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Address manuscript to:
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Annals of Dentistry
University of Malaya
50603 Kuala Lumpur
Malaysia
Tel: 603-79674800
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SUBSCRIPTIONS

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MICROTOMES AND MICROTOME KNIVES – A REVIEW AND PROPOSED CLASSIFICATION


ABSTRACT

Microtome is a mechanical instrument used to cut biological specimens into very thin segments for microscopic examination. Biological specimens can be presented in many ways. But more often, these specimens are embedded in paraffin wax blocks and the commonest way of sectioning these specimens can be achieved by the microtome. The earliest form of the microtome enabled free hand sectioning of fresh or fixed material using a sharp razor. Modern microtomes are precision instruments designed to cut uniformly thin sections of a variety of materials for detailed microscopic examination. Central to the production of good sections is the microtome knife. Microtomy virtually begins and ends with a sharp, blemish-free cutting edge. The introduction of disposable blades has made easier the production of good quality, thin sections, but they are often unsatisfactory for sectioning harder tissues, especially bone. A sharp knife edge free from imperfections is essential for the production of good sections. Since many types of microtomes are commercially available in the market, choosing the right microtome is essential for producing the best result as required. A classification is proposed that unifies and organizes the various microtomes based on the mode of operation.

Key words: microtome, microtomy, knives, sharpening, biological specimens

INTRODUCTION

Microtome is a mechanical instrument used to cut biological specimens into very thin segments for microscopic examination (1). Micro = Small, Tome = Cut. Sectioning paraffin wax embedded tissue blocks is the commonest way of achieving this, but the tissue can also be presented in other ways for microtomy. The basic instrument used in microtomy is the microtome into which a cutting tool is clamped.

Cummings (1770), a manufacturing company, designed manual sectioning instruments made from wood, which were exclusively used in botany for cutting plants (2). Chevalier (1839) first introduced the term “microtome” in the scientific terminology. Rudolf Thoma, a Heidelberg pathologist in concert with Rudolf Jung, a precision engineer, designed the first microtome in series production called “Thoma microtome” enabling the beginning of a new era in histology (3).

Modern microtomes are precision instruments designed to cut uniformly thin sections of a variety of materials for detailed microscopic examination (4). With practice, sections which are quite thin and translucent could be produced (5). For light microscopy, where magnifications can reach up to 1,800x, the thickness of a section can vary between 1 and 10 microns (thin sections). For electron microscopy, where magnifications of about 10,000,000x is possible, the thickness of a section is usually of the order of 10 nanomicros (ultra-thin sections).

Parts of a microtome

Microtomes consist of three main parts:
• Base (microtome body)
• Knife attachment and knife
• Material or tissue holder

With most microtomes a section is cut by advancing the material holder towards the knife whilst the knife is held rigidly in place. The cutting action which can be either in a vertical or horizontal plane is coupled with the advance mechanism, so that the material holder is moved after each cut. The distance moved is pre-selected using a scale setting on the microtome body and usually extends between 0.5 and 50 microns on microtomes cutting thin sections and from less than...
60 nm to over 500 nm on machines cutting ultra-thin sections (6).

**Classification of microtomes (Proposed)**

There are different types of microtomes available, depending on the manufacturer’s specifications and based on the application required for sectioning of the samples. But the previous literature doesn’t cite any classification of microtomes, thereby making it difficult to select an appropriate microtome. So an attempt is made to classify these commercially available microtomes according to their mode of operation.

Microtomes can be classified as:
- Manual microtomes
- Semi-automatic microtomes
- Automatic microtomes

**Manual microtomes:**
- Rocking microtome
- Rotary microtome
- Sledge microtome
- Freezing microtome
- Vibrating microtome
- Ultra microtome
- Cryostat
- Sliding microtome
- Saw microtome
- Hand microtome

**Automatic microtomes:**
- Laser microtome
- Computer microtome
- Ultra-thin computer microtome

**Rocking Microtome**

This instrument is one of the oldest in design, relatively cheap, and is exclusively designed for sectioning paraffin blocks (Figure 1). The name of the microtome derives from the rocking action of the cross-arm and is simple to use. This microtome comprise of three moving parts, which is extremely reliable and requires minimum maintenance. The tissue moves through an arc as it advances towards the knife (the slightly biconcave Heifflor knife) which is held rigidly causing the sections to be cut in a curved plane (7).

However, the disadvantages are its tendency to move during cutting because of the lightness of the frame and very thin sections are difficult to obtain. The rocking microtome has largely been replaced by the more precise rotary microtome although it is re-appearing in portable cryostats.

**Rotary Microtome**

This microtome derives its name from the rotary action of the hand-wheel which actuates the cutting movements. Machines of this sort are general purpose microtomes for cutting semi-thin to thin sections for light microscopy (Figure 2). The operation is based upon the rotary action of a hand wheel activating the advancement of a block towards a rigidly held knife. The block moves up and down in a vertical plane in relation to the knife and therefore cuts flat sections. It has the advantage of being heavier and therefore more stable than the rocking type, and is ideal for cutting serial sections (8). Larger blocks of tissue may be cut on this machine, and the cutting angle of the knife (tilt of knife) is adjustable. Since a heavier and larger knife is used with this type of microtome, there is less likelihood of vibration when cutting.

Section thickness settings range from 0.5μm to 60μm on most machines. Sections of paraffin wax embedded tissues are normally cut within the range 3 to 5μm, while resin sections are attained between 0.5 to 1μm (9).

**Sledge microtome**

This microtome was originally designed for cutting sections of very large blocks of tissue (e.g. whole brain). The sledge microtome has become a popular machine for routine use since the World War
II (Figure 3). The block-holder is mounted on a steel carriage which slides backwards and forwards on guides against a fixed horizontal knife. This microtome is heavy and consequently very stable and not subjected to vibration. A large knife is used (24 cm in length) and the knife is usually wedge-shaped which reduces the possibility of vibration and requires less honing. The knife-holding clamps are adjustable and allow the tilt and the angle (slant) of the knife to the block to be adjusted with ease (10). Its action is much slower when compared to rocking or rotary microtome which is its major disadvantage.

**Sliding Microtome**

In a sliding microtome, the knife is moved horizontally against a fixed block which progresses against it in an inclined plane (Figure 4). The sliding microtome can be used for paraffin-wax embedded sections although it was designed for cutting celloidin-embedded sections (11).

**Freezing Microtome**

Although other microtomes can be modified for cutting frozen sections, this type of microtome was known to be efficient, producing the best results and was used almost universally. The machine is clamped to the edge of a bench and is connected to a cylinder of CO₂ by means of a specially strengthened flexible metal tube (Figure 5). The cutting action of the freezing microtome differs from those described previously as in this case the knife is moved whilst the tissue block remains static. The block moves by a pre-set amount, in microns, at the end of each cut. However, consistent, high quality, thin sections are very difficult to obtain with this type of microtome (10).

This device enables tissue to be frozen without the necessity of solid carbon dioxide or liquid nitrogen. This method has acquired popularity before the cryostat became widely used for frozen sections. In this instrument, two dissimilar metals are placed in apposition with one another and when a direct electric current passes through them, heat is generated on one surface and lost from the other. This phenomenon is known as ‘*Peltier* effect’ (12).

**Vibrating Microtome**

Originally thought to replace the hand microtome, the vibrating microtome was conceived as a microtome which could produce high quality sections of fresh, unfixed material from animal or botanical sources (Figure 6). This instrument has been designed to cut tissue which has not been fixed, processed or frozen and has the greatest application in enzyme histochemistry and ultrastructural histochemistry.

The name of the instrument was derived from the high speed vibration produced by a safety razor blade which provided the cutting power. The amplitude of vibration is adjusted by altering electrical voltage applied to the ‘knife’ (10). To prevent tearing, soft
material is cut whilst immersed in a fluid which also aids in dissipating heat produced at the vibrating edge of the razor during cutting.

**Ultra Microtome**

The ultra-microtome is used to prepare ultra-thin sections for light and electron microscopy (Figure 7). Very small samples of tissue or industrial product are usually embedded in hard resin before cutting. It has been reported that sections can be cut as thin as 10 nanometers (13). Two forms of advance mechanism have been developed in this style of microtome.

The thermal mechanism relies upon heat induced expansion in a bifurcated metal strip. Whereas in the mechanical form a microprocessor coupled to a precise stepping motor controls the advance mechanism (14). The cutting stroke is motor driven to provide a regular, smooth motion for sections of even thickness and constant reproducibility. Knives are usually made from glass, diamond or sapphire. The block is brought to the knife edge under microscopical control and as each section is cut it is floated on to a water bath adjacent to the knife edge (10).

**Saw Microtome**

Saw microtomes cut sections from very hard material such as undecalcified bone, glass or ceramics (Figure 8). The samples, commonly embedded in resins, are moved extremely slowly against a diamond coated saw rotating at approximately 600 rpm. It is possible to produce sections of 20 μm or greater, provided the saw blade is in perfect condition (15). The saw microtome is not capable for producing very thin sections (9).

**Hand Microtome**

The hand microtome is limited to sectioning intrinsically rigid botanical material and it is difficult to obtain thin sections from animal tissues (Figure 9) (10).

**Cryostat**

The introduction of fluorescent antibody staining techniques by Coons, Creech and Jones in 1941 led to a need for thin sections (3-5 μm) of fresh frozen tissue free of ice crystal defects (16). To satisfy these criteria the tissue must be snap frozen at a very low temperature.

Linderstrom-Lang and Mogensen designed the first cryostat in 1938. Coons and his colleagues redesigned it in 1951 (Figure 10) (17).

It consists of a microtome of any type but preferably rustproof, which is enclosed and operated within a deep freeze cabinet. The cabinet is fitted with a double glass window, and a door through which material may be passed in and out. The cabinet is equipped with a fluorescent light and a fan to ensure the circulation of cool air (15). The temperature may be regulated between -10ºC to -40ºC. Any cryostat can
be used as an alternative to a freezing microtome for rapid sectioning. Some microtomes have been designed for this purpose by incorporating an internal Freon quick freeze stage. This stage holds four block holders which after the tissue is frozen on them, are transferred to the microtome. Because the sections adhere firmly to the warm slides, the staining and mounting may be carried out more rapidly. The microtome may be adjusted to cut sections from 2-16μm (11).

**Laser Microtome**

Laser microtome is used for precise, non-contact sectioning (Figure 11) and was designed to slice samples with high precision. It's equipped with state-of-the-art femtosecond laser technology. It enables non-contact cutting inside biological tissues and various materials without causing thermal damage (18). Depending on the material being processed, slice thicknesses of about 5 to 100 μm are feasible (18).

Non-contact processing, sub micrometer precision, cutting of the tissue in its native state, no thermal damage, fewer artifacts and less time consumption in tissue preparation are the added advantages of this laser microtome (19).

**Computerized Microtome**

It is equipped with the advanced rapid thermostatic switch, semiconductor freezing, cryoscalped and cryoplate (Figure 12). The computerized microtome can carry out the rapid freezing section or routine paraffin section (dual-purpose). This microtome attains slice thickness in the range of 1-25μm with least slice adjusting graduation of 1μm and a maximal slice section of 32x32mm. The temperature of cryo scalpel and cryoplate range between 0°C ~ -18°C and -10°C ~ -40°C respectively (20).

**Microtome Knives**

Central to the production of good sections is the microtome knife. The first attempt to produce an adequate cutting surface was a sharpened razor blade; however these became blunt very quickly. In 1950 Latta and Hartmann discovered edges fine enough by using freshly cut glass (21).

Microtomy virtually begins and ends with a sharp, blemish-free cutting edge. The introduction of disposable blades has made easier the production of good quality, thin sections, but they are often unsatisfactory for sectioning harder tissues, especially bone. As these tissues constitute the greatest challenge to the microtomist, the necessity for maintaining a sharp knife has not been diminished. Microtome knives can be classified according to the material used for making the knife or based on the shape of the knife edge.

**Based on material of the knife:**

- Steel knives
- Non-corrosive knives for cryostat
- Disposable blades
- Tungsten carbide knives
- Glass knives
- Diamond knives
- Sapphire knives

**Based on shape of the knife edge (Profile):**

- Profile A: Strongly plano-concave/biconcave
- Profile B: Plano-concave
- Profile C: Wedge Shaped
- Profile D: Plane Shaped

**Steel Knives**

Steel microtome knives are manufactured from high quality carbon or tool grade steel which is heat treated to harden the edge (Figure 13) (22). The steel should be rust resistant, free from impurities and contain anti-corrosives.

The best knives are those that are fully hardened. Those which are only surface hardened lose the cutting edge very quickly once the hardened area is removed through repeated re-sharpening (10).
Non-Corrosive Knives For Cryostats
These are manufactured from hardened, heat treated stainless steel free from all impurities. They contain 12 to 15% chromium (10).

Disposable Blades
These are essentially refined, thickened razor blades (Figure 14). When these are held in a specially adapted knife holder the blades consistently produce high quality sections. These have replaced conventional microtome knives in many instances. All disposable blades are manufactured from high quality stainless steel. The edge of disposable blades can be coated with platinum or chromium to enhance strength and prolong cutting life (23-24).

Teflon coated blades are particularly suitable for use in cryostats as these offer reduced cutting resistance and minimal friction. The smaller, thinner disposable blade also reaches cryostat chamber temperature more rapidly than a conventional knife, **minimizing the delay** during blade exchanges or temperature adjustments. Disposable blades need to be held rigid in a special holder to prevent vibration during the cutting stroke (10).

Tungsten Carbide
Knives manufactured from high quality tungsten carbide (Figure 15) are practically nonmagnetic and 100 times harder than hardened tool steel (25). The knives have excellent resistance to wear but are brittle because of their extreme hardness and should be handled carefully. But, it has been reported that up to 30,000 serial sections of undecalcified bone embedded in methacrylate can be obtained per sharpening (26).

Glass Knives
The cutting edge of glass knives (‘Ralph knives’ with edges of 25 or 38 mm) used for conventional sectioning is parallel to one surface of the glass while knives used for ultramicrotomy is positioned against / across the thickness of the glass (Figure 16). Different profiles of ‘Ralph knife’ for cutting sections from different embedding media can be produced very quickly. Glass knife holders are available so that ‘Ralph knives’ can be used with a rotary microtome. Glass knives are hard but brittle and therefore require care when handling. These knives deteriorate with storage due to changes in the ‘flow’ or ‘strain’ of the glass after fracture and from oxidation impurities remaining in the hardened glass after manufacture. Knives should thus be prepared immediately before use (27).
The ralph knives can be classified according to the profile of the cutting edge.

- **High profile**: 1.0 to 1.5 cm cutting edge.
  - Suitable for cutting sections from soft embedding medium such as waxes.
- **Medium profile**: 0.5 to 1.0 cm high cutting edge.
  - Suitable for cutting sections from soft and hard plastics.
- **Low profile**: less than 0.5 cm high cutting edge.
  - Suitable for cutting sections from hard plastics.
- **Front profile**: The sloping edge artifact makes the knife unsuitable for use—this is caused by inadequately securing the glass strip at each end (10).

### Diamond Knives
Diamond knives are manufactured from gem quality diamonds without flaws. Although the diamond knives are very expensive, the knives are extremely durable, due to its hardness. These knives are used primarily for cutting very thin sections (28-29).

### Sapphire Knives
These knives are manufactured from one piece of solid sapphire artificially produced from an alumina monocystal under computer controlled thermal conditions (24). Sapphire is harder than tungsten carbide or glass which ensures high durability of the cutting edge for all types of material. The only restriction when using a sapphire knife is the block size as the knife edge is limited to 11 mm. A special knife holder is required for this kind of knife (10).

The knife edge can be classified according to its shape / profile.

