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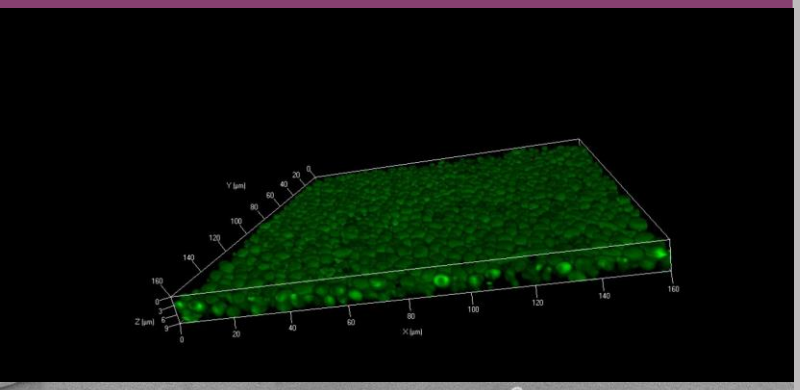

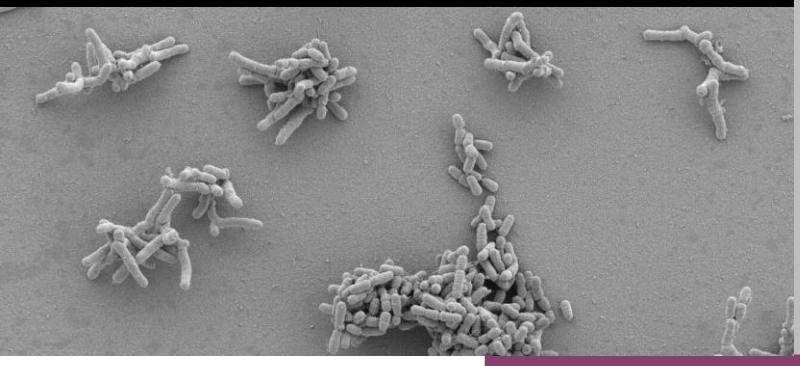

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Aims and Scope:

International Journal of Orofacial and Health Sciences (IJOHS) is a peer reviewed biannual international journal dedicated to publish high quality of scientific research in the field of orofacial sciences, health sciences and interdisciplinary fields, including basic, applied and clinical research. The journal welcomes review articles, original research, case reports and letters to the editor. Areas that are covered include but are not limited to dental sciences, oral microbiology and immunology, oral maxillofacial and craniofacial surgery and imaging, dental stem cells and regenerative medicine, dental biomaterial, oral maxillofacial genetic and craniofacial deformities.

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EDITORIAL

Introduction to IJOHS

Zainul Ahmad Rajion

Kulliyyah of Dentistry, International Islamic University Malaysia (IIUM)

It gives me great pleasure to write the foreword for the inaugural issue of the International Journal of Orofacial and Health Sciences (IJOHS).

The field of orofacial and health sciences is not static and the demand for studies addressing the large variety of current issues continues to grow. As an example, in medicine and dentistry, the planning and evaluation of maxillofacial surgery are dependent on advances in biomedical imaging for defining the underlying bony structures and their relationship to overlying soft tissue. Recently, the availability of state-of-the-art computed tomography (CT) has altered our approach to the analysis of complex craniofacial anomalies. Furthermore, the sophistication of medical imaging of the head and neck has advance significantly as a result of the marriage of computers and radiology and their close research collaboration between researchers and scientist, engineers and clinicians.

In view of this demand and the fact that numerous research findings published, there is a need for this journal, aims to bring together dentist, doctors and scientists, and other disciplines including computer expert and engineers to work together. Therefore, this journal hopes to create a medium for sharing ideas and importantly to provide a springboard for the application of multi-disciplinary and trans-disciplinary approaches with the common interest to share their knowledge and experience in many aspects of orofacial and health sciences. In addition to recognize, nurture and encourage scientific thinking that is required for the development and application of expanding biomedical knowledge and to foster scholarly interaction between them therefore contributing to the creation and improvement of sciences.

IJOHS is proud to launch its inaugural issue to keep informed of the activities and progress made. The editorial team believed that IJOHS will become the important source for the continuous research and commentary by offering an exceptional forum for the ongoing activities of the above professionals and to keep abreast of current trend and future developments.

We look forward to working together to achieve this important goal.

