed to have extraction of at least one lower first molar tooth were enrolled in this study. All patients were good in health as determined by a health history and oral questioning. Exclusion criteria included any reported allergy to local anesthetic agents, unstable cardiovascular disease, pregnancy, apical abscess and neurological disorders with sensory disturbance.

The study was designed as a randomized controlled clinical trial. Total 64 patients were equally and randomly divided into 2 groups, articaine group and lignocaine group. At the appointment, the 64 patients randomly allocated either conventional inferior nerve block (IANB) of 1.8 ml of 2% lignocaine with 1:100,000 epinephrine or buccal and lingual infiltration anesthesia of 4% articaine with 1:100,000 epinephrine for extraction of mandibular first molar. Randomization was performed by the lottery method.

Before the injection, each patient was given a hybrid visual analog scale (Helf-Parker VAS) and faces pain scale (Wong – Baker FPS). This VAS consisted of a 170 mm horizontal line with demarcations at each end with words such as “no pain” and “the worst pain imaginable”. Faces pain scale (FPS) consisted of a series of facial expressions drawing to illustrate a spectrum of pain intensity.

![Visual Analog Scale (Heft-Parker VAS)](image)
The subject was informed of the rating scales to be utilized and asked to mark a separate VAS and FPS to rate the pain they experienced at each stage of the infiltration injection, epithelial attachment breaking and tooth extraction. Using the VAS, the patient indicated the intensity of pain by marking a line on a horizontal scale. The VAS score was determined by measuring the distance in mm from the left end of the line to the point that the patient indicated.

Anesthetic injection was administered by the investigator. For the articaine infiltration anesthesia, 1.8 ml of 4% articaine with 1:100,000 epinephrine (Articaine 1:100,000; DFL, Brazil) was given by using infiltration method (1.6 ml of cartridge for the buccal infiltration and 0.2 ml of cartridge for lingual infiltration) with a dental aspirating syringe fitted with (27 G, 21 mm) disposable short needle at a rate of 1.8 ml per 60 seconds. The mandibular buccal infiltration was administered at mucobuccal fold adjacent to the selected lower first molar bisecting the approximate location of the mesial and distal roots. The needle was gently placed into the alveolar mucosa and was advanced until the needle was estimated to be at or just above the roots of the first molar. The lingual infiltration was administered to the lingual gingival adjacent to the extracted tooth.

For the lignocaine nerve block, 1.8 ml of 2% lignocaine with 1:100,000 epinephrine (Alphacaine 1:100,000; DFL, Brazil) was given by using conventional IANB method (0.9 ml of cartridge for inferior alveolar nerve, 0.45 ml of cartridge for lingual nerve and 0.45 ml of cartridge for long buccal nerve) with a dental aspirating syringe fitted with (27 G, 30 mm) disposable long needle at a rate of 1.8 ml per 60 seconds.

Following the completion of anesthetic injection, the patient received a post injection pain survey with VAS. The onset of lip numbness was also recorded. After 10-15 minutes of LA injection, detachment of epithelial attachment by probing on both buccal and lingual sides was done and extraction performed. If the patient felt pain, the treatment was immediately stopped and the patient rated their discomfort using the VAS and FPS. The success of anesthesia was defined as the ability to extract the tooth without pain or mild pain (VAS score of 0 to ≤ 54mm and FPS score of 0 to ≤ 4). In anesthetic failure case, tooth extraction was continued by using additional conventional IANB with 2% lignocaine.

Data were analyzed by Chi-square, Fisher’s exact test and Student’s t test, performed using a statistical analysis package (SPSS 15.0 software).

**Results**

The study was performed over a one year period. 36 women (56.25%) and 28 men (43.75%) with a
mean age of 39.38 years (range, 18-59 years) were included. There were no significant differences (p>0.843) between the two groups. The distribution of teeth and diagnosis of teeth are outlined in Table 1 and 2. There were no significant differences (p>0.073) between the two groups.

No significant difference was noted in injection discomfort between articaine mandibular infiltration (mean VAS 9.72 mm) and lignocaine IANB (mean VAS 11 mm) in 64 patients (p=0.734) (Fig.3). All patients reported lip numbness after articaine mandibular infiltration and lignocaine IANB, with a mean onset of 3.07 minutes (SD, 2.37 minutes) and 3.26 minutes (SD, 2.15 minutes), respectively.

The anesthetic success determined by extraction pain (VAS) is presented in Table 3. The success rate of articaine mandibular infiltration was 84.4% and that of lignocaine IANB was 87.5%. There was no significant difference (p=0.719) between the two groups. The percentage of successful anesthesia in the two study groups determined by extraction pain (VAS score) are summarized in Table 3.

According to the FPS, 29 out of 32 patients (90.6%) achieved successful anesthesia after articaine mandibular infiltration and 27 out of 32 patients (84.4%) had anesthetic success after lignocaine IANB (FPS= 0 to 4). The failure rate were 9.4% (3 out of 32 patients) and 15.6% (5 out of 32 patients) for articaine and lignocaine groups respectively (FPS= > 4). Statistical analysis value showed that there was no significant difference among study groups (p=0.453) (Fig.4).

The mean duration of soft tissue anesthesia was 134 minutes (SD, 62.55 minutes) after articaine mandibular infiltration and 181.67 minutes (SD, 47.06 minutes) after lignocaine conventional nerve block. The difference was not significant (p=0.253) (Fig.5).

Table (1) Distribution of extracted teeth for articaine and lignocaine groups

<table>
<thead>
<tr>
<th></th>
<th>Articaine Group</th>
<th>Lignocaine Group</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower left first molar</td>
<td>9</td>
<td>28.1%</td>
<td>16</td>
</tr>
<tr>
<td>Lower right first molar</td>
<td>23</td>
<td>71.9%</td>
<td>16</td>
</tr>
<tr>
<td>Total</td>
<td>32</td>
<td>100.0%</td>
<td>32</td>
</tr>
</tbody>
</table>

p = 0.073

Table (2) Relation between Diagnosis of extracted teeth and study groups

<table>
<thead>
<tr>
<th></th>
<th>Articaine Group</th>
<th>Lignocaine Group</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute exacerbation of chronic pulpitis</td>
<td>1</td>
<td>3.1%</td>
<td>0</td>
</tr>
<tr>
<td>Chronic apical periodontitis</td>
<td>27</td>
<td>84.4%</td>
<td>25</td>
</tr>
<tr>
<td>Chronic periodontitis</td>
<td>3</td>
<td>9.4%</td>
<td>5</td>
</tr>
<tr>
<td>Chronic irreversible pulpitis</td>
<td>1</td>
<td>3.1%</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>32</td>
<td>100.0%</td>
<td>32</td>
</tr>
</tbody>
</table>

p=>0.10
Table (3) Comparison of the percentage of successful anesthesia in two study groups determined by extraction pain (VAS score)

<table>
<thead>
<tr>
<th>VAS</th>
<th>Articaine Group</th>
<th>Lignocaine Group</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-54 mm (success)</td>
<td>27</td>
<td>28</td>
<td>55</td>
</tr>
<tr>
<td>&gt; 54 mm (Fail)</td>
<td>5</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>32</td>
<td>32</td>
<td>64</td>
</tr>
</tbody>
</table>

P = 0.719

Figure 3. Mean VAs (mm) for injection discomfort

Figure 4. Comparison of anesthetic success determined by extraction pain (FPS)

P = 0.453
Discussion

This study was conducted to compare the local anesthetic efficacy of two techniques, 4% articaine mandibular infiltration versus 2% lignocaine conventional IANB in the extraction of mandibular first molars.

The patients’ age (p=0.843), gender (p=1.000), tooth type and diagnosis (Table 1 and 2) were not significantly different between the two study groups. Therefore, there were no significant differences of demographic data of the patients among these study groups and the effect of age, gender and diagnosis would be minimized between the two groups.

Pain of anesthetic injection may be influenced by site of the injection, use of topical anesthesia, injection rate, injection volume, the type of the solution used due to its acidic pH (Malamed, 2004). Previous studies have reported that the inferior alveolar block injection yielded significantly more discomfort than local infiltration injection (Kaufman et.al., 2005; Sharaf, 1997). In the current study, anesthetic injection pain for articaine infiltration group resulted in a mean pain VAS of 9.92mm and 11.00mm for lignocaine IANB. Both mean values were in the mild pain category. Statistical analysis of anesthetic injection discomfort found no significant differences between the two solution groups (p=0.723). Corbett et.al., (2008) reported that the injection pain of articaine buccal infiltration (BI) and lignocaine IANB were in mild pain categories. Although BI cannot be directly compared to our pain measurement scale for the reason as we used 1.2 ml of 1.8 ml cartridge for the buccal infiltration and 0.6 ml of cartridge for lingual infiltration, the values found in this study correlate to the “mild” pain levels found in Corbett study.

Reporting of subjective lip numbness was similar after infiltration and IANB. The mean onset of lip numbness was 3.07 minutes (range 1-10 minutes) for articaine infiltration and 3.26 minutes (range 2-10 minutes) for lignocaine IANB. Several studies have examined the anesthetic efficacy with the aid of pulp tester. Although all subjects felt profound lip numbness, pulp testing revealed that subjects did not always have pulpal anesthesia because the onset of successful pulpal anesthesia started after 5 to 15 minutes of injection (Jung et.al., 2008). Thus, lip numbness only indicated soft tissue anesthesia but did not guarantee successful pulpal anesthesia (Mikesel et.al., 2005). These results suggest that a period of 5 to 15 minutes should elapse before commencing a potential painful procedure.