#### PROFILE – A: Strongly plano concave/biconcave
One surface of the plano concave knife is straight whilst the other is hollow ground (Figure 17). The bi-concave knife has two hollow ground surfaces. Both knives are extremely sharp and are used for cutting soft, celloidin embedded material or foam compounds (30). These knives are not suitable for relatively hard materials, which cause the edge to vibrate and produce the phenomenon known as chattering. To obtain the best result the knife should always be oblique to the object when cutting sections (10).

#### PROFILE – B: Plano concave
This knife is similar to a profile – A knife but has a thicker back. It is used for cutting sections from material which is too hard to cut with a profile – A knife but can also be used for softer materials embedded in paraffin wax. This knife should be positioned obliquely to the material being sectioned. Plano-concave knives are available with varying degrees of concavity (28).²⁸

#### PROFILE – C: Wedge Shaped
The wedge shaped knife has more rigidity than profile – A or B knives and can therefore be used for cutting harder materials. Because of the extra thick nature of the wedge at the tip, this type of knife cannot be ground as sharp as profile – A or B knives. It is commonly used for cutting sections from paraffin wax embedded material, frozen sections, cryostat sections and for small, synthetic resin embedded material. With this style of knife the cutting plane is transverse to the object (15).

#### PROFILE – D: Plane Shaped/Tool edge shaped
This knife will cut hard and tough material as it has greater stability than any of the other profile knives. As only one bevel provides the cutting edge this knife is the least sharp of all of the knife profiles. It is commonly used for cutting synthetic resin blocks, hard materials embedded in paraffin wax and large wax blocks. The cutting edge of all steel knives is produced by grinding a bevel on each side of the knife for profiles – A, B and C, or onto the angled surface of a profile – D knife. The bevel faces enclose a sharper angle than the main surfaces of the knife (15).

### CONCLUSION
Many types of microtomes and knives are commercially available in the market and the selection of which microtome to be used will depend on the types of tissue sample and the requirement needed. Understanding the microtomes, its components and the cutting mechanism will be useful for selecting the suitable microtome required for cutting of the tissue samples and can minimize damage or distortion to the tissue sample.

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ABSTRACT

Background: In an academic setting due to financial constrain, it is not uncommon during non-surgical procedures dental students and clinical supervisors wash their gloved hands with disinfectants in between patients or when touching on non-contaminated objects. Whether this practice could cause any deterioration of the glove and expose clinicians and patients to infectious micro-organisms was a concern.

Aim: The aim of this study was to investigate the effect of multiple washes of gloved hands with a disinfectant on the integrity of the gloves.

Methods: Three brands of commonly used gloves in a dental school were tested for leaks after multiple washes with a disinfectant. Thirty pairs of each type of gloves were subjected to 0, 1, 5, 10, 20 and 30 washes with a disinfectant solution at a 5-minute interval between each wash. After each washing cycle, the gloves were filled with 1L of water and hanged for 2 minutes to observe any signs of water leaks.

Results: The results showed that the type of gloves and number of washes were significantly associated with the leakage rates (p<0.001). Washing of gloves for more than 5 times were at least 6 times higher to suffer from leakage (OR=6.23, 95% CI=2.14–18.08). Powdered gloves were almost 13 times higher to leak in all washes (OR=12.78, 95% CI= 4.40–37.14) and were almost 25 times more likely to leak when washed for more than 5 times (OR = 24.92, 95% CI = 5.79 – 107.21) when compared to the non-powdered gloves.

Conclusion: The practice of washing gloved hands with a disinfectant deteriorates the integrity of the gloves.

Key words: Cross infection, disinfectant, glove, leakage, micropores

INTRODUCTION

The use of examination gloves in clinical practice has risen dramatically in response to an increased need to protect health care workers and patients from the possibility of transmission of various infectious diseases through contact with body fluids and also against hazardous chemicals (1-6). Various materials have been used in manufacturing gloves such as natural rubber latex or synthetic materials like nitrile, polyvinyl chloride or polyurethane (7). Some chemicals that come in contact with the gloves may react with the glove material, causing damage to the glove surface, creating micropores which may lead to penetration of harmful bacteria or viruses to the skin, and subjected the operators or patients to contamination (1-6, 8).

It is a standard infection control policy that the clinical gloves should be disposed after use (9). However, in some teaching institutions due to costing factors, during non-bloody procedures, the students and staff would wash their gloved hands with a disinfectant, rather than dispose it every time when touching on non-contaminated surfaces such as pen, patient’s record, working bench and drawers. Thus, a single pair of gloved hands could have been washed several times during the non-surgical procedures. Although hand wash disinfectant is a relatively mild and non-corrosive agent, a study had detected that the ethanol component from the hand disinfectant permeated through latex, vinyl, nitrile and synthetic elastomer after 10 mins of exposure (2). The aim of this study was to evaluate the effect of multiple washes of gloved hands with a hand disinfectant on the integrity of 3 types of non-sterile latex gloves, and to determine the number of washes that result in a positive leak of gloves.

MATERIALS & METHODS

A modified water-leak test based on Food and Drug Administration (FDA) protocol was used to determine the leakage of non-sterile latex gloves after few washes with disinfectant (10).
testing protocol where the gloves are first inspected visually and then subjected to a water-leak test. Three brands of non-sterile latex examination gloves namely Vandaier Latex® (VL) (powdered, smooth), I-Vision Latex® (IV) (non-powdered, rough) and Cross Protection Latex® (CP) (non-powdered, rough) were tested. All gloves were manufactured in Malaysia. Before the experiment, the gloves were visually inspected and tested with air leaking test by inflating the gloves with air from the dental chair to a size of about a 5-cm diameter, and then felt for any air leakage. Any defective gloves were discarded. Five volunteers were asked to remove all rings and cut their fingernails short, polished and smoothened. All hands were washed and dried with a clean towel before wearing the gloves. The volunteer wore a pair of the gloves and washed their hands with 2ml of disinfectant solution (ESEMTAN® Wash Care, Schulke & Mayr Pte Ltd, Asia) and rinsed under running water in a standard manner. Thirty pairs of gloves were used in each washing cycle, i.e. 0, 1, 5, 10, 20, and 30 times, with a 5-min interval in each wash, resulting in a total of 180 pairs of gloves were used for each brand of gloves.

After the required number of washes, the gloves were removed inside-out and tested for water leakage. The gloves were filled carefully with 1L of water. After 2 minutes, water absorbent paper was used to wipe on the glove surfaces to detect any signs of water leaks. Data was analysed using SPSS version 12.0. Descriptive analysis was performed to provide general frequencies and percentage of gloves showing sign of water leakage. Gloves were categorized into non-powdered (IV and CP) and powdered (VL), while the number of washes was categorized into five times or less (≤5) and more than five times (>5). Chi square or Fisher exact test was used to compare leakage rates between the different type of gloves and the different number of washes. Odd ratios (OR) were used to assess the effect of both variables on the integrity of the gloves. Finally, stratified analysis was carried out to determine the association between the type of gloves and glove leakage percentage when the number of washes was controlled. Level of statistical significance was set at 0.05.

RESULTS

Following initial screening of the gloves with air-leaking test, the IV latex gloves showed no sign of air leaking, while 0.5% of VL and 3.0% of CP latex gloves were rejected due to presence of air-leaking. The finding from the water-leaking test after multiple washes was presented in Table 1. There were 6.7% of VL (n=24), 1.1% of CP (n=4) and none in IV group showed sign of water leakage through the gloves. Most water leakage happened after 20 washes (Table 1) and occurred on the thumb and index fingertips region of the gloves.

Table 2 shows the associations between types of gloves, number of washes and water leakage rates. In total, 24 leaks were observed in powdered gloves compared to 4 leaks in non-powdered gloves whose difference was statistically significant (p < 0.001) (Table 2-a). The odds ratio (OR) indicates the odds of having leaked gloves were almost 13 times greater for powdered gloves (OR=12.78, 95% CI=4.40–37.14). In other words, powdered gloves were almost 13 times more likely to leak when washed with a disinfectant compared to non-powdered gloves. Regardless of the types of gloves, leakage rates were also found to be significantly higher when the gloves were subjected to more than 5 washes with disinfectant (p <0.001) (Table 2-b). The OR of having leaked gloves were at least 6 times greater when the gloves were subjected to more than 5 washes (OR=6.23, 95% CI: 2.14 – 18.08). Between the 2 types of gloves, frequent washes for more than 5 times led to significantly higher leaks in powdered gloves compared to non-powdered gloves (p< 0.001) with the risk of leakage increased for almost 25 times more for powdered gloves (OR =24.92, 95% CI=5.79–107.27) (Table 2-b-i). However, no significant
Table 2. Associations between types of gloves, number of hand washes and glove leakage rates

<table>
<thead>
<tr>
<th>Variables</th>
<th>Glove Leakage</th>
<th>OR (95% CI)</th>
<th>p-value&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Types of Gloves</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Powdered (VL)</td>
<td>24 (6.7)</td>
<td>336 (93.3)</td>
<td>12.78 (4.40 – 37.14)</td>
</tr>
<tr>
<td>Non Powdered (IV,CP)</td>
<td>4 (0.6)</td>
<td>716 (99.4)</td>
<td></td>
</tr>
<tr>
<td>(b) Frequency of washes</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>More than 5 times</td>
<td>24 (4.4)</td>
<td>516 (95.6)</td>
<td>6.23 (2.14 – 18.08)</td>
</tr>
<tr>
<td>5 times and less</td>
<td>4 (0.7)</td>
<td>536 (99.3)</td>
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<tr>
<td>[i] More than 5 times</td>
<td></td>
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</tr>
<tr>
<td>Powdered (VL)</td>
<td>22 (12.2)</td>
<td>158 (87.8)</td>
<td>24.92 (5.79 - 107.27)</td>
</tr>
<tr>
<td>Non Powdered (IV,CP)</td>
<td>2 (0.6)</td>
<td>358 (99.4)</td>
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<tr>
<td>[ii] 5 times and less</td>
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<tr>
<td>Powdered (VL)</td>
<td>2 (1.1)</td>
<td>178 (98.9)</td>
<td>2.01 (0.28 – 14.39)</td>
</tr>
<tr>
<td>Non Powdered (IV,CP)</td>
<td>2 (0.6)</td>
<td>358 (99.4)</td>
<td></td>
</tr>
</tbody>
</table>

Level of significance was set at 0.05
<sup>a</sup> Chi square test was used
<sup>b</sup> Fisher exact test was used

The findings in this study shown that multiple washes of gloved hands with a disinfectant (ESEMTAN<sup>®</sup> Wash Care) affecting the integrity of the gloves, as shown in the water-leaking test in the VL and CP latex gloves. The disinfectant solution used in this study is a common disinfectant used in dentistry for hand washing. According to the manufacturer’s manual, it contains active agents such as sodium chloride, sodium hydroxide, citric acid, lactic acid, undeclenic acid, lauric acid and phenoxyethanol. The actual chemical agent in the disinfectant solution that causes damage on the surface of gloves was not identified. In dentistry, the gloves also often in contact with other chemical agents such as acrylate monomers (4), alcohol (2), cresophene and sodium hypochlorite (13), which were reported to have damaging effect causing permeability on the gloves.

The quality of glove appeared to be closely related to the manufacturer’s production quality, as this was evident when 0.5% and 3% of VL and CP gloves were rejected respectively before the experiment due to defect detected through the air test. In contrast, none was rejected when the 180 pairs of gloves were selected from the IV gloves for the experiment. In this study, the powdered smooth VL gloves suffered the highest rates of perforations (7%) after multiple washes compared to the non-powdered rough latex gloves (CP and IV). One possible explanation to this finding was the presence of the powder in the latex gloves might damage the integrity of the gloves during the washing actions.

It was noticed in this study that the thumb and finger index areas of the gloves were the most frequent leakage areas. This could be due to the standard hand wash procedure during the experiment, in which the physical rubbing movement in these areas might damage the integrity of the glove. It is also worthy to...
note that there are other procedures in clinical situations such as holding dental instruments, stretching of the gloves while retracting the mouth or coming in contact with dental materials that could have resulted in ‘greater stressed’ gloves, which may subsequently amplify the deleterious effect on the gloves when washed with disinfectant. The results of the study underline the need to evaluate infection control inclination among students and clinical supervisors in terms of glove usage. This finding indicates that washings of gloved hands with a disinfectant run a risk of damaging the protective barrier of the gloves, which subsequently exposes clinicians to chemical agents and infectious pathogens. Hence, the practice of ‘recycling’ of gloves by multiple washing of the gloved hand should be avoided. However, due to financial constraints, the ‘reuse’ of gloves was practiced in some developing countries, despite evidences against that practice (14). A study reported that the use of autoclaved reused gloves had a defect of 13% and a perforation rate as high as 82% (14).

Some clinicians, who succumb to washing their gloved hands, wear double or triple gloves, glove liners for better protection. However, this does not necessarily guarantee safety against cross infection, as reported in a systematic review that although the additional barriers significantly decreased the perforations rate to the innermost gloves, the evidence on the reduction of infection rates in patients was not clear (15).

In clinical practice, the cross infection protocol should always be strictly followed. Gloves should be removed before touching on non-contaminated surfaces such as pen, clinical models or records and then a new pair of gloves should be used for the subsequent procedures. However, if this ideal protocol could not be implemented, clinicians should at least consider changing to a new pair of gloves when a more contaminated procedure such as scaling or extraction is going to be carried out, rather than wearing the same gloves which had been washed multiple times. It is advised that changing gloves should be practiced when carried out long clinical procedures (16). In essence, the clinicians should be aware that they are not fully protected by wearing gloves especially after multiple washes of the gloved hands.

CONCLUSION

Within the limitations of the study, multiple washes of gloved hands with a disinfectant solution for more than 5 times lead to deterioration of the integrity of the gloves, which caused water leakage in the latex gloves in the powdered latex Vandier® and non-powdered latex I-Vision® groups.

ACKNOWLEDGEMENT

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Note: The authors do not have financial links to any of the glove or disinfectant companies.

REFERENCES


MALAYSIAN SENIOR DENTAL OFFICERS’ PERCEPTIONS OF THE EMPLOYABILITY OF MALE DENTAL NURSES AND POSSIBLE POLICY IMPLICATIONS


ABSTRACT

Background: In Malaysia, training to enter dental nursing profession is only open to women. Ironically, there are no such gender restrictions on training for any other health related professions in Malaysia. 

Aim: Therefore this study aims to assess the perceptions of Malaysian Senior Dental Officers (SDOs) towards the employability of male workers in the dental nursing profession and to compare findings from male and female SDOs. 

Methods: This cross sectional study was carried out on all SDOs in Ministry of Health, Malaysia, using a self-administered questionnaire. Descriptive statistics and a chi square test were used to address the study objectives. 

Results: Of the 112 participants, 78 SDOs returned the questionnaire, yielding a response rate of 70%. The majority of SDOs had positive perceptions of the employment of male dental nurses. It was indicated that gender is an important indicator for workforce development, and that the employment of both male and female dental nurses would enhance productivity. Almost 70% of SDOs perceived that the productivity of oral health service would be enhanced by having male and female dental nurses but 84.6% disagreed that male dental nurses would be more productive than female. Two thirds of SDOs disagreed that male dental nurses would increase satisfaction among male patients. About 64% of male SDOs disagreed that dental nursing profession is associated with female traits. There was no significant difference between perceptions by male and female SDOs for any statements. 

Conclusion: The majority of Malaysian SDOs have positive perceptions towards the employability of male dental nurses, and perceived dental nursing as a suitable profession for both genders. Training for the dental nursing profession should therefore be made available for men.