May I also take this opportunity to extend my grateful thanks to the Dean of the Kulliyyah of Dentistry, Dr. Salwana Supa'at for electing me as Chief Editor.

REVIEW ARTICLE

Genetics of malocclusion: A review

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Abstract

Malocclusion is one of the most common craniofacial problems observed worldwide. Affected individuals suffer not only from aesthetic concerns but also from functional problems, such as with mastication and pronunciation. The prevalence of malocclusion in East Asians is higher than in other races. Reports have shown besides environmental factors, there is association between certain types of malocclusion with specific genes. Positive association of mandibular prognathism has been implicated to genes such as *Matrilin-1;* while mutation in *DUSP6* has also been shown to contribute to the incidence of malocclusion. This review aimed to briefly discuss the involvement of other additional genes such as *MYO1H* and *PAX9* in the incidence of malocclusion as observed from our local institution.

Keywords: malocclusion, genes

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Introduction

Malocclusion is one of the most common dental problems in mankind, together with dental caries, gingival disease and dental fluorosis (Dhar *et al.,* 2007). A malocclusion is defined as an irregularity of the teeth or a mal-relationship of the dental arches beyond the range of what is accepted as normal (Walther *et al.,* 1994). Malocclusion should not be considered as abnormal or pathological, instead as a variation of occlusion in a continuous multifactorial trait (Nishio *et al.,* 2016).

Nonetheless, the condition of malocclusion may lead to distorted facial appearance, limited masticatory function, increased risk of dental trauma and compromise the quality of life (Claudino *et al.,* 2013).

Classification of skeletal and dental malocclusion

The deviations from normal occlusion can be presented clinically from skeletal and/or dental. Skeletal discrepancy is caused by the distortion of the proper mandibular and/or maxillary growth during fetal development (Joshi *et al.,* 2014). This can occur in any three plan of space:

anteroposterior, vertical and transverse (Alhammadi, 2019). Salzmann in 1950 was among the first to classify to the underlying skeletal structure into Class I, Class II (convex profile) and Class III (concave profile).

Dental malocclusion may be classified according to several classifications. One classification is by British Standard Institution (BSI, 1983) classifying the occlusion according to the incisor relationship, into Class I, Class II Division 1, Class II Division 2 and Class III. Class I incisor relationship is when the mandibular incisors edges occlude with or lie immediately below the cingulum plateau of the maxillary central incisors. Class II incisor relationship is subdivided into division 1 and division 2 according to the inclination of the upper incisors. Class II division 1 occurs when the maxillary central incisors are proclined (or with average inclination) with an increased overjet. Whereas, class II subdivision 2 happens when the maxillary central incisors are retroclined and the overjet can be minimum or maybe increased. Class III is when the mandibular incisors edges lie anterior to the cingulum plateau of the upper central incisors with the overjet reduced or reversed.

Angle classification used occlusal relationship of the first molar to classify type of malocclusion into three classes that are Class I, Class II and Class III (Weinberger, 1993). Class I molar relationship is characterized by normal mesio-distal relation of the jaws and dental arches, as indicated by the normal locking on eruption of the first permanent molars, at least in their mesio-distal relations, though one or more may be in buccal or lingual occlusion. Class II molar relationship can be explained by the molar relationship shows the buccal groove of the mandibular first molar distally positioned when in occlusion with the mesio-buccal cusp of the maxillary first molar.

On the other hand, Class III molar relationship is classified when the molar relationship shows the buccal groove of the mandibular first molar mesially positioned to the mesio-buccal cusp of the maxillary first molar when the teeth are in occlusion.

Prevalence of malocclusion

The prevalence of dental malocclusion in East Asians especially Class III is higher than in other races (Soh *et al.,* 2005). This has been supported by a finding by Chu *et al.* (2007), which compared their study with those from surveys of young Caucasians, Africans and Asians. This study showed that the prevalence of Class I malocclusion in Chinese adults was higher than that in Caucasian adults (48% versus 23%), but was similar to that of Asian (48%) or African (50%) young adults. The prevalence of Class III malocclusion in Chinese and in Asian adults is higher than that in African adults (20% versus 14%). Although Class II malocclusion is less common in the Chinese young adults, a study using peer assessment rating index reported that Class II malocclusion being more severe than Class I or III malocclusion in young Asian males (Soh *et al.,* 2005). While in the Northern part of Saudi Arabia, Class I malocclusion was dominant, followed by Class II and Class III, respectively (Alajlan *et al.,* 2019).