In the present study, the successful anesthesia in two study groups determined by extraction pain (VAS) was shown in Table 3. Comparing the articaine infiltration data with the lignocaine IANB data, of the 64 patients, 27 (84.4%) experienced anesthetic success after articaine infiltration compared with 28 (87.5%) after the IANB. The failure rates were 5 (15.6%) and 4 (12.5%) for articaine and lignocaine groups respectively. Success was defined as extraction discomfort of no or mild pain (VAS = 0 - ≤ 54mm) and failure was defined as moderate or severe pain (VAS > 54mm). No statistically significant difference was noted between the techniques (p=0.719). Our findings corroborate those of

Figure 5. Duration of soft tissue anesthesia
Corbett et al. (2008) who also conducted a clinical study evaluating articaine infiltration technique and lignocaine IANB by using electric pulp tester.

According to FPS definition, 29 patients (90.6%) experienced anesthetic success after articaine infiltration compared with 27 patients (84.4%) after the lignocaine IANB. There was no statistically significant difference in anesthetic success of the two groups (p=0.453) (Figure 4).

We found that articaine infiltration was as effective as lignocaine IANB in the extraction of mandibular first molars. A possible mechanism of articaine is that it has better bone penetration efficacy. Articaine contains a thiophene ring instead of benzene ring like other amide local anesthetics, which may allow the molecule to diffuse more readily. This speculation is corroborated by the claims that articaine is able to diffuse through soft and hard tissues more reliably than other local anesthetics and that maxillary buccal infiltration of articaine provides even a palatal soft tissue anesthesia (Haas et al., 1990)

There are possible mechanisms of action by which buccal infiltration of articaine achieves its effect as an alternative to regional block methods in the first molar region. It may be assumed that infiltration through to the inferior alveolar nerve canal and blocking the inferior alveolar nerve distal to that point or infiltration through the mental foramen to produce a modified mental and incisive nerve block (MINB).

These possibilities can be tested in clinical investigation. Meechan and colleagues (2011) conducted a trial in which they compared buccal infiltration with lingual infiltration of the same dose of anesthetic whether this blockade was the result of infiltration through the cortex to the mandibular canal or entry into the canal via the mental foramen. In this investigation, healthy adult participants received either a buccal or a lingual infiltration of 1.8 ml of 4% articaine with 1:100,000 epinephrine opposite to the mandibular first molar. The results of this study showed that the buccal infiltration for the first molars, first premolars and lateral incisors, was more successful (P < .001) than the lingual infiltration only. This is evidence that the mental foramen is important in the mechanism of action of a buccal infiltration at the mandibular first molar. But the lingual infiltration only cannot access the mental foramen and any effect would be caused by diffusion through the cortex.

Martin and coworker (2011) showed that dose dependent effectiveness of BI anesthesia of 4% articaine with 1:100,000 epinephrine at the mandibular first molar. The 1.8 mL provided anesthesia in 50% of the cases and 3.6 mL provided anesthesia in 70% of the cases. Hence, it can be suggested that additional dose of articaine may provide more anesthetic success in mandibular infiltration.

The mean duration of soft tissue anesthesia was 134 minutes (SD, 62.55 minutes) after articaine mandibular infiltration and 181.67 minutes (SD, 47.06 minutes) after lignocaine conventional nerve block. Although the duration of soft tissue anesthesia of two group were not statistically difference, articaine infiltration group had rapid anesthetic wear off. Articaine benefits from short plasma half-life compared with other amide local anesthetics (Mikesell et al., 2005; Malamed et al., 2005) and when gives as an infiltration should circumvent the possible concentration related neurotoxicity associated with regional blocks methods.

In addition, articaine infiltration technique may be preferred in certain patients such as those suffering from hemophilia in order to reduce the chances of dangerous hemorrhage. Trismus and nonsurgical paresthesia as a result of damage from the needle to the inferior alveolar or lingual nerves is avoided. Articaine infiltration anesthesia does not required the specialized equipment needed for intraosseous delivery and less destructive to the periodontium that follows intraligmentary injections.
Conclusion

Anesthetic efficacy of 4% articaine with epinephrine mandibular infiltration was similar to that of an IANB using 2% lignocaine with epinephrine in extraction of mandibular first molars. Thus, articaine mandibular infiltration method can be useful alternative to 2% lignocaine IANB in achieving anesthesia of mandibular first molar extraction.

References


Comparison of semilunar coronally advanced flap alone and in combination with button technique in the treatment of Miller’s Class I and II gingival recessions: A Pilot Study

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Abstract

Background: Gingival recession is one of the most common aesthetic and functional concerns associated with periodontal disease. A variety of surgical procedures have been introduced to the field of cosmetic periodontology for treatment of gingival recession.

Aim: To evaluate and compare clinical outcome of semilunar coronally advanced flap with and without button technique in the treatment of Miller’s Class I and II gingival recessions.

Study Design: A total of 12 subjects with bilateral single Miller’s Class I and II recession were selected for the study. Split mouth design was used.

Materials and Method: Surgical sites were randomly divided into test and control groups. In control sites SCAF alone was done where as in test site a combination of SCAF and button technique was performed. The clinical parameters including gingival recession, periodontal pocket depth, clinical attachment level and width of keratinized gingiva were recorded at baseline and 6 months postsurgery. Data so collected was put to statistical analysis.

Statistical Analysis: Student t-test was used to find significance of parameters between baseline and 6 months. For intergroup comparisons paired t-test was performed.

Results: Statistically significant improvements were recorded in both groups from baseline to 6 months. Intergroup comparison yielded statistically significant differences in GR and CAL in favor of test group.

Conclusion: Combination of SCAF and Button technique resulted in statistically significant improvements in clinical parameters as compared to SCAF alone. Future clinical studies with much larger sample size and longer follow up periods are warranted.

Key words: Semilunar coronally advanced flap, button technique, gingival recession, root coverage.

Introduction

Gingival recession is one of the most common aesthetic and functional concerns associated
with periodontal disease.[1] It is the displacement of soft tissue margin apical to cementoenamel junction[2] and is very common in patients having good oral care standards as well.[3] It may be associated with inflammatory periodontal disease, mechanical trauma or with the presence of factors such as tooth malposition, root prominences, aberrant frenulum attachment and orthodontic tooth movements etc.[4,5] It is aesthetically undesirable condition that may lead to root dentine hypersensitivity and root caries.[6] A variety of surgical procedures have been introduced to the field of cosmetic periodontology which include free gingival grafts, guided tissue regeneration, pedicle flaps such as semilunar coronally advanced flap, lateral sliding flap, double papilla flap etc. for treatment of gingival recession.[7,8] Semilunar coronally advanced flap (SCAF) was introduced in 1986 by Tarnow.[9] Major advantage of this flap technique are that no sutures are required because of lack of tension on the tissue being coronally positioned. But concerns regarding flap stability without sutures have been raised in previous studies particularly in teeth with highly scalloped gingiva. Thus various modifications of this flap technique have been introduced.[10] A previous study had compared SCAF in treatment of gingival recession with and without tissue adhesive. Tissue adhesive was used with an aim to improve stability of the flap.[11] The objective of our study was to evaluate and compare clinical outcome of semilunar coronally advanced flap with and without button technique in treatment of Miller’s Class I & II gingival recession.

**Materials and Method**

The study was conducted in Genesis Institute of Dental Sciences and Research, Ferozepur, India. The clearance for the study was granted by Institutional Ethical Committee. 12 subjects with bilateral single Miller’s Class I and II recession defects were selected for the study. Selected subjects were non smokers, systemically healthy and had acceptable levels of oral hygiene standards. A written informed consent was obtained from all the participants. Miller’s Class III and IV recession defects, smokers, teeth with cervical caries or restorations were excluded. Selected subjects underwent phase I periodontal therapy. The surgical sites were randomly divided in to control group and test group by coin flip method. In control site, SCAF was performed using Tarnow’s technique. In this flap was advanced coronal to CEJ as far as possible and positioned accordingly without any tension. A gentle pressure was applied to ensure proper adaptation and stabilization of flap. Periodontal dressing was placed. In test sites, same technique was followed but the flap was stabilized by suture using button technique. Sutures were removed after 10 days. (Figure1-4)
Results

All the subjects completed the follow up. There was no post-operative complication in any of the subjects. Healing was uneventful. Mean plaque scores were maintained throughout the study period indicating good standard of plaque control. Mean and standard deviation of clinical parameters for both groups at baseline and after 6 months are shown in Table I and II respectively.

A statistically significant reduction in gingival recession was observed in both test group and control group from baseline to 6 months. An overall reduction of 2.35+0.22mm was reported in test group, whereas it was 1.38+0.17 for control group. When reduction in gingival recession was compared among groups, a statistically significant result was obtained in favor of test group (p<0.001) [Table 3]. Clinical attachment level gain of 2.92+0.21mm and 2.17+0.13mm was obtained in test and control group respectively after 6 months. Intergroup comparison for CAL yielded a statistically significant result, which was in favour of test group (p= 0.024) [Table 3]. In test group a mean probing pocket depth reduction of 0.42+0.07mm was recorded after 6 months. For control group mean reduction of 0.08+0.27mm in probing pocket depth was reported. Statistically non significant results were obtained on intergroup comparison of probing pocket depth (p=0.73) [Table 3]. An increase of 0.58+0.01mm in keratinized gingiva was reported after 6 months in test groups which was statistically significant. For control group a statistically significant gain of 0.33+0.01 mm was reported in keratinized gingiva.