Key words: dentists’ perceptions, male dental nurses, policy implication, Malaysia

INTRODUCTION

There has been a rise in the number of female workers in many sectors, including medicine and dentistry (1-3). Globally, many issues have been debated in relation to the implications of gender imbalance in the healthcare workforce such as discrepancies between male and female workers in their working hours, productivity, choice of practice location and salary schemes (3-5). Many countries have therefore made extra efforts to promote gender mainstreaming and equality in the labour force (5,6). Various strategies have been carried out by planners to improve gender employability in the health care sector by encouraging gender equality in career opportunities and development, creating support groups for career planning at school and university level and through promotional activity in the media (7).

Dental auxiliaries worldwide are predominantly female (6,8). Approximately 96.5% of European dental auxiliaries are female (6). Traditionally, women are believed to be more caring and compassionate and this supposedly makes jobs such as dental auxiliary more suited to them (6,7). Indeed, it has been argued that men and women have different traits that make them suitable only for particular types of job (6). To date, the dental nursing profession in the public service in Malaysia has been restricted to women (9). Ironically, this gender restriction is only applicable to dental nursing training. There are no such gender restrictions on applications to enter other health allied science training such as for dental technologists, dental surgery assistants, medical assistants and medical nurses (9).

Gender imbalance also exists in the Malaysian medical workforce where the number of females are more than the male workers (10,11). However, recently there have been positive developments in the medical
Malaysian Senior Dental Officers’ perceptions of the employability of male dental nurses and possible policy implications

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qualifications in Dental Public Health. Part B consisted of questions on the respondent’s demographic profile including their gender, age, working experience and postgraduate qualifications in Dental Public Health. Part B consisted of two parts. Part A consisted of questions for the questionnaire. The questionnaire was developed on the basis of a literature review and an expert group discussion involving two dentists and two public health specialists to decide on the items for the questionnaire. The questionnaire consisted of two parts. Part A consisted of questions on the respondent’s demographic profile including their gender, age, working experience and postgraduate qualifications in Dental Public Health. Part B consisted of seven close-ended questions (Table 1) with a five point Likert-scale ranging from strongly disagree (scale 1) to strongly agree (scale 5). The questionnaire was face-validated by three dental public health experts; two from the University of London and one from the University of Malaya. They were asked to provide feedback independently on the overall content and structure of the questionnaire. The feedback received from the experts only resulted in minor structural adjustments. The questionnaire was then piloted on seven SDOs who were working in one of the states of Malaysia. They were invited to give comments on the clarity and relevance of each question in the questionnaire. The response obtained was mostly positive and no additional changes were needed to the questionnaire.

Ethical approval was obtained from the Research Ethics Committee of the University of London (QMREC0449) and permission to conduct this study on Malaysian dentists was granted by the Senior Director of the Malaysian Oral Health Division. After the permission had been obtained, another letter was sent seeking a list of names and basic information of all the registered SDOs in the Ministry of Health, Malaysia. The State Deputy Director of Health (Dental Division) for all of the 15 states of Malaysia assisted in distributing the questionnaire to all SDOs, and the questionnaire was kept anonymous.

The questionnaires, worded in English, were posted to each SDO together with a research information sheet, a consent form and a self addressed postage paid envelope. Participants were asked to respond within two weeks of receiving the questionnaire, and a follow-up mailing was conducted one month after the date of questionnaire distribution.

Data processing and statistical analysis

Data were analysed using SPSS software version 12. Descriptive statistics and chi square test were used to address the study objectives. Results for each question were recoded into three groups: scores of 1 to 3 were classified as ‘disagree’, and scores of 4 and 5 were classified as ‘agree’. Scores of 3 (neutral) were recoded as ‘disagree’ because they were considered uncertain responses for the purposes of clear comparison with those who were in agreement. Chi square test was used to measure the association between male and female SDOs’ perceptions of the employability of men in the dental nursing profession in Malaysia. Significance level was set at p < 0.05.

RESULTS

Of the 112 participants, 78 SDOs completed and returned the questionnaire, giving a response rate of 70%. Two-thirds (68%) of the respondents were females. Their age ranged from 27 to 57 years old, with a mean age of 44.9. One-third of the participants (36%) were...
qualified with a Masters in Dental Public Health (DPH) and the rest had a dental degree as the highest level of education. More than half (64.1%) of the respondents had less than five years’ working experiences.

Overall, most of the SDOs had positive perceptions towards the employability of male workers in dental nursing (Table 1) where almost 65% of them agreed that the ‘introduction of male dental nurses into the health service should be encouraged’. Independently, 60% of male and 66% female SDOs agreed with this statement. Two-thirds of SDOs (70.5%) disagreed that ‘male patients would be more satisfied if they were treated by male dental nurses’ and a similar proportion was observed in both genders. More than half of SDOs (59.7%) believed that ‘the total number of male and female dental nurses is an important indicator in future workforce development’, with no obvious difference between male and female DDOs.

Although two-thirds of SDOs (69.2%) perceived that ‘employing both female and male dental nurses could enhance service productivity’, the majority of them (84.6%) disagreed that ‘male dental nurses can be more productive than female dental nurses’. There was slightly higher disagreement from female SDOs (86.8%) compared to male SDOs (80.0%) about this statement. Nearly half of male (48.0%) and half of female (50.9%) SDOs agreed that ‘the dental nursing profession is more suitable for females’. Slightly more than half of SDOs (55.1%) disagreed that ‘female dental nurses can deliver better quality care because they are more caring and compassionate in nature than male dental nurses’. There was a higher proportion of disagreement from male SDOs (64.0%) on this statement. Similar patterns of perception were observed between male and female SDOs for all of the seven statements in the questionnaire. No statistically significant differences were found between the perceptions of SDOs of either gender for any statements (Table 1).

**DISCUSSION**

This is the first study that has attempted to obtain an insight into the views of dentists on gender issues in the Malaysian dental nursing profession. The key finding from this study suggests that SDOs have positive views towards the employability of male dental nurses in Malaysia. There was no obvious difference between the perceptions of male and female SDOs for any statement on the questionnaire. Similarly, only a few studies have reported a significant difference in the perceptions of female and male respondents in relation to gender issues in medical nursing (4,13). This pattern may be explained by a shift towards equal gender opportunities in the health care workforce. Looking at medical counterparts, there is a growing number of male medical nurses being employed in Malaysia (10). The International Council of Nurses in 2008 showed that the number of registered

<table>
<thead>
<tr>
<th>Questionnaire Items</th>
<th>Disagree N (%)</th>
<th>Agree N (%)</th>
<th>p valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male (n=25)a</td>
<td>Female (n=53)a</td>
<td>Total (n=78)</td>
</tr>
<tr>
<td>Q1. I believe that the introduction of male dental nurses rather than female dental nurses alone in the health system should be encouraged.</td>
<td>10 (40.0%)</td>
<td>18 (34.0%)</td>
<td>28 (35.9%)</td>
</tr>
<tr>
<td>Q2. I believe that male patients would be more satisfied if they were treated by male dental nurses.</td>
<td>18 (72.0%)</td>
<td>37 (69.8%)</td>
<td>55 (70.5%)</td>
</tr>
<tr>
<td>Q3. I believe that the total numbers of male dental nurses and female dental nurses are important indicators in future workforce development*</td>
<td>10 (40.0%)</td>
<td>21 (39.6%)</td>
<td>31 (40.3%)</td>
</tr>
<tr>
<td>Q4. I believe that male dental nurses can be more productive than female dental nurses.</td>
<td>20 (80.0%)</td>
<td>46 (86.6%)</td>
<td>66 (84.6%)</td>
</tr>
<tr>
<td>Q5. I believe that the productivity of the oral health services will be enhanced by employing both female and male dental nurses.</td>
<td>7 (28.0%)</td>
<td>17 (32.1%)</td>
<td>24 (30.8%)</td>
</tr>
<tr>
<td>Q6. I feel that dental nursing is more appropriate for females because they tend to be more caring and compassionate by their inborn nature.</td>
<td>13 (52.0%)</td>
<td>26 (49.1%)</td>
<td>39 (50.0%)</td>
</tr>
<tr>
<td>Q7. I believe that female dental nurses can deliver better quality care because they are more caring and compassionate in nature than male dental nurses.</td>
<td>16 (64.0%)</td>
<td>27 (50.9%)</td>
<td>43 (55.1%)</td>
</tr>
</tbody>
</table>

* Chi-square test

Sample do not equal to N=78 due to missing data

Table 1. Comparison between perceptions of male and female Senior Dental Officers (SDOs) towards the employability of male in dental nursing profession in Malaysia (N=78)
male nurses in Malaysia had increased steadily since their first employment in 2006 (10).

There are diverse views regarding the impact of men in medical nursing. A substantial literature reported that allowing male workers into the medical nursing profession can help to balance the staff, increase the mix of skills in the team and create a better working environment (13-15,21). The employment of both male and female dental nurses might also assist in the appropriate allocation of dental workload and placements. For example, research has shown that male nurses are more suitable to work in areas that need physical strength such as the Intensive Care Units (ICU), the operating room and the emergency department (13). This pattern in the medical setting might be mirrored in dentistry where male dental nurses could work in hospital-based dental setting that involves being on-call or work in dental operating rooms. In addition, some dental tasks in school services and mobile dental squads which require physical strength such as the heavy lifting of portable dental chairs and compressors could also be delegated to male dental nurses. Another study has found that male health care providers are more likely to work in rural areas and engage in community service (4). This suggests that one of the potential contributions of men in the dental nursing profession in Malaysia might include extensive involvement in community service especially in rural areas.

Although two-thirds of the SDOs (69.2%) believed that the productivity of oral health services could be enhanced by employing both female and male dental nurses, this does not stipulate that male dental nurses would be more productive than their female counter parts. In the Ministry of Health, Malaysia, staff are required to work full time and dental tasks are usually distributed equally among workers based on their respective qualifications. Also, apart from maternity leave entitlement, it is very seldom that female staffs are away from their jobs for a long period of time. This show that there is possible similar productivity between male and female staff working in the ministry. However, in countries where there is flexibility in working practices, female workers have been shown to work fewer hours or part time and they also to tend to take early retirement (5,6,22). In this scenario, there are possibilities that male workers will have higher productivity as they spend more days at work compared to female.

A study on patients attending a dental school in Northern California concluded that there was no gender preference when it came to choosing oral health care providers (23). This is in line with the results from the present study which shows that the majority of the SDOs disagreed that ‘male patients would be more satisfied if they were treated by male dental nurses’. However the findings of this study contradict those from another local study which found that patients tended to choose health professional of the same gender as themselves (17). It is to be noted that the latter study assessed the perceptions of health care workers who worked in hospitals where most patients were adults. Dental nurses in Malaysia are only allowed to treat children below the age of seventeen, and this child population might not experience any difficulties about receiving care from someone of a different gender than themselves. This could explain the reasons why the SDOs in this study felt that no gender preferences existed.

Previous research has shown that the nursing profession in general is traditionally believed to be suitable for women because they are more caring and compassionate in nature (6,19). The findings from this study have shown that a higher proportion of male SDOs (64.0%) compared to female SDOs (50.9%) disagree that the dental nursing profession is associated with female traits. However, the difference was not statistically significant. This may suggest that respondents felt that the dental nursing profession in Malaysia is suitable for both genders and that there is a possible penetration of men in this profession in the future. It was recommended that equal opportunities be given to both genders when pursuing their career of choice (6). Looking at some other aspects of medical and dental nursing, the literature reported that recruiting men into the profession is a challenge. The potential barriers that have been stated include the image of the profession, low wages, career pathway restrictions and disapproval by peers and family members (4,6). Therefore, further research is needed before a change to the profession is made.

The results of this study should be interpreted in light of the following limitations. First, there is the possibility of respondents’ bias in any self-reporting survey. Second, participants (n=7) involved in the pilot study were included in the main study and it could be argued that contamination may have occurred during piloting. However, in this study, no changes were required following the piloting, and the questionnaires were distributed to different states separately, so it is unlikely that any contamination occurred. Despite these limitations, this study has good external validity and the results represent national data which might be generalizable to oral health planners working in Ministry of Health, Malaysia.

Possible policy implications and future research recommendation

The results of this study demonstrated that oral health planners have positive attitudes towards the potential employment of male dental nurses. In policy terms, this suggests that the opportunity should be taken to introduce male dental nurses in Malaysia. Ensuring equal gender opportunities in Malaysia falls within the remit of the Public Services Commission, which should therefore work closely with the Ministry of Health to abolish the gender restrictions currently placed on the training of dental nurses, so as to bring
the dental nursing profession into line with other health-related professions. Discussion is needed of a policy framework to establish career pathways for both male and female dental nurses in order to maximise gender diversity within the profession. This in turn will improve the career prospects and development of non-operating auxiliaries, such as dental surgery assistants, by opening up to them the additional possibility of an alternative career in dental nursing. However, further study is needed to confirm the finding of present study. Future research should also explore the employability of men in the dental nursing profession in relation to the following issues; barriers to the recruitment of male dental nurses in Malaysia, factors influencing male students to choose dental nursing as a career, the social acceptability of male dental nurses among different stakeholders in Malaysia and to compare findings with other countries.

CONCLUSION

The majority of SDOs have positive perceptions of the employability of male dental nurses. There was no statistically significant difference between perceptions by male and female SDOs for any of the questionnaire items. Dental nursing in Malaysia is perceived to be a suitable profession for both women and men. Hence, policy changes to open the recruitment of Malaysian dental nurses to both genders should be considered.

ACKNOWLEDGEMENT

We would like to thank all Senior Dental Officers in Malaysian Ministry of Health who had participated in this study and the Senior Director of the Malaysian Oral Health and the State Deputy Director of Health (Dental Division) for their valued administrative support during data collection period.

REFERENCES


DETERMINANTS OF DNA YIELD AND QUALITY FROM DIFFERENT NON-INVASIVE SAMPLING METHODS


ABSTRACT

Aim: The purpose of this study was to determine the DNA yield and quality from different non-invasive sampling methods and to identify the method which gave the highest DNA yield. Method: Thirty-eight volunteers had been recruited in this study where blood, buccal cells and saliva were collected using various collection techniques. Buccal cells were collected by 1) cytobrush and 2) saline mouth rinsing or “swish”. Meanwhile saliva was collected by passive drooling method. Upon processing the white blood cell (WBC), buccal cells and saliva samples, DNA extraction was performed according to the manufacturer’s protocol. Quantification and quality (DNA ratio at A260/A280) of the extracted DNA were determined using NanoDropND-1000®. T-test was performed to compare means between DNA obtained from various collection methods.

Results: DNA yields from buccal cells collected with cytobrush, “swish”, saliva and WBC (mean ± SD) were (8.2 ± 5.9)ng/μl, (28.2 ± 14.9)ng/μl, (5.9 ± 9.5)ng/μl and (105.3 ± 75.0)ng/μl respectively. Meanwhile the mean DNA ratio at A260/A280 for cytobrush, “swish”, saliva and WBC were 2.3, 2.0, 1.7 and 1.8 respectively. Post hoc test with Bonferroni correction suggested that DNA yield from “swish” technique exhibited the least mean different as compared to the DNA extracted from WBC (p<0.05). There was no significant difference in the mean quality of the DNA extracted from WBC, saliva and buccal cells collected in these non-invasive methods (p=0.323). Conclusion: The “swish” technique of obtaining buccal cells yielded the highest amount of DNA and was of the quality of DNA extracted from blood sample.