Hardy *et al.* (2012) through his meta-analysis study reported that, Chinese from Hong Kong and Malaysian showed a relatively higher prevalence of Angle Class III malocclusion. In addition, Indian populations showed a relatively lower prevalence as compared to other races (Hardy *et al.,* 2012). Our own demographic study showed that Class III

(according to BSI Incisor Classification) represents the majority of malocclusion cases observed in our local setting, whereby Malays constitute the highest number of orthodontic patients followed by Chinese and Indians (Ismail *et al.,* 2017). The prevalence data indicated that the occurrence of different types of malocclusion varies according to geographical location.

Genetics and malocclusion

Aetiologically, skeletal malocclusions arise from skeletal disharmonies. Thus, it is essential to have a good understanding of the skeletal growth in general. In orthodontics, one of the most challenging aspects in treating patients is predicting their craniofacial growth patterns. In this respect, it is important to understand how genetic factors and their interactions with environmental factors affect facial growth in a particular individual.

Study of the aetiology of malocclusion is a complex subject since both genetic and environmental factors may affect craniofacial development (Mossey, 2014). Several studies have shown that there is a strong link of malocclusion especially skeletal malocclusion Class III or mandibular prognathism (MP), with both genetic as well as environmental factors (Jena *et al.,* 2005; Chaturvedi *et al.,* 2011; Hartsfield *et al.,* 2012).

The relative genetic contribution to Class III malocclusion has been the subject of interest of many researchers. Some evidence has been found suggesting that genetic factors contribute to the malocclusion susceptibility. In a review article, Moreno *et al.* (2015) mentioned that association studies have found positive correlations for mandibular prognathism and genes *EPB41, SSX21P* and *PLXNA,* located within the locus 1p22-p36, while genes *COL2A1, TGFB3,* and *LTBP2* within the 12q13-q24 locus. MATRILIN-1 is a cartilage matrix protein and its polymorphism has been shown to be associated with mandibular prognathism in Korean population (Jang *et al.,* 2010). Genotyping results showed that the *Matrilin-1* polymorphism haplotype TGC had a pronounced risk effect for mandibular prognathism compared with controls which suggest that polymorphisms in *Matrilin-1* could be used as a marker for genetic susceptibility to mandibular prognathism.

The mutation in *DUSP6* has also been identified in cases of malocclusion and reinforces that the 12q22-q23 region is biologically relevant to craniofacial development and may be genetically linked to the Class III malocclusion (Nikopensius *et al.,* 2013). Very recently, Nowrin *et al.* (2019) detected a missense mutation in EXON 3 of *DUSP6* gene in three members of a Malaysian Malay family with Class III malocclusion. This study further acknowledged the importance of *DUSP6* gene in skeletal functions (Nowrin *et al.,* 2019).

With the advancement of dentofacial phenotyping and the availability of large-scale genomic data analysis, the fundamental aspect of genetic mechanism which underlies the developmental process of craniofacial complex is unravelled. Additionally, available genetic analysis such as linkage analysis, whole exome sequencing, polymorphism or mutational analysis has enabled genetic association study to be performed on malocclusion cases, hence broadened the knowledge on the involvement of certain genes with the incidence of malocclusion.

Pax9 Genes

Alterations in genes which are important during the process of craniofacial development have been associated with the incidence of craniofacial abnormalities. Paired Box 9 gene (*PAX9 gene)* located at chromosome 14 (locus 14q13.3) is a gene family which is responsible in tooth as well as skeletal development (Ghergie *et al.,* 2013). Anne *et al.,* (2015) claimed that *PAX9* gene regulates cell proliferation, migration and determination in multiple neural crest-derived lineages, such as cardiac, sensory, and enteric neural crest, pigment cells, glia, craniofacial skeleton and teeth, or in organs developing in close relationship with the neural crest such as the thymus and parathyroids. *PAX9* gene is a protein encoding gene that encodes the transcription factor that is important for craniofacial and dental development (Seo *et al.,* 2013). Krivicka-Uzkurele *et al.,* (2016) stated *PAX9* gene is expressed in the developing facial processes and influence the formation of lower face. Kavitha *et al.,* (2010) found that *PAX9* gene has 4 exons which are highly conserved in human being. Mutated *PAX9* gene is frequently associated with oligodontia or hypodontia as well as Class II/Division 2 malocclusion (Ghergie *et al.,* 2013a). Animal studies conducted by Peter *et al.,* (1998) and Nakatomi *et al.,* (2010) found that mutated or absence of *PAX9* gene shown poor development of skeletal and odontogenesis with lack of coronoid process formation. Peter *et al.,* (1998) added this particular gene was highly expressed at the region of pharyngeal pouches, mesenchyme of nasal processes, maxillary and mandibular arches, as well as at the area of developing tooth buds hence supporting the importance of *PAX9* in craniofacial, tooth and skeletal development.