Discussion

The changing face of dentistry has ushered in a new era where the present day aim is to have a healthy and esthetically pleasing dentition. Thus esthetics has become an essential criterion of the overall treatment plan in dentistry, which comprises of a healthy and beautiful smile at any age.[12] Gingival recession is of great esthetic concern associated with periodontal...
disease. Coronally advanced flap is one of the most reliable techniques for treatment of single gingival recessions and different surgical flap designs have been proposed overtime increasing the possibility of achieving root coverage.[13,14] A semilunar coronally advanced flap has various advantages such as no tension on flap, no suture requirement and no vestibular shortening and moreover papilla remain unchanged.[9]

The present study was done to compare the clinical outcome of SCAF alone and in combination with button technique in treatment of gingival recession. In button technique, orthodontic buttons/brackets are used as a passive component for holding sutures so as to provide maximum stability to the flap in coronally displaced position.[15] A previous study had compared SCAF in the treatment for gingival recession with and without tissue adhesive and concluded that SCAF followed by application of EPIGLU is an effective procedure for root coverage.[11] Some other studies had also warranted the fixation and stabilization of flap for attaining better results.[10,16]

In present study, a split mouth designed was used and sites were randomly assigned to two treatment groups (Test and Control groups). Eight out of twelve sites in test group gained complete root coverage whereas in control group seven sites achieved complete root coverage. These findings are in agreement to previous studies of Sandro-Bittencourt et al 2006[17] , Sandro-Bittencourt et al 2009[18] A statistically significant reduction of 2.35+0.22mm and 1.38+0.17mm was recorded in gingival recession in both test and control group respectively. Decrease in gingival recession may be due to the formation of new connective tissue attachment and epithelial attachment.[19] Intergroup comparison yielded statistically significant results in favor of test group. This may be attributed to the use of button technique in test sites as suturing the flap with button offer better stabilization in desired location.[10] Less amount of recession reduction in control group may also be due to lack of stability of coronally positioned flap to counteract wound contraction. This may also be the reason for greater gain in clinical attachment level in test sites as compared to control sites. Gain in clinical attachment level was statistically significant for both the groups after six months which was in accordance to previous clinical trials.[17,18] Intergroup comparisons revealed a statistically non significant results for probing pocket depth. A statistically significant increase in width of keratinized gingiva was reported in both groups after 6 months but intergroup comparison revealed a statistically non significant difference. In SCAF granulation tissue that fills the semilunar area will generally turn into same type of tissue that was present before repositioning the tissue. Increase in width of keratinized gingiva is due to the tendency of coronally displaced mucogingival line, to regain its original position. Plaque scores for both groups remained constant through out the study period as the patients were reinforced towards better oral hygiene at regular intervals.

Some previous studies have compared coronally advanced flap with and button technique and reported better results with use of an orthodontic button.[17,18,20,21] To best of our knowledge, this is the first study which compared SCAF with and without button technique. Limitations of this study included smaller sample size and short follow up period (6 months). The present study also hinted us regarding the use of combination of SCAF and Button in lower teeth for root coverage as well. As SCAF is a simple procedure in comparison to highly expensive alternatives so future studies with larger sample size and longer follow up period are warranted for better exploration of the findings.

**Conclusion**

Combination of SCAF and Button technique resulted in statistically significant improvements in reduction of gingival recession and clinical attachment level gain as compared to SCAF alone. Future clinical studies with much larger
sample size are warranted and longer follow up periods are warranted.

References


### Tables

**Table I: Clinical parameter changes in Test Group.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>No.</th>
<th>Baseline</th>
<th>After 6 months</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean +SD</td>
<td>Mean +SD</td>
<td></td>
</tr>
<tr>
<td>GR</td>
<td>12</td>
<td>2.45+0.42</td>
<td>0.10+0.21</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>CAL</td>
<td>12</td>
<td>4.17+0.71</td>
<td>1.25+0.62</td>
<td>0.000*</td>
</tr>
<tr>
<td>PPD</td>
<td>12</td>
<td>1.25+0.45</td>
<td>0.83+0.38</td>
<td>0.339</td>
</tr>
<tr>
<td>WKT</td>
<td>12</td>
<td>0.92+0.52</td>
<td>1.5+0.51</td>
<td>0.027*</td>
</tr>
</tbody>
</table>

*Statistically significant.

**Table II: Clinical Parameter changes in Control Group**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>No</th>
<th>Baseline</th>
<th>After 6 months</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean +SD</td>
<td>Mean +SD</td>
<td></td>
</tr>
<tr>
<td>GR</td>
<td>12</td>
<td>2.35+0.22</td>
<td>0.72+0.58</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>CAL</td>
<td>12</td>
<td>3.67+0.65</td>
<td>1.59+0.52</td>
<td>0.000*</td>
</tr>
<tr>
<td>PPD</td>
<td>12</td>
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<td>1.00+0.02</td>
<td>0.017*</td>
</tr>
<tr>
<td>WKT</td>
<td>12</td>
<td>1.50+0.67</td>
<td>1.83+0.83</td>
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</tbody>
</table>

* Statistically significant

**Table III: Intergroup Comparison**

<table>
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<th>Parameter</th>
<th>Group</th>
<th>No.</th>
<th>Mean + SD</th>
<th>t-value</th>
<th>p-value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
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<td>GR</td>
<td>Test</td>
<td>12</td>
<td>2.35+0.22</td>
<td>0.692</td>
<td>0.001</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>12</td>
<td>1.38+0.17</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAL</td>
<td>Test</td>
<td>12</td>
<td>2.92+0.21</td>
<td>2.429</td>
<td>0.024</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>12</td>
<td>2.17+0.13</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPD</td>
<td>Test</td>
<td>12</td>
<td>0.42+0.07</td>
<td>0.312</td>
<td>0.73</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>12</td>
<td>0.08+0.27</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WKT</td>
<td>Test</td>
<td>12</td>
<td>0.58+0.01</td>
<td>-1.431</td>
<td>0.167</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>12</td>
<td>0.33+0.01</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

S- Statistically significant
NS- Statistically Non Significant
Dental Caries Status and Oral Health Behavior among Myanmar Migrant Workers in Mae Sot District, Tak Province, Thailand

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ABSTRACT
This cross-sectional study was aimed to assess dental caries status, knowledge, attitudes and practices in oral health, and their associations among Myanmar migrant workers in Mae Sot district, Tak province, Thailand. A total of 130 Myanmar Migrant workers participated in an oral examination and were interviewed by structured questionnaires.

The prevalence of caries among this group of workers was 86.9% with a mean DMFT of 2.09±1.39. The majority of them had a good score on level of knowledge (83.1%), but a poor score on level of practices (60.8%) in oral health and an equal proportion of good and poor level of attitude scores (53.1% and 46.9%, respectively). Those who had poor scores on knowledge, attitude and practice in oral health, had a higher prevalence of caries than those who had a good score on knowledge, attitude and practice in oral health (poor score: 16.9% on knowledge, 46.9% on attitude, 60.8% on practice, good score: 83.1% on knowledge, 53.1% on attitude, 39.2% on practice).

This study suggests on oral health promotion program along with oral health care services for this group of Myanmar migrant workers. Simple treatment for scaling, filling, and extraction should be available for them for better oral health status.

KEY WORDS: MYANMAR MIGRANTS/ ORAL HEALTH STATUS

Introduction
Oral Health can be described as “the normality and functional efficient of teeth, supporting structures of the teeth, jaws and structures related to mastication and maxillofacial complex.”(1) It also describes the well-being of the oral cavity, including the absence of oral disease, the dentition and its supporting structures and the optimal functioning of the mouth and its tissues and it is a fact which preserves the highest level of self-esteem and interpersonal relationships.(2) Oral health problems affect the quality of human life. It is mainly as a result of two major oral diseases: dental caries and periodontal disease. Since dental caries and periodontal diseases are the multi-factorial origin, the contributions made by epidemiological studies render more understanding of the factors related to these major oral diseases.(3)

Dental caries is one of the major causes of tooth loss in the world. It is also one of the most common disorders of mankind, starting at an early age, affecting children and young adults but can occur any age. (4) A number of factors have been put forward to explain the variation in prevalence, extent and severity of dental caries, not only between developing and developed countries but also between rural and
urban populations.

Many people claimed that they knew a lot about dental facts, including how to keep their oral hygiene to the best level. However, most of the studies reported a lot of cases pertaining to dental caries and periodontal diseases, which is mainly related to poor oral hygiene and lack of dental knowledge. In addition, it has been observed that numerous campaigns in promoting oral health awareness to the public by dental health care providers have always received poor responses and yielded results less than hoped for. (5, 6) Kiyak (1981) found that there was a significant relation between Decayed, Missing and Filling Teeth Total (DMF-T) scores and awareness of oral health among Asian people i.e., lower DMF-T in an individual with higher awareness level.(7)

Health Education attempts to change behavior by altering an individual’s knowledge, attitude, belief and practice about oral health matters. Education of general public is an integral part of a preventive oriented approach to oral health and disease problems. Education can help to increase knowledge of public. It is often assumed that knowledge determines attitude and attitude determines behavior.(8) When knowledge (K), attitudes (A), and practices (P) of a person towards oral health is profound, the oral health status of this person is good (KAP control oral health). KAP take part in the major role in the promotion of oral health; since prevention of oral diseases entirely depend on the individual awareness. Within KAP model, the change from an unhealthy attitude to a health attitude will occur if adequate information, motivation and practice of the measures to be adopted by the subject are given.(9) Information means that the subject has all the data necessary to understand what oral disease is and how it arises, as well as to understand the protective measures that need to be adopted (Knowledge). This knowledge will, in theory, lead to changes in attitude, which will in turn lead the subject to make changes in their daily life (practice).(9)

Working population usually is majority of population who need to have good health for good quality of life. In Myanmar, proportion of working population was about 53.10 % (2010). (10) Myanmar had to face with problem in moving out of population, especially working group to nearby country such as Thailand. Main reasons for migration are known to be better work, civil war, food and job insecurity, or having suffered from military acts of tyranny. In addition to cross-border trade, the town’s main industries comprise sweat shops and factories.(11)