Key words: buccal cells, non-invasive methods, cytobrush, “swish”, saliva, DNA yield, DNA quality

INTRODUCTION

Buccal cells and saliva are fast becoming an attractive alternatives source of genomic DNA to large scale epidemiological genotyping projects than the more traditional and more invasive phlebotomy-derived DNA from whole blood sample because it is cost-effective and yields sufficient quantity and quality DNA for polymerase-chain-reaction (PCR) -based biomarker assays (1-5). In addition, due to the less invasive, simple, and sometimes easy-to-self-administered methods of collecting the buccal cells, there is a better participation rate from the subjects (6). Methods for collecting the buccal cells, among others, include the use of cytobrush and mouthwash. Saliva has also emerged as a potentially good source of genomic DNA as it contains a vast number of epithelial cells (7,8). While all the aforementioned studies have described successful PCR applications using DNA extracted from buccal cells, none has, thus far, compared all collection methods in the same individual. Hence, it is the objective of this study to compare the yield, and quality, of genomic DNA derived from saliva and buccal cells collected via cytobrush and mouthwash from the same individual.

MATERIALS AND METHODS

Participants
Thirty eight in-house staffs and colleagues from the Faculty of Dentistry, University of Malaya, were recruited for this randomized study during the period of 11th to 22nd June 2007.

Sample Collection
Participants were advised to refrain from brushing their teeth and food/fluid (except water) ingestion, as well as gum-chewing, for at least 1 hour before the sample collection. They were then required to rinse their mouth vigorously with tap water for 5 times before sampling.
Saliva collection method: passive drool
Saliva was collected from participants by accumulating the saliva at the base of their mouth and tilting their head downwards to allow the secreted saliva to be drawn off from the lower lip. Sterile funnel was attached to the opening of a 15ml Falcon’s tube and gently the saliva was spitted into the tube until 4ml of saliva was obtained. Samples were then centrifuged for 20 seconds at 2000rpm in room temperature and 1ml of the supernatant was transferred as aliquots before storing at -20°C.

Buccal cells collection using cytobrush (BCC)
Participants were instructed to rub their cheeks against the teeth for 30 times before a Cytobrush plus Medscan® was used to brush against the inner cheeks. The brush was then twirled upward and downward with counter pressure for 10 times and left to air dry on the table in the open area for 15 minutes. After that, each of the brush was put inside a 15ml Falcon’s tube and stored immediately at -80ºC without any processing. Before DNA extraction, the brush handle was cut off with a wire cutter to ~20mm from the bristle and put inside another 15ml Falcon’s tube.

Buccal cells collection using mouthwash (BCS)
Participants were instructed to rub their cheeks against the teeth for 30 times before normal saline mouthrinse was used. Each participant was instructed to swish 10ml of the mouth rinse vigorously left and right while rubbing their cheek against the teeth again for 30 seconds. With a sterile funnel, the mouth rinse was expectorated into a 15ml Falcon’s tube and stored immediately at -80ºC without any processing. After DNA extraction, the brush handle was cut off with a wire cutter to ~20mm from the bristle and put inside another 15ml Falcon’s tube.

Blood samples collection
3ml of blood from the volunteers were drawn into two blood tubes (plain and EDTA) and the tubes were then carried and transported to the lab for processing on ice packs keeping the blood chilled at 4ºC. In the lab, the blood tubes were centrifuged at 1500rpm for 15 minutes at room temperature. Serum and plasma were aliquoted 300μl each into 0.5ml microcentrifuge vials before storage while the white blood cells (WBC) layer was scrapped from the EDTA tube. The WBC was the layer between red blood cells and serum. All serum, plasma and WBC were stored at -80ºC.

DNA extraction and Quantification
DNA of all samples was extracted using QIAamp Blood Mini Kits (Qiagen Inc., Valencia, CA) according to the manufacturers’ protocol with some modifications.

Blood, saliva and mouthwash
4ml of PBS was added to the sample and centrifuged to get the pellet. The pellet was then lysed with 20μl of protease and incubated at 56ºC for 10 minutes. 200μl of ethanol (100%) was added to the pellet and then loaded onto a spin column. Before the final centrifugation, incubation at room temperature was extended to 15 minutes to increase DNA yield. DNA was then absorbed, by short centrifugation, onto the QIAamp silica membrane, washed and eluted with 100μl of Buffer AE (supplied together in the QIAamp Blood Mini Kit).

Cytobrush
DNA extraction from cytobrush was done by incubating with protease at 56ºC and the period was extended to 30 minutes to make sure that cells, attached to the brush, were lysed fully. The final elution volume obtained was 150μl.

Concentration measurements
DNA quantification was done using NanoDropND-100 spectrophotometer (NanoDrop Technologies, USA). Readings were taken at wavelengths 260 and 280nm. The ratio of the readings at 260 and 280nm (OD260/OD280) provided an estimate of the purity of the DNA. A good quality of DNA would have an OD260/OD280 ratio between 1.8 to 2.0.

Statistical Analysis
Data analysis was done using Statistical Package for Social Sciences (SPSS) version 12.0. To compare means of DNA yields and quality between different collection techniques (WBC, saliva, buccal cells via “swish” and cytobrush), t-test was employed and p<0.05 was considered statistical significant. Post hoc test was done using paired t-test with Bonferroni correction was performed to determine which means from these various collection methods differ from each other.

RESULTS
The mean (± SD) DNA yield from cytobrush, “swish”, saliva and WBC was 8.2 (± 5.9)ng/μl, 28.2 (± 14.9) ng/μl, 5.9 (± 9.5)ng/μl and 105.3 (± 75.0)ng/μl respectively.

The mean differences in DNA yield were 77.0ng/μl, 97.1ng/μl, and 99.4ng/μl for WBC versus “swish”, WBC versus cytobrush and WBC versus saliva respectively. Meanwhile the mean differences in DNA yield for “swish” versus cytobrush were 20.0ng/μl, “swish” versus saliva 22.4ng/μl and cytobrush versus saliva 2.4ng/μl. DNA yields between WBC and “swish” techniques exhibited the least mean difference (p=0.000) after post hoc test with Bonferroni correction (Table 1).
The mean ratio (± SD) of the DNA quality were 1.8 (± 0.1), 2.0 (± 0.3), 2.3 (± 3.1) and 1.7 (± 0.9) collected from WBC, “swish”, cytobrush and saliva respectively (Table 2). However, repeated measure showed no significant differences in DNA quality between different collection techniques (p = 0.323). Therefore, no comparison was made between different collection techniques.

**DISCUSSION**

Direct comparison among studies are difficult, however, Lum and Le Marchand (9) found an average of 49.7μg of DNA collected from 64 individual of diverse ethnic origins using the “swish” technique. Abouta et al. (1) and Garcia-Closas et al. (7) also found a higher mean amount of DNA collected from the “swish” technique, (15.8μg and 56.7μg, respectively) than the amount of DNA collected by using cytobrush (11.95μg and 2.4μg, respectively). Though lower than the aforementioned studies, current study indicated “swish” technique was able to yield a higher amount of DNA with 28.2 (± 14.9)ng/μl (which equivalent to approximately 1.64μg). These findings were in concordance with studies conducted by Heath et al. (10) and Garcia-Closas et al. (7) that collection of buccal cells using mouthwash gave greater DNA yields and quality than cytobrush. The higher DNA yield from “swish” technique could possibly include the presence of non-human DNA such as from oral bacteria or food remnants (11) as compared to buccal cells collection through cytobrush only brush from a single site of the inner cheek.

The way in which the buccal cells collected through cytobrush procedure may have shown to affect DNA yield. Cells recovered from improper brushing of the inner cheek could likely be superficial ones in the process of apoptosis. About 30% of buccal cells collected from persons with healthy, non-inflammatory oral mucosa showed apoptotic signs (12). Hence, DNA from certain individuals may be prone to lower quality and yield.

Previous studies had shown high DNA yield obtained from buccal cells using the “swish” technique (1,7,9). The difference in DNA yield in this study using the “swish” technique compared to previous works carried out by others could be due to the type of mouth rinse used between these studies. The Scope mouthwash which had been used by Abouta et al. (1) and Garcia-Closas et al. (7) was reported to be the most suitable, commercially available mouthwash for obtaining DNA for clinical and research applications (10). The current study however used normal saline mouthrinse (0.9% NaCl) as the collecting medium. The normal saline was used because of its minimum chemical composition of the solution and also the mild and gentle taste as mouth rinse. Most of the commercial mouth rinses such as Scope, Listerine, Colgate and Oral-B tasted palatably pungent contained substantial chemicals components (with the presence or absence of alcohol) which may influence the stability of DNA quality and the downstream analysis using the DNA. Study done by Heath et al. (10) has proven that DNA was found to be stable in normal saline at room temperature for up to 4 days. As evidenced in another study in which the subjects used sterile water as the collecting medium, the mean amount of DNA extracted was 4μg/μl (13). Besides, in terms of cost, same amount of normal saline is much cheaper and cost effective than commercial mouth rinses. Because DNA could be stable in normal saline and it’s cost effective, normal saline used as mouth rinse was deemed suitable in this study.

The average amount of DNA obtained from saliva in this study was much lower than those reported by a number of other studies. Rylander-Rudqvist and colleagues (14) reported an average DNA yield of 135.9μg from 90 samples while Hansen et al. (6) recorded an average of 10.8μg in their study. Dissimilarity in each individual’s oral flora, dietary/lifestyle habits or because of other reasons such as whether rinsing protocol was performed prior to saliva
collection and whether actual saliva and not sputum/phlegm was obtained, all of which could probably explain the differences among these studies. Individuals who smoked often experienced xerostomia which could lead to dry mouth and reduced salivary flow. Storage conditions could also affect the reported lower range in the current study (16, 17).

Nevertheless, the quality of the DNA extracted from these non-invasive methods used in this study corresponded to those studies which had also demonstrated the feasibility of using the DNA in PCR-based applications (1,17).

**CONCLUSION**

The results showed that “swish” technique employed in this study yielded higher amount of genomic DNA from buccal cells and could be further optimized in the future for PCR-based applications. This non-invasive approach and easily collected source of cellular material as in comparison to blood is definitely more cost effective in a large cohort molecular epidemiological study.

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ABSTRACT

Introduction: Diagnosis and management of orofacial pain of non-odontogenic origin has always been a challenge to dentists. Inaccurate diagnosis would result in delay of treatment and in cases of orofacial pain, affects patient’s quality of life. Temporomandibular pain dysfunction syndrome is the most common temporomandibular disorder that presents to dental clinics. Trigeminal neuralgia, also known as tic douloureux is a relatively rare condition that causes electric shock-like pain when the trigger zone is stimulated by triggering factor. Case report: A case of temporomandibular pain dysfunction syndrome in a 52 years old Indian lady that was managed as trigeminal neuralgia for 7 years is presented. Conclusion: The aim of this case report is to make dentists aware of the signs and symptoms of different orofacial pain, so that early and accurate diagnosis can be made and appropriate treatment instituted.

Key words: orofacial pain, temporomandibular pain dysfunction syndrome, trigeminal neuralgia

INTRODUCTION

Chronic orofacial pain can be a frustration to both the clinician and patient. Dentists are often challenged with the task of diagnosing odontogenic-like pain originating from the various anatomical structures on facial region. They have to aware that oral cavity is not the source of all orofacial pain but only as the site of pain (1). It is important for dentists to be aware of the signs and symptoms of different orofacial pain so that unnecessary invasive investigation and treatment can be prevented.

The lack of diagnostic laboratory tests in majority of cases of orofacial pain has further results in delay in achieving accurate diagnosis. In many situations, the key of differentiating source versus site of orofacial pain depends heavily on thorough history taking, comprehensive clinical examination and sound knowledge of clinical epidemiology (2-3). Failure in doing so can lead to misdiagnosis, unnecessary, inappropriate and irreversible treatment.

The purpose of this case report is to remind dentists of the signs and symptoms of temporomandibular pain dysfunction syndrome (TMJPDSD) and trigeminal neuralgia so that accurate diagnosis can be made and appropriate treatment instituted without delay.
thought it was the cause of the pain. The remaining upper posterior teeth were caries free; none of the teeth were tender to percussion. There were no signs of attrition on the teeth; neither were signs of clenching on the buccal mucosa. Three sets of muscles of mastication, i.e. the temporalis, lateral pterygoid and medial pterygoid on the left side were tender to palpation. The score of pain at verbal rating scale was 9 out of 10.

Dental panoramic tomography was taken on the same day to exclude causes of orofacial pain of hard tissue origin. No abnormality was detected. Both condyles appeared normal.

A diagnosis of TMJPDS was made based on the clinical examination and history. Other non-odontogenic orofacial pain that might occur in that area of face, namely atypical facial pain, migraine and sinusitis were taken into consideration. Patient management including reassurance in which she was explained of the possible etiologies of the condition was mentioned. She was told that her teeth are not supposed to be in contact except when eating, swallowing and speaking. She was advised not to clench her teeth whenever she is aware of it. In addition, she was also advised to stop taking all the medication that was given earlier except tramadol if in pain. Patient was asked to have hot towel massage on the pain area for 15 minutes, 3 times a day and avoid hard food and wide mouth opening.

On one week review, patient appeared more cheerful and upon questioning, she had not taken any medications that were given earlier, only hot towel massage was used as advised. The score of pain on verbal rating scale has dropped to 4. On examination, only the lateral pterygoid muscle was tender to palpation but it was not as tender as before. She was advised to continue with hot towel massage and tramadol was prescribed to be taken when necessary.

On one month review, pain had further decreased to 2 on verbal rating scale, but the lateral pterygoid muscle was still tender to palpation. However, patient had not taken any medication to control the pain since the first visit. The face now appeared symmetrical. The same advice was given as in the last visit.

On two months review, patient claimed that now pain only occurs when chewing hard food. She only does hot towel massage when there is pain. All muscles of mastication were not tender to palpation and she had normal width of mouth opening. Patient was advised to carry on with hot towel massage when necessary.

Six months after the patient’s first visit, she was asymptomatic and none of the muscles of mastication were tender to palpation.

Patient’s complaint and response to treatment are summarized in Table 1.

**DISCUSSION**

Differential diagnosis of orofacial pain can be divided into odontogenic and non-odontogenic origins. Odontogenic origin can be broadly divided into pulpitis, periodontitis and cracked tooth syndrome; whereas non-odontogenic origin could originate from a wide variety of disorders, including but not limited to tumours, infection and abnormalities of anatomical structure on the facial region, such as: salivary glands, sinuses, ears, temporomandibular joint, nose, eyes, and vascular. In addition, pain on orofacial region could be psychogenic in origin or referred from other parts of the body such as cardiac or intracranial structure.

Temporomandibular joint is a hinge and gliding joint, consisting of condylar head of the mandible and glenoid fossa of the skull. This joint is unique due to the presence of a fibrocartilage articular disc interposed between (4). In addition, the articulation between the the upper and lower teeth and the related muscle of mastication further contributes to its complexity.

TMJPDS, together with internal joint derangement and degenerative joint disease are conditions that grouped under the umbrella of temporomandibular disorder. Patient’s common complaint of limitation of mouth opening, joint clicking and pain on the preauricular region are universal to these temporo-mandibular disorders. It was Costen in 1934 who first described the nature of TMJPDS (5). Although much research has been done, there is still much to be understood regarding the exact etiology and treatment of temporo-mandibular disorder (6).