Polymorphism in *PAX9* gene; SNP marker rs8004560, has been suggested to have an association with Class II/Division 2 malocclusion with hypodontia except the third molar (Wall *et al.,* 2009). Ghergie *et al.,* (2013a) also found association between *PAX9* SNP (rs8004560) with Class I malocclusion patients. We have performed sequencing analysis on patients with Class II skeletal base malocclusion for *PAX9* SNP (rs8004560). However, no significant association of *PAX9* SNP (rs8004560) with Class II skeletal base was observed from our local population (Saad *et al.,* 2018). This might be due to small number of samples recruited in our study.

Myo1H Genes

Another gene which has been shown to be associated with malocclusion is *MYO1H*. *MYO1H,* located at 12q24.11 is a class 1 myosin that is in a different protein grouping than the myosin heavy chain isoforms found in the skeletal muscle sacromeres, which are the basis of fibre typing. Myosin is superfamily of motor proteins that involve in generating force and movement along actin filaments (Mooseker and Cheney, 1995). Class 1 myosin is necessary for cell motility; phagocytosis and vesicle transport (Rowlerson *et al.,* 2005). Myosin heavy chain isoforms was revealed to be found in the masseter muscle via immunohistochemical staining and gene expression studies (Arun *et al.,* 2016). Few studies suggest that muscle affect the skeletal growth during embryonic stage, postnatal stage, and homeostatic relationship in adult and aging process (Brotto, 2015). Therefore, genetic alteration in genes responsible for muscle function will also affect the skeletal growth. In a recent article, Sun *et al.* (2018) have shown that the expression of *MYO1H* orthologous genes were detected at mandibular jaw of zebrafish model, whereby jaw cartilage defects were demonstrated in the *MYO1H* knockdown

model. These developmental and functional studies strongly demonstrate the importance of *MYO1H* gene for proper jaw growth and development and its contribution towards the pathogenesis of mandibular prognathism and mandibular retrognathism in human (Arun *et al.,* 2016; Sun *et al.,* 2018).

Tassopoulou-Fishell *et al.* (2013) reported significant association between *MYO1H* SNP (rs10850110) with mandibular prognathism patients whom are mostly Caucasian. Ghergie *et al.* (2013b) also performed single nucleotide polymorphism analysis of *MYO1H* gene (rs10850110) on malocclusion Class I, II and III from Romanian population. Their study also detected association of *MYO1H* SNP (rs10850110) allele and genotypes with different malocclusion cases. Arun *et al.* (2016) studied genetic association by performing PCR-RFLP methods on three SNP markers of *MYO1H* on mandibular retrognathism cases. These markers include rs10850110, rs11611277 and rs3825393. The SNP rs3825393 showed a statistically significant association with mandibular retrognathism, while no association was detected in other two polymorphism markers with mandibular retrognathism (Arun *et al.,* 2016). Due to these findings, we also initiated a preliminary analysis of *MYO1H* single nucleotide polymorphism of rs10850110 on mandibular prognathism cases, but no significant association was observed. Again, small sample size might contribute to this finding (Yahya *et al.,* 2018). Thus, we are proposing for larger number of samples to be recruited for future genetic association study. In addition, the criteria for inclusion and exclusion to fulfil the exact classification of the malocclusion must be followed strictly.

To date, most of the genetic studies looking into the polymorphism of these genes with malocclusion have been done in other parts of the world. As far as we are aware, scanty data regarding dental malocclusion and its genetic analysis is available from our local population (Esa *et al.,* 2001). Thus, we hope that the ongoing studies carried out in this institution could provide new scientific information for the betterment of the knowledge in the management and treatment of malocclusion in this population. This could attribute to clinicians and researcher in the field of craniofacial research.