Migrants belong to the difficult-to-reach population in health and preventive care. Essential criteria for the sustainable effectiveness of preventive and health promotion consist of the proper selection of target groups and successfully approaching them. Some of the barriers such as low literacy and language barrier make the access to preventive care and health promotion more difficult. In addition, there are only a few preventive offers which are target group focused. The use of native speaking preventive consultant is an effort to improve the access to preventive care for migrants by low threshold come and access-structures.(12)

Mae Sot is one of nine districts in Tak province where many foreign migrants (mostly Myanmar) have been staying. Those migrants are with or without registration with the Office of Provincial Administration, Ministry of Interior. The migrant population was about 524,897 and only 124,618 foreign migrants are registered (Ministry of Interior, 2004). Since a lot of Myanmar, Laos and Cambodian migrants have stayed at Mae Sot without precise health data, the health planning for those foreign migrants has not been universally covered yet and those foreign migrants could not reach their equitable right. The migrants’ health strategy was prepared by Mae Sot Health Office to allow them to receive the integrated and universal public health service. (13)

Although the Thai Government provides the health care services for the migrants, even
documented Myanmar migrants still cannot reach these health services. (13)

There is limited information in exploring the factors which make migrants not to access these health care services. The mortality and morbidity of Myanmar migrants will be increased in the future if the health problems of those migrants cannot be solved. Though health care services are availability for Myanmar migrants, there is still big gap between migrants and utilization of these services. Only if we narrow down this gap, we can save many lives of migrants. (13)

Another factor influencing the utilization of general health and dental health care services may be their health perception, which is so poor that utilization of general health and dental services are not very common in these populations compared to their concern on housing and foods. Some of Myanmar migrants do not want to consult the health care personnel when they get sick for a number of reasons. It may be because of Language barrier and socioeconomic factors. (13)

Regarding the dental health of the migrant students, the school health team visits schools for displaced children in and near Mae Sot to provide a number of services such as screening, water/sanitation assessment, first aid supplies, polio vaccinations, biannual prophylactic de-worming and vitamin A supplementation and dental and oral care.

For adult, Mae Sot General Hospital and Mae Tao Clinic provide general and dental health care services for them. (14)

The aims of this study was to assess caries status and knowledge, attitude and practices regarding oral health including utilization of oral health care services, and their association among Myanmar migrant workers in Mae Sot district, Tak province, Thailand.

Materials and Methods

A cross-sectional study was carried out in Myanmar migrant workers in Mae Sot District, Tak Province, Thailand during January to February 2013. Study Population was Myanmar migrant workers who lived in 14 communities of Mae Sot District, Tak Province, Thailand.

Inclusion Criteria

(1) Migrants who were working in Mae Sot District, Tak Province, Thailand.
(2) Migrants who agreed to participate in this study under consent.
(3) Migrants who were Burmese.

Exclusion Criteria

(1) Migrants who were ill with physical or mental disorders which cannot participate in oral health examination.
(2) Migrants who were unable to answer questionnaire by making, writing and interviewing.

Based on the household data of Myanmar migrants available from International Organization of Migration (IOM) in March 2006, there were total of 1337 households with 6152 Myanmar migrants workers in 14 communities of Mae Sot community area in Mae Sot District. It consisted of 14 municipal communities which considered as 14 clusters. Two communities or clusters were selected by purposive sampling. One was congested community and one was non-congested community. From these communities, the respondents were selected according to the inclusion criteria. As the migrants were staying without registration, it is better to estimate the number of households. Therefore convenience sampling was applied in selecting the respondents or study sample.

The sample size of the study was 129 respondents, calculating from the following formula (Cochran, 1963),

\[ n = \frac{z^2 \alpha/2 P(1-P)}{d^2} \]

\( n \) = sample size
\( z \) = value from normal distribution associated with 95% confident level = 1.96 = Significant level, set at 0.05
P = Anticipated proportion of individuals in the study population on “A pilot study of dental caries status in relation to knowledge, attitudes and practices in oral health in Myanmar, Asia Pac J Public Health.(2003)” =86 %

d = maximum allowable error = 0.06

\[ \alpha = \text{significant level} \]

\[ n = \frac{(1.96)^2 (0.86) (0.14)}{d^2} = 128.5 \]

Minimum sample size = 129 subjects.

A total of 130 subjects participated in this study.

Questionnaires were constructed by researcher. The contents of the questionnaires were divided into 5 parts.

For pretesting of questionnaires, they were translated into Myanmar language. Pre-test of questionnaires were performed for content validity by dental expert. Reliability test was done among 30 migrants Myanmar, and alpha’s Cochran was calculated. Improving questionnaires were done.

Oral health examination form was modified from the WHO Record Form for DMFT Index and treatment needs.

Dental caries status was measured as decayed, missing and filling (DMFT) in permanent dentition. It was detected by the naked eyes under the natural light.

Individual DMFT = DT+MT+FT in severity of dental caries

Mean DMFT = Total individual DMFT/Number of persons examined

Mean DMFT = \( \sum \) Individual DMFT

Total population of examined

High affected group = DMFT >13.9

Moderately affected group = DMFT 9.0-13.9

Low affected group = DMFT 5.0-8.9

Very low affected group = DMFT <5.0

Survey team was consisted of 1-2 examiners, 1-2 recorders and 1 dental assistant. Meeting of team was scheduled before working to calibrate and to standardize oral health examination.

Data Collection

The survey protocol was reviewed by ethical committee. Data was collected by face to face interview using the structure questionnaires after taking informed consent and also explained about the study to get high validity. Questionnaires were asked by the researcher only, so as to prevent interpersonal bias and using local language (Myanmar Language) to eliminate misunderstanding so as to establish least errors. Validity of questionnaires was reviewed by advisor and co-advisor.

For standardization and calibration, before data collection period, intra-examiner calibration was performed by examining a group about 60 school children twice on following day and the consistency of examination was determined. The kappa statistics result was 0.80 showing strong agreement.

The consistency of examination was determined by examining a group about 20 migrant workers twice on following days. This study involved two examiners. The examiner was able to obtain an estimate of the extent and nature of the diagnostic errors by comparing two times of examinations within one month. Duplication of examination was performed in second time. Kappa statistics or trust of agreement was calculated. Teeth showing disagreement were subjected to reexamination. The Kappa value was 0.92, showing almost perfect agreement according to the World Health Organization, Calibration of Examiners for Oral Health Epidemiological Surveys.

Procedure for answering the questionnaires for on Knowledge and Practice of migrant workers in Oral Health

The questionnaires were translated to Myanmar
Language and distributed to the community. The migrant workers were explained how to answer for each segment of questionnaires properly. Questionnaires were collected when they had been finished to answer all the questionnaires. After finishing answering questionnaire, migrant workers were asked to participate in oral examination.

**Procedures for Oral Health Examination**

Oral health examination was carried out by the researcher and recorded by one trained recorder. Oral examination was performed by using codes and criteria described by Oral health Survey Basic Methods of WHO (1997). Examination was done under daylight reflected through a plane mouth mirror, with a worker seated on a chair. The researcher was dictated findings to the recorder who sited beside the subject. This was account for checking whether all data entry was correct. Then, 20% of samples were reexamined to check the examiner’s consistency.

**Data Analysis**

After data entering and editing by SPSS 11.5 (Statistical Software), the following statistical analysis was calculated.

For Descriptive Statistics, frequency of distribution, proportion and mean with standard deviation were used to describe the general characteristics, prevalence of dental caries status (DMFT), oral behavior.

To assess the associations between dental caries status and dental behaviors, Chi-square test and Mann-Whitney test were used. A p-value less than 0.05 was considered as statistically significant.

**Result**

Total number of participants with complete information was 75 from congested and 55 from uncongested area in Maesot District, Tak Province, Thailand.

<table>
<thead>
<tr>
<th>Dental Caries Status</th>
<th>Number</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caries free</td>
<td>17</td>
<td>13.1</td>
</tr>
<tr>
<td>At least 1 cavity</td>
<td>113</td>
<td>86.9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Caries status</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>DT</td>
<td>113</td>
<td>2.06</td>
<td>1.39</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>MT</td>
<td>0</td>
<td>0.00</td>
<td>0.00</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>FT</td>
<td>0</td>
<td>0.00</td>
<td>0.00</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>DMFT</td>
<td>113</td>
<td>2.06</td>
<td>1.39</td>
<td>1</td>
<td>5</td>
</tr>
</tbody>
</table>

Table - 1. Proportions of Caries Free among Myanmar migrant workers (n=130)

Table -2. Mean and SD of DMFT and each component of DMFT (n=130)
### Table 3. Comparison of average score of DMFT by general characteristics

<table>
<thead>
<tr>
<th>Variables</th>
<th>DMFT</th>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean</td>
<td>SD</td>
<td>z-value</td>
<td>p-value*</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 30 yrs</td>
<td>93</td>
<td>1.16</td>
<td>1.07</td>
<td>-7.9</td>
<td>0.01</td>
</tr>
<tr>
<td>31-40 yrs</td>
<td>37</td>
<td>3.38</td>
<td>1.14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>65</td>
<td>2.08</td>
<td>1.59</td>
<td>-2.17</td>
<td>0.03</td>
</tr>
<tr>
<td>Female</td>
<td>65</td>
<td>1.51</td>
<td>1.30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Educational Status</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Middle School Level</td>
<td>84</td>
<td>2.02</td>
<td>1.57</td>
<td>-1.99</td>
<td>0.05</td>
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<tr>
<td>High School Level</td>
<td>46</td>
<td>1.39</td>
<td>1.20</td>
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</tr>
<tr>
<td>Living Place</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Congested Area</td>
<td>75</td>
<td>2.13</td>
<td>1.55</td>
<td>-3.49</td>
<td>0.01</td>
</tr>
<tr>
<td>Non Congested Area</td>
<td>55</td>
<td>1.33</td>
<td>1.25</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*P-value from Mann Whitney test