TMJPDS have a multifactorial etiology, including local mechanical factors, psychological and co-morbidities (7). Prolonged muscle contraction such as in parafunction habit and muscle hyperactivity plays an important role (2). It is seen more common in

<table>
<thead>
<tr>
<th>Time</th>
<th>Pain on verbal rating score</th>
<th>Muscle that are tender to palpation</th>
<th>Width of mouth opening</th>
</tr>
</thead>
<tbody>
<tr>
<td>First visit</td>
<td>9</td>
<td>temporalis, medial pterygoid, lateral pterygoid</td>
<td>normal</td>
</tr>
<tr>
<td>One week review</td>
<td>4</td>
<td>lateral pterygoid</td>
<td>normal</td>
</tr>
<tr>
<td>One month review</td>
<td>2</td>
<td>lateral pterygoid</td>
<td>normal</td>
</tr>
<tr>
<td>Two months review</td>
<td>1</td>
<td>none</td>
<td>normal</td>
</tr>
<tr>
<td>Six months review</td>
<td>0</td>
<td>none</td>
<td>normal</td>
</tr>
</tbody>
</table>

Table 1. Comparison of patient’s pain and muscle tenderness on first visit and after treatment
females than males. The pain of TMJPDS may be worse in the morning, in the afternoon, during period of stress or in cold weather. It can be uni or bilateral and can spread to the temporal area or the body of mandible (4). Patient commonly complaint of frequent headaches with history of facial trauma, teeth grinding, sleep problems, pain elsewhere in the body and high levels of psychological distress (7). The most common complains of patients with TMJPDS are pain in the preauricular region, limitation mouth opening and noises, either clicking or crepitus from the joint when in function (4,6). However, absence of one of these symptoms does not exclude the diagnosis (6). Patient presented in this case although did not presented with the cardinal triad symptoms of TMJPDS, the sign of tenderness of three of the muscle of mastication on palpation excludes it from other orofacial pain.

Treatment for TMJPDS is targeted at breaking the cycle of muscle hyperactivity, pain, more muscle hyperactivity and so forth (4). The treatment options includes, but not limited to: explanation and reassurance (make the patient aware that muscle tension contributes to symptoms), hot towel massage, transcutaneous electrical nerve stimulation (TENS), bite plate appliance/splint, medication such as muscle relaxant, analgesic and anti-depressants.

Hyperactivity of temporalis or masseter muscle is easily relieved with heat massage but lateral pterygoid is more difficult to assess, hence more difficult to relieve (4). This is demonstrated in our present case whereby after one week of hot towel massage, temporals and maseter muscle were no longer tender to palpation but lateral pterygoids took 2 months to be pain free. Patient’s facial asymmetry is probably due to hypertrophy of muscle of mastication which turned normal when the muscle hyperactivity cycle is stopped.

However, one must keep in mind that regardless of the type of treatment provided; about 80% of patients with TMJPDS get better suggesting the possibility of placebo effect of treatment. However, at least 20% of the patients do not get better or may be non-responsive to treatment due to psychosocial factors (8). Hence, non-surgical management should be the primary approach for the management of myofacial pain (9). From author’s experience, treatment of patients with TMJPDS varies from one to the other. Treatment that works well for one patient might not work for the other with similar complaint and clinical presentation. Hence, several treatment options may need to be explored in finding out which is the best for the patient.

Gabapentin, which was prescribed to the patient from the neurological department, is used either alone or in combination with other medications to prevent and control seizures and to control the pain in cases of post-herpetic neuralgia. It may also be used to treat other nerve pain conditions such as diabetic neuropathy, and peripheral neuropathy. In cases of orofacial pain, it is used for treatment of trigeminal neuralgia if carbamazepine is ineffective. Therefore it is possible that the previous physician was treating the patient for trigeminal neuralgia.

Trigeminal neuralgia has the characteristic features of electric-shock like pain, intermittent temporal pattern, unilateral and lasts from a few seconds to two minutes, within the distribution of trigeminal nerve with maxillary branch affected most commonly. It has a trigger point, and only occurs when the stimulating factor stimulates the trigger point. The stimulating factor can be any routine daily activity, such as washing face, opening mouth, brushing teeth or eating. The pain is excruciating, and frequently described by patients as stabbing or throbbing in nature. However, it does not disturb sleep and patient is pain free between episodes. The pain that was suffered by our patient did not have any of the characteristic features mentioned except throbbing pain. However, the pain lasts for hours which are uncommon with trigeminal neuralgia.

Difference in the quality of pain described for trigeminal neuralgia (throbbing and excruciating) and musculoskeletal pain (mild to moderate aching pain) should assist the clinician in making the correct diagnosis (3). In some cases of trigeminal neuralgia, the pain does not fit exactly into the criteria mentioned above. This is called atypical or mixed trigeminal neuralgia with presence of persistent ache between paroxysms or mild sensory loss (10). The etiology of most cases of trigeminal neuralgia is idiopathic although some are associated with tumour and vascular compression (11). In young patients with symptoms of trigeminal neuralgia, multiple sclerosis needs to be excluded. Table 2 compares the signs and symptoms of the differential diagnosis of the present case.

Medication is often the first line of treatment with trigeminal neuralgia. Although trigeminal neuralgia is not life threatening, the pain and its effect on life is distressing.

Carbamazepine is the treatment of choice for trigeminal neuralgia. If pain relief is incomplete, a second drug such as phenytoin can be added. Failure of medical therapy should prompt the clinician to review the diagnosis (12). Surgical option is considered if pain control cannot be achieved with drugs. However, even with surgery, pain may not be permanently eliminated and recurrence is unpredictable (10).

CONCLUSION

Dentists should be competent to differentiate the signs and symptoms of different orofacial pain so that an accurate diagnosis is established as early as possible and appropriate treatment or referral is instituted to prevent unnecessary investigation and treatment, and patient’s quality of life is not affected. Clinicians should rule out all possible diagnosis before deciding on treatment options as many other conditions can also present as pain in the orofacial region.
Table 2. Comparison of signs and symptoms of non-odontogenic orofacial pain

<table>
<thead>
<tr>
<th>Feature</th>
<th>Trigeminal neuralgia</th>
<th>TMJPDS</th>
<th>Atypical facial pain</th>
<th>Migraine</th>
<th>Sinusitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>female</td>
<td>female</td>
<td>female</td>
<td>female</td>
<td>male and female</td>
</tr>
<tr>
<td>Age (years)</td>
<td>more than 40 years</td>
<td>20-30</td>
<td>middle age</td>
<td>adolescence</td>
<td>any age</td>
</tr>
<tr>
<td>Pain Type</td>
<td>lancinating, stabbing, burning</td>
<td>aching (mild to moderate)</td>
<td>throbbing, deep, diffuse, boring(^{13})</td>
<td>throbbing</td>
<td>aching, pressure across the midface</td>
</tr>
<tr>
<td>Severity</td>
<td>worst pain in the world</td>
<td>worst in the morning or evening</td>
<td>debilitating (moderate to severe)</td>
<td>dull, irritating</td>
<td></td>
</tr>
<tr>
<td>Location</td>
<td>division of trigeminal nerve, unilateral</td>
<td>preauricular, uni or bilateral</td>
<td>deep non-muscular areas of face, uni or bilateral, does not follow nerve distribution(^{13})</td>
<td>mostly unilateral</td>
<td>Uni or bilateral; upper posterior teeth and/or maxilla</td>
</tr>
<tr>
<td>Duration of episode</td>
<td>few seconds to 2 minutes</td>
<td>last for few minutes</td>
<td>Continuous; can have pain free periods</td>
<td>4 to 72 hours function</td>
<td>on palpation or</td>
</tr>
<tr>
<td>Provoking and associated factors</td>
<td>trigger factors (daily activities, for example talking, shaving or eating) at trigger zone</td>
<td>no specific triggering factors; associated with limitation of mouth opening, anxiety, parafunction(^{13})</td>
<td>no specific triggering factors; associated with anxiety, depression, other bodily pain(^{13})</td>
<td>frequently on arising in the morning with preceding visual, sensory, motor or speech disturbance; associated with photophobia, phonophobia, osmophobia and nausea; seek dark, quiet room</td>
<td>worst with head movement; history of recent fever and blocked nose; associated teeth are tender to percussion; usually more than one tooth is affected</td>
</tr>
</tbody>
</table>

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Early Childhood Caries, Dietary Habits and Nutritional Status of Toddlers: An Intervention Study in Kelantan

Ruhaya Hasan
Department of Community Dentistry

The aim of this study was to assess the effectiveness of a health promotion intervention program (TIPTOP package) in preventing early childhood caries (ECC), to improve nutritional status and dietary habits among toddlers. The study design was an intervention prospective study and conducted in Tumpat and Pasir Mas districts in Kelantan. The intervention group was 2-3 year old toddlers in Tumpat and received the TIPTOP package, while the control group was in Pasir Mas and received the normal toddlers’ oral health program under the MOH. Baseline information on a proxy population of 527 preschool children (5-6 years old) showed that almost every child in both districts was affected by caries (97.9%) and three-quarters have more than 7 teeth affected by caries. The majority of preschool children had malnutrition; 36.1% who were underweight (WAZ), 40.1% were stunted (HAZ) and one-third (31.1%) were underweight (BMI-for-age). Majority of nutrients intake were not adequate as compared to RNI (2005). Added sugar consumption was three times higher (205.1%) than the recommendation. Dietary habits of sugary foods and drinks showed that “milo” consumption was the highest scored (84.6) followed by “cokelat” (sweets) (75.3) and ice-cream (67.5). More than one-half of parents scored in the “moderate” category of knowledge, attitude and practices. At the intervention phase, 519 of toddlers aged 2-3 years old from Tumpat and Pasir Mas were screened for their caries status and only 15.8% (n=82) were caries free. There was only one drop out and the final sample consists of 40 toddlers from Tumpat and 41 from Pasir Mas who participated until the end of study period. After 18 months, majority of toddlers in intervention group had low or no caries (77.5%), the percentage of caries-free was 37.5%, the odds ratio of having ECC was lower (OR=0.805). About three-quarters (72.5%) had normal of WAZ, more than three-quarters (80%) had normal of HAZ and majority (87.5%) had normal of BMI-for-age. Added sugar intake was reduced, most of sugary foods and drinks consumption improved in intervention than control group. There were improvements in energy, protein, calcium, iron, zinc and vitamin C intake and was a large improvement of fruits and cariostatic foods consumption in intervention group than control group. The majority of mothers’ knowledge scores was high (82.5%), 65% scored high attitude and almost all (95%) had high practices scores. In the control group, only 58.5% had low or no caries, one-third (39%) were normal of WAZ and (34.1%) normal of HAZ and more than one-third (39%) normal of BMI-for-age. Only protein, iron, zinc, vitamin C and vitamin A intake were improved. Added sugar intake increased about one-third. Only one-third (39%) with high knowledge, nearly one-half (43.9%) with high attitude and more than one-half (58.5%) with high practices score at the end of the study period. We conclude that the combination of nutrition and oral health promotion intervention in the TIPTOP program meant to prevent ECC, could have a positive influence in producing good general health outcomes. We recommend that the current Ministry of Health toddlers’ health program should therefore adopt the effective elements of the TIPTOP health promotion package.
Microbial Assessment of Dental Unit Waterline System (DUWS)

Chua Chong Sing
Department of Oral Biology

Introduction: Dental Unit Waterline System (DUWS) is an interconnected water network found inside a dental chair unit (DCU) that allows water to pass through and deliver to the patient’s mouth during treatment. Many previous studies demonstrated the DUWS water was often heavily contaminated and might pose a risk of infection to the patients as well as dental personnel.

Objective: To determine the sanitary level of output water from DCUs and assess the effectiveness of silver-coated tubing used in DCU in reducing microbial counts in DUWS water.

Methods: Water from sources which include the air-water syringe, low speed handpiece, high speed handpiece and distilled water (control) were sampled from 13 DCUs. The temperature and pH of each sample were measured and the microbial counts of the total aerobic bacteria, total coliform, faecal coliform, Escherichia coli, faecal streptococci and Pseudomonas aeruginosa were determined using conventional microbiological procedures. Based on PCR products, the 16S rDNA gene sequence of bacteria isolated from the water samples were determined and used for identification. An in vitro model simulating the tubing of a DCU was setup in the laboratory using silver-coated and conventional polyurethane tubes. A microbial suspension comprising of similar bacteria earlier identified in the DUWS outgoing water was passed through the tubing in cycles of stagnation and flushing to mimic the routine operation of a DUWS. The effectiveness of the tubing in preventing biofilm formation was compared and assessed by the counts of adhering bacteria and observed under scanning electron microscope.

Results: The average pH of the outgoing water was slightly acidic at pH 5.4-5.5 at an average temperature of 23°C. The outgoing water was found free of pathogenic contaminant but highly loaded with four types of bacteria identified as Sphingomonas rhizogenes (17.9%), Sphingomonas dokdonesis (79.5%), Sphingomonas mucosissima (1.1%) and Methylobacterium radiotolerans (1.5%). The interior surface of both polyurethane and silver-coated tubes showed extensive biofilm formation and the outgoing water was heavy with bacterial counts. No significant difference in biofilm formation and bacterial contamination in the outgoing water were found in both types of tubing (P>0.05).

Conclusion: The microbial load in the outgoing water from DUWS in the clinic under study was high and failed to meet the recommendation by American Dental Association. Silver-coated tubing was not effective in preventing biofilm formation nor reducing microbial load in DUWS water when compared with polyurethane tubings.

The Effect of Glass Fibre Post Diameter on the Fracture Resistance of Endodontically Treated Teeth

Dr Tey Kuan Chuan
Department of Conservative Dentistry

Objectives: To determine the effect of glass fibre-reinforced composite (FRC) posts of different diameters on the failure load of endodontically treated teeth with different remaining dentine and reinforcing resin composite (RRC) thicknesses and the mode of failure in each group.

Methods: Fifty extracted intact human maxillary central incisors were decoronated 2 mm incisal to buccal CEJ and endodontically treated. The teeth were randomly assigned to one of the five groups (n=10): Group B (Blue), post space prepared with size 0 post drill (Ivoclar Vivadent, Liechtenstein)/size 0 FRC post/no RRC; Group W (White), size 1 post space/size 1 FRC post/no RRC; Group R (Red), size 3 post space/size 3 FRC post/no RRC; Group WR, size 3 post space/size 3 FRC post/size 1 RRC; Group BR, size 3 post space/size 0 FRC post/size 0 RRC. Ferrule of 2 mm and 0.5 mm were prepared at facio-lingual and proximal margins respectively. All specimens were restored with Ni-Cr crowns, thermocycled and loaded at 135° from the long axis in a universal testing machine at a crosshead speed of 0.5 mm/min until fracture. Data were analyzed using One-way ANOVA followed by post-hoc comparisons (Bonferroni) with α=0.05.

Results: Mean failure loads (N) for Groups B, W, R, WR and BR were: 1406.05 (SD=376.14), 1258.76 (379.35), 1084.54 (528.37), 958.79 (199.87) and 815.91 (298.31). Significant differences were found between Group B and BR. Group B has the highest favourable failure mode.

Conclusion: Within the limitations of this study, the remaining dentine is the most important factor in fracture resistance of endodontically treated teeth and produces the most favourable fracture if failure occurs (Group B). Therefore, the use of smaller FRC post and RRC is recommended rather than enlargement of post spaces to fit accurately larger FRC posts as the enlargement of post spaces increases the risk of unfavourable failure.
Association Between Dietary Pattern and β-carotene Intake with Oral Cancer Risk

Helen Ng Lee Ching
Department of Community Dentistry

Background: The role of diet in cancer risk has mainly been investigated based on the intake of each food item. However, food consumption of an individual is usually complex and is made up of a combination of different food items. Intakes of β-carotene from food sources have also been reported to reduce cancer risk.

Objectives: This study aims to identify dietary patterns and β-carotene intake of Malaysians, and to determine their association with the risk of oral cancer.

Methods: A hospital-based, case-control study was conducted on 306 Malaysians who sought treatment at participating centers/hospitals. The subjects were randomly selected from the OCRCC-UM database and consisted of 153 cases and 153 controls that were matched for gender, age (±5 years) and ethnicity. Dietary intake was measured using Food Frequency Questionnaire (FFQ). Factor Analysis (FA) was performed to identify dietary patterns based on the intake of 9 major food groups. NutrieMart Version 2.0.0 software was used to generate β-carotene value from the food consumption of each subject. Logistic Regression was conducted to compute the odds ratio (OR) for intake of β-carotene and also the food components retained by FA.