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ORIGINAL ARTICLES

Potential antibacterial effects of flaxseed and *Nigella sativa* extracts on *Streptococcus pyogenes*

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Abstract

Antibiotic resistance is a major global problem, associated with inadvertent drug usage. Herbal interventions are a therapeutic strategy that warrants greater research attention. Flaxseed and *Nigella sativa* are well recognized original super foods that have demonstrated potent anti-microbial and anti-biofilm activities. In the oral cavity, the bacterial population is a result of the dynamic relationship between pathogens and commensals *Streptococcus pyogenes* is an important global human Gram-positive pathogen that causes a wide variety of acute infections, it is highly virulent since it has the ability overcome the host defence system. This in vitro study aims to evaluate antimicrobial activity of flaxseed and *Nigella sativa* extract against *S. pyogenes*. Ethanolic extract of flaxseed and *Nigella sativa* extracts were prepared and the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against *S. pyogenes* was estimated. The results of this study show that both extracts exhibited antibacterial activity against *S. pyogenes*. Present study demonstrated the bactericidal activity of both extracts which can be an adjunct to the future natural anti-bacterial therapy.

Keywords: Antibacterial effect, flaxseed, Nigella sativa, Streptococcus pyogenes

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Introduction

Nowadays, there is a consumer preference for natural products over synthetic drugs. One of the main reasons for the same is to avoid the adverse effects of synthetic medications and the risks of bacterial resistance (David & Gordon, 2012). In the oral cavity, the bacterial population is a result of the dynamic relationship between pathogens and commensals *Streptococus pyogenes* may contribute to many human diseases, ranging from mild superficial skin infections to life-threatening systemic diseases. Infections typically begin in the throat or skin. Infections due to certain strains of *S. pyogenes* can be associated with the release of bacterial toxins that can lead to scarlet fever (Hammer, 2007). Other toxigenic *S. pyogenes* infections may lead to streptococcal toxic shock syndrome, which can be life-threatening (Hammer, 2007). The increase in the incidence of invasive *S. pyogenes* infection has frequently been associated with specific clones, which raises the

possibility that the rise of particularly virulent clones was responsible for this re-emergence - in particular, the MT1 clone which is dominant among invasive *S. pyogenes* isolates in most developed countries (Luca-Harari *et al.*, 2009). Variation in the distribution may lead to fluctuations in the severity of infections and in overall mortality rates. *S. pyogenes* infection may be observed in persons of any age, although the prevalence of infection is higher in children because of the combination of multiple exposures (in schools or nurseries, for example) and host immunity (Martin *et al.,* 2004). The prevalence of pharyngeal infection is highest in children older than three years and has been described as a 'hazard' in school-aged children (Martin *et al.,* 2004). Contemporary data suggested that invasive *S. pyogenes* infections incidence is around 2 to 4 per 100,000 population in developed countries (Steer *et al.,* 2012).

Numerous observational studies have described the frequencies of potential risk or predisposing factors in patients with invasive *S. pyogenes* disease, rigorous assessment through analytical means have been limited. The relative importance of these factors may change over time as the prevalence of the acute or chronic predisposing factors changes in frequency, such as influenza activity (Zakikhany *et al*., 2011). Infection of *S. pyogenes* in people lacking of teeth causes oral and maxillofacial cellulitis prior to sepsis. In this case, *S. pyogenes* originated from sinusitis leaked to oral cavity thus, leading to systemic infection through wounding of oral cavity mucosal lining. The study found that, the risk of odontogenic infection still there even among edentulous patients (Inagaki *et al.*, 2017). Penicillin remains the drug of choice for the empirical treatment of *S. pyogenes* infection, despite over sixty years of use. *S. pyogenes* has also remained uniformly susceptible to penicillin and resistance towards penicillin or other β-lactams which has been approved for the treatment of *S. pyogenes* (Spellerberg & Brandt, 2016).

Flaxseed and flaxseed oil (also called linseed oil) originated from the flax plant (*Linum usitatissimum*). Flaxseed protein extracts have demonstrated antibacterial activities against most tested microorganisms, especially Gram-negative bacteria. Meanwhile, flaxseed oil has been shown to have antibacterial potential against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* K-12 (Kaithwas *et al*., 2011). Evidently, flaxseed contains the highest content of lignin and secoisolariciresinol diglucose (SDG) among all grains, and is the richest dietary source of plant-based SDG (Liggins *et al*., 2000; Zhang & Xu, 2007). Flaxseed derivatives, such as defatted flaxseed meal or flax hulls, have higher concentrations (2.3 % and 4 % respectively) of SDG (Gaafar *et al*., 2013). Their usage as a dietary supplement is becoming more popular nowadays as a series of researches have highlighted its multitudinous effect on human health. However, there are still a lot of ongoing studies on the means of optimizing the beneficial effects of this called magic plant (Pan *et al*.,2009).