### Table 4. Comparison of average score of DMFT by level of knowledge, attitude and practice of oral health

<table>
<thead>
<tr>
<th>Variables</th>
<th>DMFT</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean</td>
<td>SD</td>
<td>z-value</td>
<td>p-value*</td>
</tr>
<tr>
<td>Knowledge</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Good (8-10 marks)</td>
<td>108</td>
<td>1.31</td>
<td>1.06</td>
<td>-7.22</td>
<td>0.01</td>
</tr>
<tr>
<td>Poor(1-7 marks)</td>
<td>22</td>
<td>4.14</td>
<td>0.89</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Attitude</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Good (31-50 marks)</td>
<td>69</td>
<td>0.97</td>
<td>0.71</td>
<td>-6.66</td>
<td>0.01</td>
</tr>
<tr>
<td>Poor (10-30 marks)</td>
<td>61</td>
<td>2.72</td>
<td>1.57</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Practice</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Good (7-10 marks)</td>
<td>51</td>
<td>0.78</td>
<td>0.58</td>
<td>-6.67</td>
<td>0.01</td>
</tr>
<tr>
<td>Poor (1-6 marks)</td>
<td>79</td>
<td>2.44</td>
<td>1.52</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*P-value from Mann Whitney test

### Table 5. Comparison of average score of DMFT by accessibility of oral health care services

<table>
<thead>
<tr>
<th>Variables</th>
<th>DMFT</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean</td>
<td>SD</td>
<td>z-value</td>
<td>p-value*</td>
</tr>
<tr>
<td>Convenient</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>82</td>
<td>1.74</td>
<td>1.35</td>
<td>-0.29</td>
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</tr>
<tr>
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<td>48</td>
<td>1.87</td>
<td>1.68</td>
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</table>

*P-value from Mann Whitney test
Table - 6. Association of dental caries status by level of knowledge, attitude and practice in oral health

<table>
<thead>
<tr>
<th>Variables</th>
<th>DMFT</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean</td>
</tr>
<tr>
<td>Knowledge</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Good (8-10 marks)</td>
<td>17(15.7%)</td>
<td>91(84.3%)</td>
</tr>
<tr>
<td>Poor (1-7 marks)</td>
<td>0(0.0%)</td>
<td>22(100.0%)</td>
</tr>
<tr>
<td>Attitude</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Good (31-50 marks)</td>
<td>13(18.8%)</td>
<td>56(81.2%)</td>
</tr>
<tr>
<td>Poor (10-30 marks)</td>
<td>4(6.6%)</td>
<td>57(93.4%)</td>
</tr>
<tr>
<td>Practice</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Good (7-10 marks)</td>
<td>14(27.5%)</td>
<td>37(72.5%)</td>
</tr>
<tr>
<td>Poor (1-6 marks)</td>
<td>3(3.8%)</td>
<td>76(96.2%)</td>
</tr>
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</table>

*p-value from chi-square test

**Discussion**

The aims of this study was to assess caries status and knowledge, attitude and practices in oral health including utilization of oral health care services, and their association among Myanmar migrant workers in Mae Sot district, Tak province, Thailand. This study was conducted by using face to face interviewed questionnaires and doing oral health examination.

Results showed that the proportion of Myanmar migrant workers with caries free was 13.1% while the remaining (86.9%) was affected by dental caries. Similarly, 113 Myanmar migrant workers were affected by dental caries with at least 1 cavity whereas 17 Myanmar migrant workers were free from dental caries. The percentage of dental caries status was a little bit higher than those in the percentage of (86.0%) the pilot study of dental caries status in relation to knowledge, attitudes and practices in oral health in Myanmar. The differences of the results of these two studies can be caused by three reasons.

- The pilot study of the dental caries status in Myanmar was conducted in 2003 and this study was carried out 10 years later. (16)
- The lifestyle and socio-economic status was changed within 10 years.

Regarding the associations of the dental caries status with age, it showed that there was significant association with age and dental caries status by chi-square test. According to the Mann Whitney test, it also showed that it was significant association with age and dental caries status. Prevalence of having at least 1 DMFT was lower in ≤ 30 years of age group than 31-40 years of age group with significant association between caries status and age group. It means that ≤ 30 years of age group was less caries than 31-40 years of age group. The mean age is 26.83± 5.77 and caries prevalence was 86.9%. There was evidence to show that in the study of dental caries experience, prevalence and severity in Mexicans adolescents and young adults (mean age = 18.20±1.65, caries prevalence=74.4%) which was significantly associated with age and dental caries status. (15)

According to the results of the knowledge, attitude and practice of Myanmar migrant workers in oral health, answered by migrant but this study was conducted in Maesot (Thailand-Myanmar border area.)
workers were quite good but some questions had low percentage in correct answers. For some particular questions like “When you suffered from toothache, you usually go to see dentist to relieve the symptoms”, “Every carious tooth needs to be extracted” and “Dental caries is a disease which cause only to children and young person”, only 46.2%, 57.7% and 57.7% respectively can give correct answers. This result showed that the lack of knowledge in certain aspect of dental caries regarding etiology and prevention. It was due to lack of dental health education in the community.

Attitude questions regarding to oral health, the questions like “The pain in the tooth will go forever after it is properly treated by a dentist” can answer that strongly agree and agree are only 3.8% and “Filled teeth by dentist cannot guarantee for future caries if no proper cleaning teeth” can answer that strongly agree and agree are 10.7. The attitude of the migrant workers in the community should be improved although almost all of Myanmar migrant workers can answer satisfactory in attitude questions.

For practice questions regarding to oral health, the questions like “Do you often rinse your mouth with antiseptic (medicated) mouthwash?” and “You change your toothbrush when it becomes too old at least every 3 months”, only 29.2% and 30.0% can answer correctly. But for the question which was “You go to see dentist every 6 months for cleaning teeth and for regular check up”, no one can answer correctly. It means that they didn’t go to see dentist every 6 months for check-up. It may be due to factors such as socio-economic status. Their practice should be improved and health promotion programs also need to be implemented.

For the utilization of oral health care facilities which is quite important thing among these groups of study population, most of the migrant workers can answer satisfactory. Almost all of the questions answered by migrant workers were quite good according to accessibility, affordability and availability. But regarding the convenience to reach to oral health care facilities, one third of the migrant workers answered “inconvenient”. They gave the reasons that they cannot go to the oral health care facilities because they didn’t have identity card. They stayed in Thailand as non-registered and polices will catch them. Regarding the comparison of mean between convenient in utilization of oral health care or dental visit and dental caries status, there was no significant difference. Mean DMFT of the convenient group was 1.74±1.35 and the inconvenient group was 1.87±1.68. According to the results of this study from mean DMFT, it was found that no one had filled teeth and missing teeth due to caries, only decayed teeth. It was also found that the oral health services in clinics nearby was scaling or cleaning the teeth or treatment of gingivitis and periodontitis.

Regarding the associations between level of knowledge, attitude and practice, there was association between knowledge, attitude and practice of oral health by chi-square test. By calculating with the Mann Whitney test, it also showed that there was significant difference of means scores between level of knowledge, attitude and practice of oral health. According to the score, 108 of them received good score in knowledge of oral health in which 17 of them were caries free and 91 of them were affected by dental caries. The rest 22 Myanmar migrant workers, who got low score in knowledge of oral health, all of them had at least 1 cavity. Regarding the attitude of oral health, 69 Myanmar migrant workers received more than 30 marks (good score) in attitude while 61 Myanmar migrant workers got less than 30 marks in it. Among 69 Myanmar migrant workers who got good score in attitude, 17 of them were free from caries while 91 were affected by dental caries. The rest 22 Myanmar migrant workers got less than 30 marks in it. Among 69 Myanmar migrant workers who got good score in attitude, 17 of them were free from caries while 91 were affected by dental caries. For the oral hygiene practice, 51 of them received good score in oral hygiene practice in which 14 of them were caries free and 37 of them were affected by dental caries. The rest 79 migrant workers, who got low score in oral hygiene practice, 3 of them were caries free but 76 of them had at least 1 cavity. In the pilot study of
dental caries status in relation to knowledge, attitudes and practices in oral health in Myanmar, there was also significant association between knowledge, attitude and practice of oral health.

To sum up, this study showed association with dental caries status and level of knowledge, attitude and practice. For the comparison means between convenient and dental caries status, there was no significance difference of means. Even though the results showed high proportion of good knowledge (81.3%), but the proportion of good practice was just only one third. This means that good knowledge could not lead to a good practice. Therefore, oral promotion program should be focused in the community.

On the other hand, the caries prevalence was 86.9% and it was quite high in the community. Therefore, the reduction of high caries prevalence rate can be achieved by a preventive and oral hygiene promotion program in order to improve oral health status of this population.

Barriers to assess the oral health care facilities

Most of the Myanmar migrant workers were unregistered and thus they felt they were insecure to going to use the oral health care facilities. From the study, it was found that most of them could not afford the registration fees and that led to delay in getting health insurance. They did not aware of their registration status and health insurance system because of lack of knowledge and lack of money to make the registration. Therefore, information and importance of registration and oral health insurance should be educated to Myanmar migrant workers.

This study was a cross sectional survey with purposive sampling which demonstrate an association not a causation. Besides, this study was conducted in only 2 areas of Mae Sot District, Tak Province, Thailand. Moreover, sample size was not big enough for referring this result to the whole study population in areas of Mae Sot with the limitation of time. Thus, the result of the study was not implying the general population of migrant workers as a whole.

Conclusion

Although knowledge in oral health was good among the majority of working age group of Myanmar migrant workers, the high prevalence of dental caries with low to moderate level of attitude and practice suggested that oral health promotion program along with oral health care services should be provided especially effective oral health education, regular dental check-up and simple treatments.