Results: FA identified four patterns that accounted for 69.4% of total variability of food intake within the sample. The first pattern labeled as ‘modern’ was loaded with processed foods and snacks whereas the second pattern termed as ‘prudent’ was characterized by intake of fruits and vegetables. The third pattern labeled as ‘traditional’ consisted of beverages and starches while the fourth pattern termed as ‘combination’ was loaded with intake of dairy, fermented/salted and meat/byproducts. A significant reduced oral cancer risk was found for ‘prudent’ (OR 0.53, 95% CI: 0.28-0.98, p<0.05), whereas a significant increased risk was found for both ‘combination’ (OR 2.43, 95%CI: 1.33-4.45, p<0.05), and ‘traditional’ (OR 2.32, 95%CI: 1.23-4.25, p<0.05) patterns. However, after adjusting for risk habits of tobacco smoking, alcohol consumption and betel-quid chewing, only ‘combination’ (aOR 2.99, 95%CI: 1.55-5.75, p<0.05) and ‘traditional’ (aOR 2.08, 95%CI: 1.09-3.97, p<0.05) patterns remained significant. High intake of β-carotene was not significantly associated with reduced risk of oral cancer (OR 0.83, 95%CI: 0.42-1.66, p>0.05).

Conclusion: The findings suggest that the highest consumption intake of both ‘traditional’ and ‘combination’ patterns may induce twice and thrice the risk of oral cancer respectively. No significant association was found between high intakes of β-carotene with oral cancer risk. This study had also shown meaningful association between dietary pattern and oral cancer risk which was possible to established using Factor Analysis.

Effects of Brucea javanica and Piper betle Extracts on Oral Candida spp.

Mohd Al-Faisal bin Nordin
Department of Oral Biology

Candida species is an opportunistic microorganism residing in the human oral cavity. There is increasing prevalence of candidal infections in the oral cavity largely because of the increasing size of the population at risk. The rise in diagnosed cases of oral candidiasis is also due to the immunosuppressive effect of prescribed antifungal agents on resistant hosts. The ability of Candida species to adhere on mucosal tissues and/or denture surfaces, and the production of hydrolytic enzymes along with defective host immunity are among the known key factors of invasions and pathogenesis of oral candida. Although Candida albicans remains as the most pathogenic organism, the emergence of non-Candida albicans Candida (NCAC) species with reduced susceptibility to prescribed antifungal agents has prompted efforts to study antifungal agents from natural sources. Plants have long been known to possess medicinal values and are rich in chemical constituents that can be used in the development of antifungal products. Initially, seven local plants were systematically screened for their antifungal activity and two of them – Brucea javanica L. and Piper betle L. were selected based on their positive antifungal activities on seven Candida species tested. The specific objectives of this study were: (i) to determine the minimum inhibition concentration (MIC) and minimum fungicidal concentration (MFC) values of the extracts, (ii) to investigate the growth inhibitory effect of the extracts based on changes in the pattern of growth profile of each Candida species, (iii) to investigate the influence of extracts on adherence mechanisms which include the non-specific and specific bindings, (iv) to explore the differential expressions of multigene family of secreted aspartyl proteinases (SAPs) and hyphal cell wall protein 1 (HWP1).

Candida species purchased from the American Types Culture Collection (ATCC) were Candida albicans ATCC 14053, Candida dubliniensis ATCC MYA-2975, Candida glabrata ATCC 90030, Candida krusei ATCC 14243, Candida lusitaniae ATCC 64125, Candida parapsilosis ATCC 22019 and Candida tropicalis ATCC 13803. Growth inhibitory responses of the Candida species to the extracts include determination of the MIC and MFC, while effect on the growth curve was determined based on...
spectrophotometric assay. Deviations in the doubling time (g) and specific growth rates (μ) were computed as percentage to extract-treated cells relative to that of the total cells in the absence of extracts. In addition, the anti-adherence effect of the B. javanica and P. betle extracts which included study on the cell-surface hydrophobicity (CSH) and specific bindings on pellicles, ultrastructure and the regulations of SAPI-10 and HWP1 were also analysed. 0.12% w/v chlorhexidine gluconate-containing mouthrinse was used as a reference.

In preliminary screening, the diameter of inhibition zone (DIZ) values showed that B. javanica and P. betle aqueous extracts exhibited a wide range of antifungal activities over the seven Candida species with C. dubliniensis identified as the most sensitive. Of the seven Candida species, C. tropicalis showed the highest growth rates (0.319 ± 0.002 h⁻¹) while the others were in the range of 0.141 ± 0.001 to 0.265 ± 0.005 h⁻¹. This indicated that different species of candida reproduce at different rate. In the presence of extracts, the lag and log phases were extended and shifted to the right. This resulted in the deviations of the g- and μ-values, indicating that the extracts may have exerted fungistatic activity towards the candidal cells. Growth kinetics of the candidal species was also elucidated based on colony forming unit (CFU) enumeration. Different Candida species have shown different CSH values and adhering capacity to the pellicle. Growth kinetics of the candidal species was also elucidated based on colony forming unit (CFU) enumeration. Different Candida species have shown different CSH values and adhering capacity to the pellicle.

In view of the CSH, C. krusei, C. dubliniensis and C. tropicalis showed the highest adsorption to hexadecane at 30.23%, 26.19% and 19.70%, respectively, while the others were much lower within the range of 7% to 10%. The CSH of all Candida species were significantly affected by these two extracts (P < 0.05), with B. javanica exhibiting more than 60% reduction of CSH than P. betle. Specific bindings of the candidal cells on the pellicles were also shown to be affected by the treatment of extracts. Exposure to P. betle-treated pellicle drastically reduced the adhering capacity of three out of seven candidal species by more than 50% (C. tropicalis 86.02%, C. albicans 61.41% and C. krusei 56.34%). Pellicles treated with B. javanica exhibited similar effect on C. tropicalis (89.86%), C. lusitaniae (89.66%), C. albicans (79.74%), C. glabrata (76.85%) and C. krusei (67.61%). Comparatively, the adherence interference effect of B. javanica towards the candidal cells was slightly higher than P. betle. In addition to the growth inhibitory and anti-adherence effects, physical changes in the cell walls of Candida species were also demonstrated following treatment of the candidal cells with the extracts. The expressions of SAPI-10 and HWP1 were affected by the extracts treatment, suggesting that the extracts have successfully penetrated and disrupted the intrinsic environment of the cells. The genes seemed to be suppressed and this may then revoke the pathogenesis of oral Candida.

As a conclusion, B. javanica and P. betle exhibited antifungal activities towards the seven oral Candida species tested. Data from this study strongly suggest the fungistatic and growth inhibitory effects of the extracts. Thus, B. javanica and P. betle extracts may be considered as promising adjuncts in oral health products.

Chromosomal Alterations in Oral Cancer for Selected Indian and Indigenous Population

Noor Julieana Mat Amin
Department of Oral Pathology, Oral Medicine and Periodontology

Introduction: In the Malaysian population, the Indians were identified as having a higher oral cancer (OC) risk compared to Malays, Chinese and other ethnicities with betel quid chewing contributing the major factor among Indian female. Cancer progresses through a series of histopathological stages where the progression is thought to be driven by the accumulation of genetic alterations (Garnis et al., 2009). Therefore, the identification of chromosomal alterations will not only enhance the understanding of the biology in this process, but will also identify important genes that might involve in oral carcinogenesis. Thus, this study utilised array Comparative Genomic Hybridization (arrayCGH) technology as a platform.

Aims: To identify chromosomal alterations leading to the identification of genes involved in the development of OC, and to determine the relationship between selected altered regions with socio-demographic characteristics which include gender, ethnicity and habits and selected clinicopathological parameters namely tumour nodes metastasis (TNM) stage, tumour size (T), lymph node status (N), tumour grade and tumour site.

Methods: A total of 20 fresh frozen tissue samples diagnosed as OC and reference DNA from peripheral blood lymphocytes were obtained from the Malaysian Oral Cancer Data and Tissue Bank System (MOCDTBS)-Oral Cancer Research and Coordinating Centre (OCRCC), University of Malaya (UM). A series of 750 μm thick tissue sections were mounted in Optimal Cutting Temperature (OCT) compound and stained with H & E. Tissue sections containing more than 70% epithelial tumour were selected and confirmed by an oral pathologist. Analysis of arrayCGH was performed using the Human Genome CGH Microarray KIT 4x44K chip platform. Graphical overview of chromosomal alterations was obtained by using the Genomic Workbench Standard Edition 5.0.14. Validation of DUSP22 gene generated from arrayCGH results was then carried out at DNA (copy number) and cDNA (gene expression) levels. Statistical analysis was performed using SPSS version 19.
Results: In this study, the occurrence of deleted regions was slightly higher than the amplified regions. The most frequently deleted regions were detected on chromosome 19p13.3, followed by 19p13-p13.11 and 19q13.3 while amplifications were most commonly detected at 8q24.3, 8q11.1-8q11.2 and 3q26 regions. The most common amplified gene detected was DUSP22, followed by KIAA0146. For the deleted regions, the most common genes detected were FBXO25, INPP5A, NX6-2, C10orf92, CDH4, TAF4, and LSM14B. With respect to the clinicopathological parameters (tumour size (T), TNM stage, lymph node status (N), tumour grade and tumour site) and sociodemographic profile (gender, ethnicity and habits), no association was found between the most amplified (8q24.3, 8q11.1-8q11.2 and 3q26) and deleted regions (19p13.3, 19p13-p13.11 and 19q13.3). Only region 19p13-p13.11 showed significant relationship ($p=0.044$) with tumour site. Validation and analysis at copy number (CN) and gene expression (GE) level for the DUSP22 gene revealed consistent results with previous arrayCGH in which tumour cells showed over-expression.

Conclusion: This study showed no association between chromosomal alterations and the clinicopathological parameters and sociodemographic profile studied. Only tumour site showed correlation with chromosomal alteration. It is recommended that a bigger study be conducted using bigger sample size in order to obtain more significant association.

**Differentiation of Oral Candidal Species Based on the Internally Transcribed Spacer (ITS) Regions of rDNA**

Raja Ahmad Zahir Raja Halinuddin
Department of Oral Biology

*Candida* is a genus of opportunistic yeast that are usually harmless residents of the oral cavity, but they become pathogenic under conditions which allow them to increase their proportion to other members of the oral microflora. Correct and accurate identification of the candidal species infecting an oral candidiasis patient is important due to antifungal drug resistences. The region in the fungal genome that contains the gene that codes for rRNA, also known as rDNA, has been found to be useful for phylogenetic studies and species identification. The purpose of this study is to compare the candidal loads of denture wearers, periodontal disease patients, and a control group, in addition to differentiating isolated oral *Candida* sp. based on rDNA, in order to assess the effectiveness of using the rDNA region for candidal species identification. Samples from the saliva and the surfaces of the palate, tongue and cheek mucosa were collected from 45 individuals consisting of three target groups: periodontal disease patients, denture wearers with healthy oral cavity, and non-denture wearers with healthy oral cavity as the control group. The samples were subjected to serial dilution and spread on agar plates, which were then scored for Colony-Forming Units (CFUs). Next, fifteen random candidal colonies were isolated and subjected to genomic DNA extraction based on glass beads disruption. ITS1, ITS2, ITS3 and ITS4 primers were used to amplify regions in the rDNA, and the ITS1-5.8S-ITS11 region was then digested by *Hinfl* and *MspI* restriction enzymes. The microbial loads on all the sites of denture wearers groups was found to be significantly higher than the control group, while only the microbial loads on the tongue surface of the periodontal disease group was significantly higher than in the control group, while at all the other sites there was no significant difference. Comparing the restriction fragment lengths of the clinical samples to that of seven ATCC control species allowed the identification of *Candida albicans*, *Candida tropicalis*, *Candida parapsilosis*, *Candida dubliniensis* and *Candida glabrata* (*C. krusei* and *C. lusitaniae* were also among the ATCC control species and could be differentiated, but none of the clinical samples were of those species). One clinical sample had a unique band and restriction fragment pattern that could not be matched to any of the control species, however based on the band sizes it is most likely to be *Candida famata*. The *MspI* restriction digest was not able to distinguish between *C. albicans* and *C. dubliniensis*, whereas the *Hinfl* digest could not distinguish between *C. tropicalis* and *C. parapsilosis*. In conclusion, candidal colonization in denture wearers, while there appears to be no significant difference in periodontal patients other than on the tongue surface. Furthermore, restriction enzyme digestion of the candidal rDNA region is potentially useful for candidal species identification.

**Evaluation of Human Platelet Lysate as an Alternative Culture Medium for Expansion and Hepatocyte Differentiation of Dental Pulp Stem Cells**

Aimi Naim Abdullah
Department of Children Dentistry and Orthodontics

The advancement of stem cells research has pushed the frontier of regenerative medicine to a different level where range of diseases including liver diseases could be potentially treated using cell therapy. The usage of stem cells offers alternative treatment to organ transplantation, wherein, organ shortage issue and possibility of rejection are major limitations. Dental pulp stem cells from deciduous teeth (SCD) have been identified as a source of adult stem cells with high proliferation activity and multilineage differentiation. Current studies showed that SCD have potential to...
differentiate into hepatic lineage and they usually cultured in fetal bovine serum (FBS) supplemented media. Thus, this may expose SCD to the possibility of contamination to virus, prions, mycoplasma and transmission of disease. It is necessary to identify animal-free serum media if cell therapy is to play a major role in regenerative medicine. Human platelet lysate (HPL) appears to be a suitable substitution to FBS for stem cells culture as it is a non-animal origin and has a lot of natural growth factors to support stem cells proliferation and differentiation. The aim of this study was firstly to evaluate HPL as an alternative culture media to FBS in the culture, expansion and differentiation of SCD. Secondly, the ability of SCD to differentiate into hepatocyte differentiation when cultured in FBS and HPL will also be assessed. The properties of SCD cultured in both media were examined through growth kinetics, trilineages differentiation and expression of specific surface markers. The differentiation potential of SCD in FBS and HPL into hepatocyte-like cells was investigated from the morphological formation, periodic acid Schiff (PAS) staining and the expression of hepatocyte specific marker with urea secretion assay at day 0, 7, 14 and 21. Results showed SCD cultured in HPL has higher proliferation rate compared to FBS and displayed similar potential of trilineage differentiation and phenotypic expressions. SCD cultured in both media also displayed potential to form hepatocyte-like cells based on the morphology changes from fibroblast to hexagonal-shaped cells and positive staining for PAS. The expression of hepatocyte specific markers and the urea secretion increased proportionally with the day of hepatocyte induction. Within the limitations in this study, it could be conclude that HPL could be an alternative to FBS as the proliferation of SCD cultured in HPL is statistically higher and exhibited similar characteristic to FBS. SCD in HPL also showed potential to differentiate into hepatocyte cells and thus may become a hope for alternative treatment of liver diseases.

Materials and Methods: The subjects for this study included imaging of 60 patients (30 males and 30 females) from the Division of Oral radiology, with ages ranging from 20 to 60 years (mean age, 47 years). The samples were selected according to gender, race and age groups. The position of the mandibular canal and mandibular canal diameter were measured at five different locations. Linear measurements were done in the coronal view just posterior to the mental foramen at 10 mm interval (D1, D2, D3, D4 and D5). Mandibular foramen diameter and incidence of bifid mandibular canal were also recorded. The samples were imaged using CBCT and SimPlant software and data analyzed through SPSS (v.12).