References:


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A Study of facial dimensions for the aesthetic replacement of maxillary anterior teeth

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²Retired Rector, University of Dental Medicine, Yangon, Myanmar

Abstract
An important element of prosthetic treatment for edentulous patient is the selection of appropriate sized anterior artificial teeth that will restore the natural harmony of dentofacial structure to achieve optimal aesthetics. The main objectives of this study were to determine the presence of proportional relationships among facial and anterior teeth measurements and to analyze the biometric ratios between facial and dental proportions in Myanmar population for anterior teeth selection. A total of 404 Myanmar subjects comprising male and female, age ranged from 18 to 25 years were studied. All facial measurements were made with face bow and digital caliper. Cast measurements were made with digital caliper having a precision of 0.01 mm. The mean values for facial dimensions were: Bizygomatic Width (BZW) = 140.24mm, Face Length (FL) = 187.75mm. The mean values for anterior teeth dimensions were: Incisor Width (IW) = 8.34mm, Anterior Six Teeth Width (ASW) = 53.88mm, Incisor Length (IL) = 10.14mm. From the results of this study the new biometric ratios between facial dimensions and teeth dimensions were: BZW:IW = 16.84, BZW:ASW = 2.6, FL:IL = 18.59. A positive significant correlation existed between facial and anterior teeth measurements (p=0.01). The regression formulae were: IW = 6.075 + (0.016 x BZW), ASW = 34.485 + (0.138 x BZW), IL = 7.133 + (0.016 x FL). The clinical significance of this study is the clinicians whose patients are of Myanmar origin who now have a biometric ratio and a formula method for determining the anterior teeth size for rehabilitating edentulous patients.

Introduction
One of the most difficult aspects during the selection of maxillary anterior teeth for a removable prosthesis is determining the appropriate size of maxillary anterior teeth. Many attempts have been made to establish methods of estimating the size of these anterior teeth, and improving the aesthetic outcome. Aesthetics is a primary consideration for most patients seeking prosthodontic treatment. One of the major hurdles in clinical prosthodontics has been the selection and replacement of maxillary anterior teeth in the absence of pre-extraction records. With respect to the appearance of face, maxillary central incisors are popularly considered to be the key teeth when treating edentulous patients (Mavroskoufis, 1980). Poor aesthetics is a common reason in failure of maxillary complete dentures.

The selection of maxillary anterior teeth for complete dentures has long posed a problem in clinical practice, and a controversy about the best method to employ still exists. Several methods are of questionable validity, and many dentures have an obviously artificial appearance. As stated by Boucher (1970), the maxillary anterior teeth must be in proportion with the size of the face and head to achieve good aesthetics. Sharry (1974), Boucher (1970) and Pound (1962) all condemned the practice of selecting teeth based on measurements on the master cast. All agreed that size selection should
be based on facial measurements and proportions.

In Myanmar, there are no studies linking facial proportions with the size of anterior maxillary teeth that could be used as guide for determining/defining the maxillary anterior teeth dimensions. Therefore, the purpose of the present study is to determine the presence of proportional relationships among facial and anterior teeth measurements and to analyze the biometric ratios between facial and dental proportions in Myanmar population for anterior teeth selection.

**Material and Methods**

18-25 years aged Myanmar subjects from Yangon and Mandalay were selected according to selection criteria. Sample size is 404.

**Inclusion Criteria**

1. Myanmar subjects aged ranging from 18 to 25 years
2. All subjects have well aligned natural maxillary anterior teeth

**Exclusion Criteria**

1. Apparent loss of tooth structure due to attrition, fracture, caries or restorations
2. Gingival or periodontal conditions or therapy that would undermine a healthy tissue to tooth relationship
3. Interdental spacing, crowding, rotation
4. Restored anterior teeth
5. History of Orthodontic treatment
6. Asymmetry of the face
7. Obvious problems that could disfigure or otherwise affect the face and dentition

All subjects were thoroughly explained about the study procedure and taken written consent to participate in this study. The study had been approved by the Ethics Committee of the University of Dental Medicine, Yangon.

**Natural tooth measurements**

Irreversible hydrocolloid impression of the maxillary jaw was made (Aroma Fine Plus, GC Co., Japan) and the cast was poured in hard stone (Fuji Rock, Type IV Die stone, GC Co., E.U.). A small quantity of the mixed impression material was applied on the labial surfaces of the anterior teeth before the bulk of the tray was inserted into the mouth. The impression was disinfected with 0.5 % NaOCl solution for 10 minutes and immediately poured in stone using a vibrator and kept until completely set. Standard proportions, mixing temperature and time were used for both the impression material and the cast material. All impression procedures were performed according to the standardized methods used in the department. Measurements were made directly on the casts using a digital caliper (0.01mm precision).

**Figure 1. Measurement on study cast by digital caliper**

Clinical crown width (IW) and height/length (IL) of the right maxillary central incisors were measured. Total width of anterior six teeth (ASW) was also measured on the cast using a transparent Mylar strip. The strip was placed over the labial surfaces of the maxillary anterior teeth from distal surfaces of left to right canine. Arc distance was marked with a 0.1 mm pointed tip permanent marker.

**Figure 2. Measurement of ASW by Mylar strip**
The strip was then kept on a flat white background, and the distance between the two marks was measured with a digital caliper.

**Facial measurements**

Facial measurements were taken with the subject seated in a dental chair, Frankfort plane parallel to the floor. Greatest bizygomatic width (BZW) of each subject was measured by using a face bow and millimeter ruler as suggested by Zarb et. al. (1990) and then accurate measurements were taken with the help of digital caliper.

![Figure 3. Measurement of Facial Dimension by face bow and digital caliper](image)

Facial length/height (FL) was also measured by digital caliper from the hairline to the lower edge of the mandible at rest. All the measurements were done for three times and average values were recorded. The recorded data was analyzed statistically.

**Results**

![Figure4. Percent distribution of male and female](image)

This figure shows the percent distribution of male and female subjects of this study. (Male = 74.50%, n = 301; Female = 25.50%, n = 103)

| Table 1. Facial dimensions and anterior teeth dimensions of study subjects |
|-------------------------------|-----------------|------------------|------------------|
| parameters | Male (mean±SD) | Female (mean±SD) | Total (mean±SD) |
| FL (mm) | 190.13±8.1 | 180.81±7.8 | 187.75±9.0 |
| IL(mm) | 10.26±0.6 | 9.8±0.7 | 10.14±0.7 |
| BZW (mm) | 141.73±5.9 | 135.89±5.3 | 140.24±6.3 |
| IW (mm) | 8.4±0.4 | 8.1±0.4 | 8.34±0.4 |
| ASW (mm) | 54.41±2.5 | 52.31±2.3 | 53.88±2.6 |

All four hundred and four Myanmar subjects’ mean values of facial dimensions and anterior teeth dimensions were shown in Table 1. It shows the parameters of this study, facial length (FL), incisor length (IL), bizygomatic width (BZW), incisor width (IW) and anterior six teeth width (ASW) in millimeter.

| Table 2. The biometric ratios between facial dimensions and teeth dimensions |
|-------------------------------|-----------------|------------------|------------------|
| Ratio | Mean±SD | 95%CI |
| BZW:IW | 16.84±1.0 | 16.7500 16.9488 |
| BZW:ASW | 2.60±0.1 | 2.5932 2.6211 |
| FL:IL | 18.59±1.4 | 18.4520 18.7386 |

Table 2 represents the analysis of biometric ratios between bizygomatic width and the width of right maxillary central incisor and the width of six maxillary anterior teeth, and also the biometric ratio between facial length and length of right maxillary central incisor.

| Table 3. Linear regression analysis (BZW vs IW) |
|-------------------------------|-----------------|------------------|------------------|
| B | Std. Error | t | P |
| Constant | 6.075 | 0.468 | 12.991 | 0.000 |
| BZW (mm) | 0.016 | 0.003 | 4.8538 | 0.000 |

IW=6.075 + (0.016 x BZW), Dependent variable: (IW) Incisor width (mm), Predictors: (Constant), (BZW) bizygomatic width (mm)

Linear regression analysis shows that bizygomatic width (BZW) is significant predictor of the incisor width (IW) (p< 0.001).
Figure 5. Linear regression analysis to evaluate the relationship between bizygomatic width (BZW) and incisor width (IW)

Scatter plot shows statistically significant positive correlation between incisor width (IW) and bizygomatic width (BZW) ($r=0.235, r^2=0.055, p<0.001$). According to linear regression, correlation is not very strong.

Table 4. Linear regression analysis (BZW vs ASW)

<table>
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<tr>
<th></th>
<th>B</th>
<th>Std. Error</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>34.485</td>
<td>2.731</td>
<td>12.628</td>
<td>0.000</td>
</tr>
<tr>
<td>BZW (mm)</td>
<td>0.138</td>
<td>0.019</td>
<td>7.111</td>
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</tr>
</tbody>
</table>

ASW = 34.485 + (0.138 x BZW), Indicates $p<0.001$, Dependent variable: (ASW) anterior six teeth width (mm), Predictors: (Constant), (BZW) bizygomatic width (mm)

In this study, it is likely that the anterior six teeth width increase by 0.138 mm for every 1mm increase in bizygomatic width (BZW).

Figure 6. Linear regression analysis to evaluate the relationship between bizygomatic width (BZW) and anterior six teeth width (ASW)

<table>
<thead>
<tr>
<th></th>
<th>B</th>
<th>Std. Error</th>
<th>T</th>
<th>P</th>
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<tbody>
<tr>
<td>Constant</td>
<td>7.1333</td>
<td>0.746</td>
<td>9.557</td>
<td>0.000</td>
</tr>
<tr>
<td>FL</td>
<td>0.016</td>
<td>0.004</td>
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</tbody>
</table>

IL=7.133 + (0.016 x FL), Indicates $p<0.001$, Dependent variable: (IL) incisor length (mm), Predictors: (Constant), (FL) face Length (mm)

Linear regression analysis shows that face length (FL) is significant predictor of the incisor length (IL) ($p<0.001$). In this study, it is likely that the incisor length increase by 0.016 mm for every 1mm increase in bizygomatic width (BZW).