Results: In this study the mandibular canal was identified in all samples with 100% good visibility. The measured data were expressed as minimum, maximum, median, K-S value and mean ± standard deviation. The results showed that the position of the right mandibular canal is similar to the position on the left side of the jaw.

Apicocoronal position of the mandibular canal showed that the superior measurements were 14.85 ± 3.64 mm at D1, 13.94 ± 3.85 mm at D2, 12.99 ± 4.08 mm at D3 and 14.22 ± 1.52 mm at D4. The inferior measurements of the canal was 9.37 ± 1.69 mm at D1, 8.24 ± 1.69 mm at D2, 7.96 ± 1.93 mm at D3, 9.65 ± 2.54 at D4 and 15.21 ± 4.18 mm at D5. The buccolingual position were 3.89 ± 1.00 mm (buccal) and 4.33 ± 1.25 mm (lingual), 5.59 ± 1.20 mm (buccal) and 3.35 ± 1.20 mm (lingual), 6.71 ± 1.34 mm (buccal) and 3.25 ± 1.32 mm (lingual), 5.68 ± 1.63 mm (buccal) and 3.08 ± 1.46 mm (lingual), 4.24 ± 1.59 mm (buccal) and 2.12 ± 1.40 mm (lingual) at D1,D2,D3,D4 and D5 respectively.

The minimum mandibular canal diameter recorded was 2.00 mm and the maximum was 3.40 mm. In this study the average mean was 2.16 ± 0.30 mm with the least mean diameter at D2 location (2.01 ± 0.42 mm) and the largest mean diameter at D1 (2.25 ± 0.47 mm) and D5 (2.25 ± 0.43 mm). The average mandibular foramen diameter was measured to be 2.55 ±0.43 mm.

The incidence of bifid mandibular canal was greatest in Malays (n=18), followed by Indians (n=9), while no bifid canal was noticed in the Chinese.

Conclusion: Position of the canal changes due to changes in the mandibular bone. Measurements showed that the mandibular canal curves toward the lingual side the more distal it is away from the mental foramen. Apicocoronal assessment of the canal reveals that it is curving downward towards the inferior mandibular border until D3 and then it curves upwards. This CBCT study reveals there are variations in the position of the mandibular canal. It is highly recommended that careful assessment and planning using computed tomographic imaging is done prior to any surgical intervention in the mandibular canal region to avoid untoward complications.

**Metrical Analyses of the Location of the Mandibular Canal Using CBCT**

Saif Yousif Abdullah
Department of Oral and Maxillofacial Surgery

Introduction: The increased neurosensory disturbances and hemorrhage after surgical intervention in the mandibular canal region increased the demand for presurgical planning and proper assessment to avoid those complications.

Aims: Determine the path and course of the mandibular canal of dentate Malaysian patients, mandibular canal diameter, mandibular foramen diameter and the incidence of bifid canal using the Cone Beam Computed Tomography (CBCT).
With the growing focus on transnational education, there is increasing student mobility around the world. The University of Malaya (UM), under the auspices of its International Student Centre (ISC), encourages its students from different disciplines to go abroad to not only gain international orientation but also to become global-ready graduates. This activity helps establish professional and career opportunities by networking with other students, academics and professional organizations. It also helps to improve their language skills, cross-cultural understanding, and cross-cultural and interpersonal communication. It provides them with an opportunity to better understand both the diversities and similarities of different cultures and experience personal growth by developing self confidence, independence, and social skills. To encourage experiential learning, during their final year, our dental students are given the opportunity to spend a period of time abroad at one of our partner universities.

During the semester year 2010-11, some of our students had the opportunity to participate in this program and the following is a report of their activity.

International University (IU), Cambodia (www.iu.edu.kh) is one of the private medical universities in Cambodia, focusing mainly on training health professionals to work in the healthcare sector. IU offers 6 years practical and clinical course of study leading to the degree of Bachelor in Medical Science (Equivalent to MBBS in other countries) and 5 years course of study leading to the degree of Bachelor of Dental Surgery (BDS). The Faculty of Dentistry at IU is located in the outskirts of Phnom Penh. The faculty shares a building with the faculties of medicine, nursing and pharmacy. The total number of academic staff is around 20-30 while the number of students is approximately 200-250.

Faculty of Dentistry, IU provides a wide range of dental services for mainly low-income Cambodian. Costs to the patients are kept as low as possible. The teaching staff are primarily Cambodian dentists, although there are several expatriate staffs. Most of the patients are treated by dental students, and volunteers will generally supervise and assist the students. There are also facilities for dentists to treat patients themselves, and students can observe and assist. The medium of instruction used at IU are English and Khmer. The old curriculum followed a 5-year BDS program while the new curriculum, follows a 7-year Doctor in Dental Surgery (DDS) program. Students at IU begin their clinical practice in year 3 (under the new curriculum) and they practice integrated dentistry rather than discipline-based practice.

Seven final year students from the Faculty of Dentistry, University of Malaya (UM) visited IU between 16th to 24th July 2011 as part of the University’s outbound activity. They were accommodated at the IU house. During their stay at IU, the students learnt about the cultures of Cambodia, history of IU and they also participated in some of the lecture and clinical sessions at the faculty. They also interacted with their counterparts at IU and they shared their knowledge through 4 sessions of problem based learning which included trigger analysis, resource, presentation and demonstration sessions. This gave them an opportunity to build network and exchange ideas with their fellow students. They also had an opportunity to interview the dean of the Faculty of Dentistry at IU, Professor Dr Callum Durward as well as the opportunity to visit interesting and historical places in Phnom Penh.

Kaohsiung Medical University (KMU), Taiwan (www.kmu.edu.tw) formerly known as Kaohsiung Medical College was established in 1954. The university is located in the city center of Kaohsiung, which has all the attractions and excitement of city life. Apart from being the first private medical university in Taiwan, the university has also become an important cradle for highly-skilled physicians and medical-related specialists. There is a wide range of medical related courses and the facilities, both for basic and clinical research are sufficient and of high quality.
After Kaohsiung Medical College became a university in the summer of 1999, she seized this opportunity to move faster and further to attain the highest standards in teaching, research and service. It was the shared vision of everyone at KMU to make the university a modern, forward-looking institution striving for excellence in both academics and medical care. Kaohsiung Medical College received permission from the Ministry of Education to change its name to “Kaohsiung Medical University” in August 1999. The university consists of six colleges: the College of Medicine, the College of Dental Medicine, the College of Pharmacy, the College of Nursing, the College of Health Science, and the College of Life Sciences.

The College of Dental Medicine of KMU was established on August 1, 1957. The teaching staff comprised nearly 34 full-time and 20 part-time dental staff. Taiwan’s first master program of dental sciences was established in 1985 followed by the Ph.D. program in 1990. This institute has cultivated many talented people in the areas of dental service, dental education and dental research. In 1992, Taiwan’s first graduate institute of oral health sciences was founded to provide a concrete basis in promoting oral health.

Six students from UM took part in the KMU outbound program between 2nd to 9th September 2011. They stayed at the Alumni House built with donations from KMU Alumni. As part of their attachment, the students visited the College of Dental Medicine, KMU and Dental division of KMU Chung-Ho Memorial Hospital, namely the departments of Periodontology, Children’s Dentistry, Prosthodontics, Orthodontics and Center for Special Needs Dentistry. The students were able to participate in the clinical sessions. The clinics were situated in the hospital and treatment was given by final year undergraduate students and postgraduate students, who work in the same clinic in every department.

The dental curriculum at KMU comprise of a 6-year program. The students begin their clinical attachment in the 5th year, where they would assist chairsides. In the 6th year, they would treat patients and are attached to the clinics full-time. They are paid a salary during the 6th year of their posting, which runs on a rotation basis, covering all the departments. Mandarin and Taiwanese Hokkien are the spoken language between lecturers, students, staff and patients in the clinic.

Universitas Airlangga (UNAIR), Indonesia (www.unair.ac.id) located in Surabaya, East Jawa, was named after the famous King Airlangga who ruled the eastern part of Indonesia archipelagoes from 1019 to 1042. The university was established in 1954. Today it has 11 faculties and one postgraduate program, ranging from basic science, medicine, and social sciences. Its vision is to become an independent, innovative, and foremost University both regionally and globally, a forerunner of science development, technology, humanities and arts based on moral and religion.

Six students from UM took part in the UNAIR outbound program between 24th to 29th April 2011. They stayed at the Ghra Ara located about 5 minutes away from UNAIR. On day one, the students visited the Dental Faculty of UNAIR and were briefed on the history of the university and faculty as well as on the education system, clinical requirements and examination standards at the university. They met the faculty dean, Professor Dr Coen Pramono and were taken on a grand tour throughout UNAIR. The next
day, they had the opportunity to observe the “Kuliah Kerja Nyata Bersama Masyarakat” (KKN) in Kota Madya. This is a common community program for all senior UNAIR graduates, whereby the dental students stay for a period of 1-3 months among the community providing dental as well as community based services.

The dental faculty has an undergraduate as postgraduate/specialist clinic and they follow well as an integrated teaching-learning module. The attendance at the clinics and classes uses the biometric system whereby they had to scan their thumbprint for verification. The cafeteria at the faculty has one of the stalls subsidized by UNAIR for entrepreneurship program by the students. Within the faculty there is a store called “Depo” which sells various type of dental accessories ranging from handpieces, burs to the laboratory and clinical instruments and materials. Cigarette manufacturing is one of the main sources of income of the people of Surabaya, so the faculty places a lot of importance for periodic dental education and screening of the population.

This outbound program has been instrumental in enabling the participants to maximize their educational success and develop higher levels of cultural empathy, open-mindedness, social initiative, flexibility and emotional stability. However, they did report some difficulties with adjusting to the differences in areas such as communication, accommodation, teaching and learning methods and developing friendship with their counterparts in the host country. It is hoped that in the future, studies examining the processes of intercultural sojourn should consider more detailed qualitative analysis of students’ outbound experiences to gain a deeper understanding of the time abroad and how it may cause changes within the individual.

ACKNOWLEDGEMENTS

We would like to thank Faculty of Dentistry for allowing the students to attend the program as well as Dr Wan Nurazreena Wan Hassan for all her help in compiling this report.
Conferences, Seminars, Lectures and Workshops organized by the Faculty of Dentistry, University of Malaya

1. Lecture: i) “Trigeminal nerve injuries: From the laboratory to the bedside” and ii) “I can see nothing wrong with your teeth - The diagnosis & management of chronic facial pain” Prof A R Loescher (School of Clinical Dentistry, Claremont Crescent, Sheffield), Lecture Hall (Level 9), Postgraduate and Research Tower, Faculty of Dentistry, UM, 3/7/12

2. Lecture: “Minimal Intervention Dentistry (MI); management of caries lesions from the early white spots to the large cavities” by Prof Hien Ngo (Kuwait University), Lecture Hall (DK3), Faculty of Dentistry, UM, 4/7/12

3. Lecture: “Coverage of denuded root surface” by Prof Esmonde Corbet (University of Hong Kong), Lecture Hall (DK3), Faculty of Dentistry, UM, 6/7/12

4. Lecture: “International perspectives on preventing childhood dental caries” by Prof Dr. Cynthia Pine (University of Salford), Lecture Hall (DK3), Faculty of Dentistry, UM, 9/7/12

5. Sandwich One-2-Two Session: Differential Expression of Stem Cell-Like Proteins in Oral Potentially Malignant Disorder” by Prof Dr. Siar Chong Huat, Department of Oral Pathology, Oral Medicine and Periodontology, Faculty of Dentistry, Lecture Hall (Level 9), Postgraduate and Research Tower, Faculty of Dentistry, UM, 11/7/12

6. Lecture: “The multifactorial nature of periodontal disease” by Prof Mark Bartold (University of Adelaide), Lecture Hall (DK3), Faculty of Dentistry, UM, 11/7/12

7. Lecture: “Oral care for the elderly: a partnership with health team and the community”, by Assoc Prof Dr. Patcharawan Srissilapanan (Chiang Mai University), Lecture Hall (DK3), Faculty of Dentistry, UM, 16/2/2012

8. Lecture & Demonstration: “Floid & TaliTM image-based cytometer” by Ms Yeoh Wei Ying (Bio-Diagnostics Sdn. Bhd.), Level 7, Postgraduate and Research Tower, Faculty of Dentistry, UM, 13/7/12

9. Workshop: “Near infrared western blotting imaging technology” by Mr Jack Yap (Research Instruments Sdn. Bhd.), Lecture Hall (Level 9), Postgraduate and Research Tower, Faculty of Dentistry, UM, 2/8/12

10. Training: “Nikon TS 100 trinocular microscope” by Mr Apandi B. Safuan (Microlambla Sdn. Bhd.), Tissue Culture Room/ Stem Cell, Craniofacial and Molecular Biology Research Laboratory (Level 7), Postgraduate and Research Tower, Faculty of Dentistry, UM, 7/8/12

11. Lecture: “Caries risk assessment” by Prof Angus Walls (School of Dental Science, Newcastle University), Lecture Hall (DK3), Faculty of Dentistry, UM, 10/8/12

12. Sandwich One-2-Two Session: “Human papillomavirus and oral mucous disease: An update” by Prof. Hatsuhiko Maeda (Prof & Chair, Department of Oral Pathology, School of Dentistry, Aichi Gakuin University), Lecture Hall (Level 9), Postgraduate and Research Tower, Faculty of Dentistry, UM, 14/8/12

13. Workshop: “Avadis NGS Training” by Mr Li Zhi Hui (Genomax Technologies Sdn. Bhd.), Bioinformatics Laboratory, Level 8, Postgraduate and Research Tower, Faculty of Dentistry, UM, 16/8/12

14. Demonstration: “LAS 4000 digital imaging system” by Mr. Gabriel Hiew (ITS – Interscience Sdn. Bhd.), Craniofacial and Molecular Biology Research Laboratory, Faculty of Dentistry, UM, 24/8/12


16. Sandwich One-2-Two: “Assessment of Demineralisation/ Remineralisation of Dental Hard Tissues with Optical Methods” by Dr. Chew Hooi Pin, Department of Conservative Dentistry, Faculty of Dentistry, Lecture Hall (Level 9), Postgraduate and Research Tower, Faculty of Dentistry, UM, 19/9/12

17. Lecture: “Biocapability of Merck Milipore Bioscience” and “Cell Health” by Mr. Asanga Halangoda (Bioscience Asia Pacific), Lecture Hall (DK3), Balai Ungku Aziz, Faculty of Dentistry, UM, 20/9/12
18. Demonstration: “2nd demonstration for LAS 4000 System” by Mr Adrian Koh (ITS – Interscience Sdn. Bhd.), Craniofacial and Molecular Biology Research Laboratory (Level 7), Postgraduate and Research Tower, Faculty of Dentistry, UM, 21/9/2012

19. Demonstration: “MUSE- Cell Analyzer” by Mr. Asanga Halangoda (Bioscience Asia Pacific), Lecture Hall (DK3), Balai Ungku Aziz, Faculty of Dentistry, UM, 19/10/2012

20. Demonstration: “Workshop For Odyssey Fc Imaging System” by Mr. Loh Chin Chieh (Research Instruments Sdn. Bhd.), Craniofacial and Molecular Biology Research Laboratory (Level 7), Postgraduate and Research Tower, Faculty of Dentistry, UM, 2/10/2012

21. Lecture: “Oral Hygiene Aids” by Mrs Noor Faizah Dalimi (Denta Medic Technology), Seminar Room, Block D, Faculty of Dentistry, UM, 16/10/2012