Figure 7. Linear regression analysis to evaluate the relationship between face length (FL) and incisor length (IL)

Scatter plot shows the relationship between the values of face length (FL) and incisor length (IL) ($r=0.197, r^2=0.039, p<0.001$).

Discussion

Decision on the selection of appropriate artificial teeth has to be based on proper dimensions, ratios and proportions. Since the position of maxillary anterior teeth has the strongest influence on aesthetics and only few guidelines exist for their ratios and proportions, it is reasonable to correlate and define the biometric ratios, average proportions and formulae in
intact dentition of Myanmar population.

**Central Incisor dimensions**

The mean incisor width and length from this study is 8.34 mm and 10.14 mm, respectively. The mean values of this result are nearly the same with previous study (ThiriKyaw, 2008), which were 8.39mm for incisor width and 10.01mm for incisor length. Scandrett (1982) studied on Caucasians and found that the maxillary central incisor width for the right side to be 8.6 mm and for the left side to be 8.5mm. MacGregor (1989) also stated that the width of most natural maxillary central incisor is over 8.5 mm and any tooth less than 8 mm is rare. Therefore, Caucasian central incisor teeth were larger than that of Myanmar.

**Facial Dimensions**

Bizygomatic width for Caucasians was 136.5 mm as described by Scandrett (1982). Al Wazzan (1995) studied on the Saudi patients and found that the mean bizygomatic width was 128.38 mm. According to Farkas (2005), in North American Whites (NAW) young adult population, bizygomatic width is 133.5 mm and face length is 180 mm. Therefore, Myanmar people have larger facial dimensions than that of Caucasians.

**Biometric Ratios**

In the study of Lucas (2012) the measurements of mid-pupillary distance and bizygomatic width of the face were compared to the width of the maxillary central incisors and found that only the bizygomatic width technique showed values similar to the real width of the maxillary anterior teeth. Al-El-Sheikh (1998) studied on the relationship of the bizygomatic width, central incisor width and the width of the maxillary anterior teeth in Saudi population. The results showed that 1:16 ratio does not exist in Saudi population and the width of maxillary anterior teeth can be estimated through the bizygomatic width and the multiplying factor of 2.2 for both females and males. Praveen (2008) also studied on the relevance of Pound’s Formula to Indian Population. The study was conducted among 240 students in a dental school representing the different ethnic groups of India selected for anthropometric study. According to this study, Pound’s formula is not applicable to Indian population. The new formula derived from the measured values is Intercanine width = Bizygomatic / 2.4 and Width of the central incisor = Bizygomatic width / 14.6.

Oshagh (2009) studied on the relation between craniofacial dimensions and teeth size and stated that proportions of bizygomatic widths to upper centrals were 14.9 on right side and 14.7 on left side. According to the present study, the new biometric ratio derived from the measured values is Intercanine width = Bizygomatic width / 2.6 and Width of the central incisor = Bizygomatic width / 16.9. It might be due to the smaller teeth size and/or larger face size of Myanmar people than those of other populations.

**Relationship between facial and anterior teeth dimensions**

When the bizygomatic width was plotted against anterior six teeth width, a fairly strong correlation coefficient of 0.334 was exhibited. A weaker but highly correlation coefficient of 0.235 was observed when the bizygomatic width plotted against the central incisor width. A linear regression analysis was used to formulate a general equation to predict the central incisor width, central incisor length and anterior six teeth width measurement successfully. The resulted linear formulae can be used to estimate the central incisor width, central incisor length and anterior six teeth width from the face dimension. In this study, width and length of the anterior teeth might be estimated from the following regression equations: width of maxillary central incisor $IW = 6.075 + (0.016 \times \text{Bizygomatic width})$; anterior six teeth width $ASW = 34.485 + (0.138 \times \text{Bizygomatic width})$; length of maxillary central incisor $IL = 7.133 + (0.016 \times \text{Face Length})$. This method produced consistent and more
objective results when compared to visual assessment or visual matching which are more subjective and depend on personal experience and taste rather than actual metric principles. Moreover, the use of regression formulae may aid in the selection of artificial teeth for complete dentures, especially for relatively inexperienced clinicians.

**Conclusion**

From the results of this study, the following conclusions might be drawn:

1. The new biometric ratios are; 1:16.84 for maxillary central incisor width with bizygomatic width, 1:2.6 for maxillary anterior six teeth width with bizygomatic width and 1:18.59 for maxillary central incisor length and face length. These can be used as a guide for the selection of the sizes of artificial teeth in Myanmar population.

2. The following regression formulae may also be used as aids in the selection of the sizes of artificial anterior teeth for Myanmar edentulous patients. Width of maxillary central incisor IW = 6.075 + (0.016 x Bizygomatic width); Anterior six teeth width ASW = 34.485 + (0.138 x Bizygomatic width); Length of maxillary central incisor IL = 7.133 + (0.016 x Face Length).

The main benefits of this study are the clinicians whose patients are of Myanmar origin who now have a biometric ratio and a formula method of determining the anterior teeth size for rehabilitating edentulous patients.

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A study on association between age, gender and type of removable prosthodontic treatment needs in University of Dental Medicine, Yangon, Myanmar

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2Department of Prosthodontics, University of Dental Medicine, Yangon

Abstract
Prosthodontic treatment needs may vary depending on age and gender of the patients. It may also reflect the general treatment trend among the specific community. A retrospective observational study was performed to evaluate the association between age, gender and type of removable prosthodontic treatment need. Patient records were examined and data was recorded concerning age, gender and removable prosthodontic treatment prescribed at University of Dental Medicine, Yangon, Myanmar in 2012-2013. There was significant association between age and type of removable prosthodontic treatment need but gender had no significant association with the type of removable prosthodontic treatment need.

Introduction
Oral diseases such as dental caries, tooth loss and periodontal disease are universally prevalent in adults and considered a major public health problem1. Since progression of the most common dental diseases that ultimately lead to tooth loss is often time dependent, it appears that with advancing age the number of people suffering from tooth loss and the number of missing teeth increase. The loss of teeth could be a disturbing emotional experience for many people 2-4 and can lead to substantial impacts on quality of life5. Prosthodontic treatment needs may vary depending on age and gender of the patients. It may also reflect the general treatment trend among the specific community. Assessment of prosthodontic needs in a special population would aid in planning the oral health service programs especially in the institutional setting. To date, there is no report concerning age, sex and related prosthodontic treatment needs. This study was aimed to evaluate the association between age, gender and type of removable prosthodontic treatment needs at University of Dental Medicine, Yangon, Myanmar in 2012-2013.

Materials and methods
A retrospective cross-sectional observational study was performed. Data on removable prosthodontic treatment needs were taken from dental records from the Department of Prosthodontics, University of Dental Medicine, Yangon, Myanmar from January 2012 to December 2013. The age, gender and the type of removable prosthodontic treatment (complete dentures, removable partial dentures, obturator prosthesis). Data was then analyzed by chi-square test to explore the association between age, gender and type of prosthodontic treatment by using SPSS version 11.1.
Results

A total of 428 and 454 patients were treated in the Department of Prosthodontics in 2012 and 2013 respectively. The distribution of age and gender is demonstrated in figure 1. It is appreciable that the trend of age and gender distribution in 2012 and 2013 were closely similar. The 45-64 yr age group was the most common age group seeking removable prosthodontic treatment. The 25-44 and 45-64 yr age group showed that female seek removable prosthodontic treatment more than male and this trend was reverse in geriatric age groups (65-74, >75 yr).

The number of patients taking various types of prosthodontic treatment was shown in figure 2 comparing between male and female. Similar trend of case distribution for each treatment type can be noticed for 2012 and 2013. Upper & lower removable partial dentures were the most common type of treatment, followed by single upper or lower partial and complete dentures. There was no significant association between gender and type of removable prosthodontic treatment.

Furthermore, figure 3 demonstrates the number of cases for each type of removable prosthodontic treatment prescribed to each age group.
The data again demonstrated the similar trend of distribution for both 2012 and 2013. It can be seen that with advancing age, there was increased number of patients wearing complete dentures and upper & lower removable partial dentures. There was significant association between age and type of removable prosthodontic treatment needs (p<0.05, chi-square test). Interestingly many of the oldest age group (>75 yr) still retain some of their natural teeth since the data showed the need for removable partial dentures apart from complete dentures.

**Discussion**

This study was aimed to report the association between age, gender and removable prosthodontic treatment in Myanmar. Although data was collected from 2012 and 2013, the trend of patient number, age group and gender distribution, and the treatment needs were similar, reflecting the current situation, at least in Yangon which is an urban area. However it should be noticed that the data was taken only from the patient population who seek treatment in the University of Dental Medicine, Yangon only. It would be more interesting if such study can be carried out to include patients seeking prosthodontic treatments in private dental clinics in both urban and rural areas. A more extensive country-wise data collection is suggested to know the situation in the different areas of the country.

Referring to the national population census 2014 data(figure 4), it is interesting that although it belongs to relatively narrower portion of the population pyramid, the 45-64 yr age group was the most common age group seeking removable prosthodontic treatment (figure 1). This clearly indicates the chronic cumulative nature of oral diseases which tends
to cause the aged individual lose more teeth. However, in accordance with the sharp narrow trend in population pyramid, the number of geriatric patients (>65 yr) decreased.

In the present study, there was no significant association between gender and type of removable prosthodontic treatment need. It is rational from the fact that dental diseases affect both male and female to the same extent. Besides this finding, upper & lower removable partial dentures were the most common type of treatment, followed by single upper or lower partial dentures and complete dentures. This might reflect the oral health status and treatment trends in the population to some extent. It is speculated from this finding that people retain natural teeth or the dental care service is emphasizing to preserve the natural teeth rather than extracting them. Since the data was taken from the urban population, it is possible because people have more knowledge about advantage of retaining natural teeth and seek restorative dental treatments especially root canal treatment to avoid extractions. It would be more interesting if the present data can be compared with that obtained from rural areas.

In the present study, there was significant association between age and type of removable prosthodontic treatment needs; with advancing age, there was increased number of patients wearing upper & lower removable partial dentures and complete dentures. Although geriatric patients (>65 yr) have to wear complete dentures, many of them still retain some of their natural teeth as demonstrated in figure 3. It is suggested to emphasize the importance of the dental care of geriatric patients since the geriatric population has expanded much more than that of 30-40 years ago.

**Conclusion**

With increasing age, the removable prosthodontic treatment needs are increased and the oral health care services are suggested to emphasize to improve the removable prosthodontic service they are providing especially to older population.

**References**


Oral malignant melanoma: early recognition and treatment emphasized

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Abstract:
Oral malignant melanoma (OMM) accounts for 5% of all oral malignancies. Primary malignant melanoma is a rare aggressive neoplasm found in the oral cavity, estimated at between 0.2 and 8 percent of all melanomas and occurs approximately four times more frequently in the oral mucosa of the upper jaw, usually on the palate or alveolar gingivae. A case of malignant melanoma is being discussed in 67-year-old female, who was initially diagnosed with chronic periodontitis with melanotic gingiva. After biopsy, the results were suggestive of primary malignant melanoma of oral cavity. This report presents a case of oral malignant melanoma and highlights the need for early identification and treatment of this lesion.

Key words: malignant melanoma, maxilla, melanin pigment

Introduction
OMM is a potentially aggressive tumour of melanocytic origin.1 Melanomas account for 0.5% of all oral malignancies and only about 1% arise in the oral mucosa.2,3,4 It occurs between 30 and 90 years of age, with a higher incidence in the 6th decade and with a mean age of 56 years. It shows higher prevalence in yellows, blacks, Japanese, and Indians of Asia due to more frequent finding of melanin pigmentation in oral mucosa of these races.5 The most frequently affected oral sites are the palate and the maxillary gingiva.3 OMMs are asymptomatic at initial stages that delays the patient concern to approach the doctor and which further delays their detection and subsequent treatment. The delay detection may be the cause for the poor prognosis with a 5-year survival being between 15% and 38%.6 We emphasize the word caution when dealing with the pigmented lesions which may present as nodular or elevated growths with ill-defined borders as these pigmented lesions can be malignant melanoma which act as silent killers and maybe we are the one to save patient’s life.

Case report
A-67-year-old Burmese woman was referred to the oral and maxillofacial department of University of Dental medicine, Mandalay, Myanmar for consultation and management of pigmented oral mucosal lesion at right anterior gingival region of the maxilla and palate. Past dental history revealed that patient noticed that her teeth were mobile in the upper right quadrant since 5 months. So she applied indigenous traditional Myanmar medicine to the swelling every night for 2-3 days. She suffered from the appearance and disappearance of swelling repeatedly and then the swelling gradually increased in size & the colour changed to black in the labial gingival region as well as in the palate and anterior part of maxilla. She made a consultation with local doctor who after routine examination diagnosed her with chronic periodontitis with melanotic gingival and referred her to University of Dental medicine Mandalay, Myanmar for necessary treatment.
Figure 1. Intra-oral photograph showing intraoral extension of the lesion from right maxillary central incisor to distal of right maxillary second premolar and superiorly to depth of maxillary anterior vestibule.

General examination and past medical history were non-contributory. On extra oral examination, patient had facial asymmetry and swelling at right canine fossa region of mid face. There was loss of nasolabial fold and tenderness on palpation. Intraoral examination showed dark bluish to black, firm and nodular swelling of approximately 3.5 x 2.5 cm in right maxilla involving maxillary gingiva and hard palate. Lesion was extending from right maxillary central incisor to distal of right maxillary second premolar and superiorly to depth of maxillary anterior vestibule, and posteriorly nearly up to the midline of the palate. (Figure 1 and figure 2) On palpation, lesion was non tender and no bleeding was encountered, and first and second maxillary right premolars had grade II mobility and the canine had grade I mobility. Cervical lymph nodes were not palpable. Also hematological, urine examination did not reveal any significant findings. Based on the clinical findings provisional diagnosis of OMM was given.

Incisional biopsy was done from upper right premolar region and tissue obtained was sent for histopathological examination. Microscopic section revealed acanthotic stratified squamous epithelium with numerous atypical melanocytes in the basilar portion of the epithelium with invasion into the superficial lamina propria. Individual cells were round to spindle shaped containing brown to black melanin pigment, which was suggestive of malignant melanoma. (Figure 3) After seven days the lesion was removed with wide excision and partial maxillectomy from maxillary right central incisor to second premolar under general anaesthesia. (Figure 4)

Primary closure with gauze pack and palatal plate closure was done. Histopathological examination of the excised segment and tissue was consistent with the initial diagnosis of malignant melanoma.

Histopathological examination revealed the following features: microscopic section showed the marginal stratified squamous epithelium showed acanthosis and free of neoplastic polygonal cells. Sub epithelial fibromuscular stroma was bearing a versatile pigmented tumor and exhibited neoplastic polygonal to spindly cells with pleomorphic vesicular nuclei, prominent nucleoli and abundant eosinophilic granules. (Figure 3)

Figure 2. Intra-oral photograph showing posterior extension of the tumour nearly up to the midline of the palate.
Histopathological examination revealed the following features: microscopic section showed the marginal stratified squamous epithelium showed acanthosis and free of neoplastic polygonal cells. Sub epithelial fibromuscular stroma was bearing a versatile pigmented tumor and exhibited neoplastic polygonal to spindly cells with pleomorphic vesicular nuclei, prominent nucleoli and abundant eosinophilic cytoplasm, most containing brown-black pigments. (Figure 5) Neoplastic cells were also observed adjacent to groups of mucous salivary glands and on the surface of adipose tissue. The healing was uneventful and patient was on follow up for one year and later was lost to follow up.

**Discussion**

Oral melanoma is an extremely rare tumor arising from uncontrolled growth of melanocytes found in the basal layer of oral mucous membrane. The etiology of oral melanoma is unknown. Exposures to sunlight, chronic irritation from ill-fitting dentures and tobacco use, have been implicated as possible risk factors. However, there has been no evidence to support these theories. Current thought is that primary oral melanomas arise either from nevus, preexisting pigmented areas, or de novo (30% cases).

OMMs are common in males whereas females are more prone for cutaneous lesions, and in rare situations their oral lesions have less aggressive course than males. The present case was seen in a female. OMMs are more commonly seen in elderly age group and rarely before 20 years of age. This case also was observed in 67 year old female patient. One-third of the patients are asymptomatic at the time of diagnosis and bleeding is the most common presenting symptom of the patient. Melanotic pigmentation prior to the diagnosis of melanoma is found in one-third of the patients. More rarely, the tumour is immediately manifested in the nodular, infiltrative stage on apparently healthy mucosa.
In the present case also patient was asymptomatic and only complained of pigmentation and bleeding from the lesion. Tanaka et al., identified five types of OMM on the basis of the clinical appearance: pigmented nodular type, non-pigmented nodular type, pigmented macular type, pigmented mixed type and non-pigmented mixed type.16,17,18

Differential diagnosis of pigmented lesions occurring in the oral cavity includes oral melanotic macule, smoking-associated melanosis, melanosis associated with drugs like antimalarial drugs and minocycline, melanoplakia, pituitary-based Cushing’s syndrome, post inflammatory pigmentation, melanocanthoma, melanocytic nevi of the oral mucosa, blue nevi, nevi of Spitz, Addison’s disease, Peutz-Jeghers syndrome, amalgam tattoo, Kaposi’s sarcoma, physiologic pigmentation and pigmentation related to the use of heavy metals.19

All these conditions give rise to diagnostic dilemma and there are chances of wrong diagnosis. Therefore careful evaluation and proper consideration of differential diagnosis is a must. In the current case also local doctor diagnosed it as chronic periodontitis with melanotic gingiva. Green et al., described criteria for diagnosis of primary oral melanoma which includes demonstration of melanoma in the oral mucosa, presence of junctional activity, inability to demonstrate extra oral primary melanoma.6

Histological features showing atypical melanocytes, usually larger than the normal melanocytes and having varying degree of nuclear pleomorphism and hyperchromatism in the epithelial and connective tissue junction is suspicious for oral malignant melanoma.21 Usually, OMM can be diagnosed with confidence on H&E-stained sections. If pigment is completely absent immuno-histochemical stains are of significant help. Useful markers include S-100 protein, gp100 (HMB-45), Mart-1 (Melan-A).21

Surgery remains the most effective treatment for malignant melanoma and aggressive surgical control of local disease may result in prolonged disease free survival.22 The current guidelines for the surgical management of primary cutaneous melanoma recommend a diagnostic excisional biopsy of the lesion followed by a wide local excision where the diagnosis is proven. However, in the oral cavity, the size of the lesion or anatomical limitations, particularly the presence of teeth, may preclude the taking of an excisional biopsy.23,24 In this case we did incisional biopsy first followed by excision of the lesion with wide margin. The prognosis of OMM is poor with a five year survival rate of 0-55% of cases.24

To conclude, OMM is a rare, aggressive and invasive tumour. Clinically these tumours are asymptomatic which can be confusing to arrive at the final diagnosis and may lead to diagnostic dilemma. Thus the significance of early detection and prompt treatment for this entity should be stressed as it can save one’s life.

References


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