23. Sandwich One-2-Two: i) “Interaction Between Caries, Pulpal Inflammation and Physiological Root Resorption in Primary Polars” by Dr. Sadna Rajan, Department of Children Dentistry and Orthodontic, ii) “RANKL, RANK and OPG Expression in Surgically Created Periodontal Defects” by Dr. Nor Adinar bt Baharuddin, Department of Oral Pathology, Oral Medicine and Periodontology, Faculty of Dentistry, and iii) “Effect of Periodontal Intervention on Periodontal Disease and Type 2 Diabetes Mellitus” by Prof. Dr. Tara Bai Taiyed Ali, Department of Oral Pathology, Oral Medicine and Periodontology, Faculty of Dentistry, Lecture Hall (Level 9), Postgraduate and Research Tower, Faculty of Dentistry, UM, 17/10/2012

24. Demonstration: “Leica Slide Scanner SCN 400” by Ms Joanne Chew and Ms Shamala a/p Nair Balan (Histocenter (M) Sdn. Bhd.), Oral Pathology Diagnostic Research Laboratory, Faculty of Dentistry, UM, 5/11/2012-22/11/2012

25. Demonstration: “Kodak CBCT 9000 3D imaging software” by Mr. Sunil Joshi (Carestream Dental), 28/11/2012


27. Colloquium: “The antiplaque activity of pure compounds on monospecies and mixed species oral biofilm model” by Ms Nurhayati Bt Mohd Zain (Graduate student), Lecture Hall (DK3), Balai Ungku Aziz, Faculty of Dentistry, UM, 22/11/2012

28. Training: “Multidisciplinary Management of Patients With Craniofacial deformities” by Prof. Dr. Lim Kwong Cheung (University of Hong Kong), Auditorium (Level 11), Postgraduate and Research Tower & Computer Laboratory, Faculty of Dentistry, UM, 20/11/2012–23/11/2012


30. Lecture: “TMA: Problems and solutions to modern TMA methods” by Dr. Tibor Krenacs (Universiti Semmelweis, Budapest, Hungary), Lecture Hall (Level 9), Postgraduate and Research Tower, Faculty of Dentistry, UM, 26/11/2012

31. Program: “OCRCC Research Day: Personalized medicine for treatment of oral cancer” by Prof Dato’ Dr. Zainal Ariff Abdul Rahman, Prof Dr. Prepageran Narayanan and Prof Dr. Anita Zarina Bustam, Bidara & Centrepoint Hall, Level 4, Faculty of Dentistry, UM, 4/12/2012

32. Lecture: i) “Mouthrinses in the management of oral candida infection” and ii) “Safe zone for orthodontic mini-implant insertion at posterior mandible” by Prof Dr. Ngeow Wei Cheong and Assoc Prof Sabri Musa, Lecture Hall (Level 9), Postgraduate and Research Tower, Faculty of Dentistry, UM, 12/12/2012

33. Training: “Course of Basic Medical Sciences Dentistry” by lecturers from UMSC, PPUM and IMU, Postgraduate and Research Tower, Faculty of Dentistry, UM, 17-22/12/2012
NEW INSTRUCTION FOR AUTHORS

The Annals of Dentistry University of Malaya (ADUM) is published annually as the official publication of the Faculty of Dentistry, University of Malaya, Kuala Lumpur, Malaysia. Since its first publication in 1994, this peer-reviewed journal strives to promote the advancement of practice, education and scientific research in Malaysia as well as elevating the quality of patient care. An emphasis is now given to publishing and disseminating research works undertaken by dental trainees, i.e. undergraduate and postgraduate dental students, locally and globally. In line with current trend, the ADUM will be publishing forthcoming articles online before they are scheduled to appear in print.

Original research articles, and critical reviews pertaining to dentistry and its allied fields will be considered for publication at the discretion of the Editor-in-chief. If in doubt, all potential authors are encouraged to write to the Editor-in-chief prior to submission. All articles will be refereed independently by at least 2 referees. Authors of all types of articles should be aware of the following guidelines when submitting to ADUM.

I. SUBMISSION

All manuscripts must be written in English (Concise Oxford Dictionary at http://oxforddictionaries.com). They must include a title page (see detailed requirement below) and be accompanied by a cover letter, a list of suggested reviewers and the Copyright Transfer Agreement. Title pages should include the title of the manuscript, the surname(s) and initial(s) of the author(s), affiliations, address, corresponding author, abstract, total word count (Text to Acknowledgments), total number of tables/figures and the number of references.

Cover letters should certify the research is original, not under publication consideration elsewhere, and free of conflict of interest. It should contain the name, address and contacts (fax, telephone number and e-mail) of the author responsible for correspondence and for proof-reading purpose and information whether the authors are willing to meet the cost of reproducing illustrations in colour prints (if any).

Authors are encouraged to submit the names, address, and email addresses of four preferred reviewers to facilitate the speedy review of their manuscript. Preferred reviewers cannot be colleagues at the contributors’ institution or present or former collaborators.

All authors must approve and sign the Copyright Transfer Agreement of the manuscript concerned. Manuscripts will not be reviewed until the completed form has been sent to the editorial office. In addition, authors will need to obtain permission to reproduce a previously published figure or table. The approval letter must be submitted to the editorial office.

Manuscripts can be submitted to the Editor-in-chief electronically. Electronic copy of the manuscript can be submitted by sending it as an e-mail attachment to the following address: editor.adum@um.edu.my

II. GENERAL REQUIREMENT

For hard copy submissions, print the manuscript on ISO A4 (212mm by 279mm) paper with margins at least 25mm. A margin of 40mm is required on the left border of the manuscript. Print only on one side of the paper and use double spacing throughout. Number pages consecutively beginning with the title page. Numbers shall be placed on the lower right corner of each page. Each manuscript component should begin on a new page in the following sequence:

1. Title page
2. Abstract, key words, running title
3. Text (inclusive of illustrations, figures, and/or tables)
4. Conclusions (where applicable)
5. Funding/Acknowledgement (where applicable)
6. References
7. Legends

Title Page

The title page should carry the title of the article, which should be concise but informative. It should include sufficient detail for indexing purposes but be general enough for readers outside the field to appreciate what the paper tries to convey.

Abstract, key words and running titles

The second page should carry an abstract of not more than 250 words and should state the purpose of study, brief materials and methods, the findings and principal conclusion. Please repeat the main title at the
beginning, and followed by a short running title. Please provide a minimum of 6 keywords. Key words should be selected from Medical Subject Headings (MeSH) to be used for indexing of articles.

Text
The main text should include Introduction, Material and Methods, Results, Discussion, Conclusion, Acknowledgement and References, in that order respectively.

a) Introduction
Begin with a concise introduction by outlining the purpose of the research and making reference to previous relevant publications. Mention any limitation or gap in existing knowledge related to the study in question. Do not review the subject extensively and do not include data or conclusion for the work reported.

b) Materials and Methods
Describe precisely the materials/subjects used. Identify the methods, apparatus and procedures used in sufficient detail so as to allow other workers to replicate the study. Give references to established method, including statistical methods. Provide reference and brief description for methods that have been published but not well known. Describe new or modified methods and give reasons for using them and evaluate their limitations. Identify all drugs and chemicals used including generic names, dose and route of administration.

c) Results
Present results in a logical sequence in the text, tables and figures. Do not repeat in the text all the data in the tables and figures. Emphasise or summarise only important observations. Specify statistical methods used to analyse results and describe them with sufficient detail to enable a knowledgeable reader with access to original data to analyse results.

d) Discussion
Emphasise the new and important aspects of the study and the conclusion that follow from them. Do not repeat in detail, data or other materials given in Introduction or Results section. Include in the Discussion section the implication of the findings and their limitation, including implications for future research. Relate the observations to other relevant studies.

e) Conclusion
Link the conclusion to the purpose of the study to avoid unqualified statements and conclusions not supported by your data.

f) Acknowledgments
Authors are required to report all sources of support for their project or study, including but not limited to: grant funds, commercial sources, funds from a contributors’ institution. Do not refer to a study being “partially funded by the cited sources.” Consultancies and funds paid directly to investigators must also be listed. Any perceived or actual conflicts of interest need to be identified in the acknowledgments section. ADUM abides by the International Committee of Medical Journal Editors guidelines for the Ethical Considerations in the Conduct and Report of Research (http://www.icmje.org/ethical4conflicts.html). Authors are requested to include this information in the acknowledgments section and the corresponding author must confirm that all co-authors have reported any potential conflicts.

g) Figures and tables requirements
Figures (including illustrations) submitted to the ADUM should be embedded in Word documents according to the sequence they appears in the text. Tables should be viewable in a portrait view. When necessary, tables can be created in a landscape view. Original drawings, figures, charts and graphs should be professionally drawn, and lettered large enough to be read after reduction. High quality computer generated diagrams are acceptable. Prints of radiographs should be sharp and clear. Photomicrographs must include a scale and magnification shall be stated. Figures and tables should be spelt out in full when referred to in the text e.g. Figure 2 or Table 3. Figures/images should be in TIFF, JPEG or EPS format in either greyscale or colour. Please ensure that photographs are at a high resolution of 300 pixels per inch. If a person is recognisable from a photograph, written consent of the patient to publication must be obtained by the author and a copy sent to the ADUM.

h) References
References should be carefully checked, as their accuracy is the responsibility of the author(s). In the text, citations should be arranged in alphabetical order by last name of the first author without numbering. When citing a reference in the text, provide attribution for the subject under discussion. “et al’’ should be used when the cited work is by six or more contributors. When the cited work is by two contributors, use both surnames cited in the following manner: Last Name1, Last Name2. When citing multiple references by the same author(s) in the same year, use “a,” “b,” etc. (e.g., Jones, 1980b). Multiple references should be listed in chronological order of publication, separated by semicolons. Avoid using abstracts as references. When citing a Web site, list the authors and title if known, then the URL, include the date it was accessed in parentheses. Include among the reference papers accepted but not yet published; designate the journal and add “in press.” Information from manuscripts submitted but not yet accepted should be cited in the text as “unpublished observations” in parentheses. The references must be verified by the author(s) against the original documents and checked for correspondence
between references cited in the text and listed in the “References” section. All items should be listed alphabetically by the author’s last name. For multiple entries by the same author/authors, they should be cited as follows:

1. One author: chronologically by year of publication
2. Two authors: alphabetical by last names
3. Three or more authors: chronologically by year of publication
4. Same author, same publication, chronologically by date of publication, using a), b), etc., to designate order

“Unpublished observations” and “personal communications” may be inserted into and cited in the text with written permission from the correspondents, but are not to be used as references.

In summary, the reference list must be attached at the end of the paper using the following format:

Journal

Book, monograph & agency publication

i) Legends
Each figure must have a legend that is clear without references to the text. All legends must be summarised and printed on a single page with corresponding figure number clearly indicated.

In summary, the abstract, text, conclusion, figure legends, and tables should be combined into a single Word document. Illustrations, figures and/or tables must be embedded into the Word document according to the sequence they appear in the text. The cover letter, copyright transfer form and permission to reprint images/figures should be submitted as separate documents.

III. SPECIFIC REQUIREMENTS

Guest Editorials*: Manuscripts are to be submitted by invitation only. A clear and substantiated position on issues of interest to the readership community can be considered for this manuscript type. Guest Editorials are limited to 1,000 words. No figures or tables are permitted.

Reviews: These manuscripts should summarize information that is well known and emphasize recent developments over the last three to five years with a prominent focus on critical issues and concepts that highlight the latest discovery and gaps of the topic being discussed. Authors interested in submitting to this section must contact the Editor-in-chief for submission approval and instructions. Manuscripts submitted are limited to 4,000 words (inclusive of the main text of the manuscript and acknowledgments; excluding figure legends and references) with a total of 8 figures or tables; up to a maximum of 60 references; and must contain a 250 word abstract. It is best that the authors summarize important concepts in tables or flow charts or show critical data in the form of figures.

Systematic Review: These manuscripts are generally reviews undertaken on topics of high clinical relevance to oral, dental and craniofacial research. Meta-analyses should be undertaken with sufficient numbers of studies. Manuscripts submitted are limited to 4,000 words (inclusive of the main text of the manuscript and acknowledgments; excluding figure legends and references) with a total of 8 figures or tables; up to a maximum of 60 references; and must contain a 250 word abstract. It is best that the authors summarize important concepts in tables or flow charts or show critical data in the form of figures.
Original Research Reports: These manuscripts are based on clinical, biological, and biomaterials and bioengineering subject matter. Manuscripts submitted as research reports must contain a 250 word abstract and have a limit of 3,000 words (including introduction, materials, methods results, discussion and acknowledgments; excluding figure legends and references). The total number of figures or tables acceptable is 6, with a maximum of 40 references.

Letters to the Editor: Letters must include evidence to support a position about the scientific or editorial content of the ADUM. Manuscripts submitted as a letter to editor have a limit of 300 words. A maximum of one figure or table is permitted. Letters on published articles must be submitted within 3 months of the article’s electronic publication date.

Case Report: Case Reports are short reports up to 1500 words in length (excluding abstract), with one table or two illustrations or diagrams, and ideally contains no more than six references. The format of the report should be as follows:

- Abstract (no more than 50 words)
- Case report (to include short relevant history of patient, examination and investigations)
- Treatment (to include clear instructions of procedure and to include materials used, dosage and approved names of drugs)
- Differential diagnosis (to mention points of interest and a few alternatives of importance)
- Discussion (to include report of progress, lesson to be learnt from case).

IV. GENERAL INFORMATION FOR AUTHORS SUBMITTING A MANUSCRIPT PRIOR PUBLICATION

Manuscripts submitted to the ADUM are accepted for consideration giving the understanding that it contains original material that has not been submitted for publication or has been previously published elsewhere. Any form of publication other than an abstract only constitutes prior publication.

V. ICMJE COMPLIANCE STATEMENT

Manuscript submission guidelines for the ADUM follow the “Uniform Requirements for Manuscripts Submitted to Biomedical Journals” set forth by the International Committee of Medical Journal Editors (ICMJE). For additional information please visit the ICMJE web site at http://www.icmje.org/.

VI. INSTITUTIONAL REVIEW BOARD AND WRITTEN INFORMED CONSENT

For protocols involving the use of human subjects, authors should indicate in their Methods section that subjects’ rights have been protected by an appropriate Institutional Review Board and written informed consent was granted from all subjects. When laboratory animals are used, indicate the level of institutional review and assurance that the protocol ensured humane practices.

VII. DEFINITION OF CONTRIBUTORSHIP IN ANNALS OF DENTISTRY

As stated in the Uniform Requirements for Manuscripts Submitted to Biomedical Journals, put forth by the ICMJE, the Journal considers the following as an accurate definition of contributorship:

VIII. COPYRIGHT TRANSFER AGREEMENT

All rights to manuscripts will be transferred to the ADUM upon submission. Submission of a manuscript will constitute each author’s agreement that the Journal holds all propriety rights in the manuscript submitted, including all copyrights. Without the completion of the Copyright Transfer Agreement for all co-authors listed, manuscripts cannot be reviewed, thus delaying publication.

IX. CHARGES ASSOCIATED WITH PUBLICATION

a) Page Charges

There is no page charge for accepted manuscript printed page in the ADUM.

b) Colour Figure Charges

The cost of colour figures in the print version will be borne by the authors. Rates for colour reproduction are RM 100 per initial page of colour and RM 50 for each additional page of colour. However, there are no charges for figures and diagrams printed in black and white. Colour figures may be included in the online version of ADUM with no extra charges.

c) Reprint Charges

Reprints can be ordered for material printed in the ADUM. Quantities of reprints can be purchased with the reprint order form sent with page proofs to the contributors. Pre-payment is required for reprints. Bank draft and check are all acceptable forms of payment. Authors must pay for colour figures in reprints. Reprints will be mailed from 6 to 8 weeks after the article appears in the ADUM.