Nigella sativa L. (*Ranunculaceae*) – commonly as "black cumin" – is a herbaceous plant that grows in the Mediterranean countries and Turkey. It is known to have therapeutic potential; in fact, *sativa*-based oils are claimed to have potent anti-inflammatory, anticancer, antidiabetic, antimicrobial, antihistaminic, and antihypotensive effects (Al-Rowais, 2002; Salem, 2005). *N. sativa* contains many components that have pharmaceutical effects such as: thymohydroquinone, dithymoquinone, thymol, carvacrol, nigellicine, nigellimine-x-oxide, nigellidine, and alpha-hedrinhave (Aljabre *et al*., 2005). Thymoquinone is one of the main components of *N. sativa*

that has anti-microbial, anti-inflammatory, anti-hypertensive, anti-carcinogenic, antioxidant, and hepatoprotective effects (Tariq, 2008 & Ahmad *et al.,* 2013).

The present study has been conducted to evaluate the antibacterial effect of flaxseed and *N. sativa* extracts against *S. pyogenes* which is believed to be resistant to different types of antibiotics, the implication of this study will be useful in propagating the use of these natural based products as therapeutic medications.

Materials and Methods

Bacterial strains

Streptococcus pyogenes (ATCC®19615™) was used in this study. The cultures as obtained from the American Type Culture Collection (Manassas, VA, USA). Bacterial strain was stored in tryptic soy broth (TSB) with 20% glycerol at -80°C and used as required. Nutrient agar and nutrient broth (Merck) were used to culture the bacterial strains.

Flaxseed and *Nigella sativa* extracts

In collaboration with Philadelphia University, 500 grams of flaxseeds were ground using a dried blender and extracted using 99.8% ethanol in a Soxhlet chamber. The extract was collected and evaporated in a rotary evaporator under pressure at 60°C. Freeze-drying of the concentrated extracts was done for about 30 minutes to remove the water residues. The crude extracts were stored at 4°C pending further use.

The extracts of flaxseed were dissolved in 20% of dimethyl sulfoxide (DMSO) and filter-sterilized using a 0.22 µm PES syringe filter. The concentrations of the flaxseed extracts were 1, 5, 10, 20, 50, and 100 mg/ml. All extracts were diluted with DMSO to achieve the desired concentrations. Similar protocol was reflected for *N. sativa*.

Antimicrobial sensitivity tests

Bacterial growth

The bacteria were cultured on nutrient agar and inoculated in nutrient broth. The plates were incubated at 37°C for 18 to 48 hours. For broth media that were incubated for 24 hours, 10 µl from the bacterial stock was revived at 37°C to be used as the inoculum. The turbidity of the suspensions were adjusted to 1.5 to 3 x 10^8 cells/ml, which corresponded to an absorbance of 0.08 – 0.10 at a wavelength of 625 nm (Vanessa Maria Fagundes *et al.*, 2014).

Disk diffusion method

The sensitivity of *S. pyogenes* to the plant extracts was determined via the Kirby-Bauer disk diffusion method (Aqueveque *et al.,* 2006; Bauer *et al.,* 1966; Devi *et al.*, 2011) as well as the European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommendations. The nutrient agar was inoculated by swabbing with a sterile cotton swab that has been soaked in a bacterial broth. With a slight modification from previous studies, aqueous extract with 100 mg/mL concentration were pipetted with different volume (1, 5, 10, 20, 50 and 100 µl) onto sterile blank discs with 6 mm diameter (Oxoid, Badhoevedorp, Netherlands) and the discs were allowed to dry in the biosafety cabinet before being impregnated onto agar plate spread with inoculum (Revathi & Malathy, 2013). A standard antibiotic, penicillin was used as positive control for all tested bacteria while DMSO was used as negative controls. All agar plates were incubated in an incubator at 37°C for 18 to 24 hours. The positive control was penicillin while the negative control was DMSO. Susceptibility testing was performed in three biological replicates. The plates were observed for the presence of an inhibition zone. The diameters of the inhibition zones were measured (in mm) for each strain, and the mean values calculated. The absence of

inhibition zone was interpreted as absence of antimicrobial activity.

Statistical Analysis

The means and standard errors (SE) were calculated using Microsoft Excel 2010 (Microsoft Corporation, Redmond, CA, USA).

Result and Discussion

In this study, flaxseed and *N. sativa* extracts at concentrations of 5 to 100 mg/ml inhibited *S. pyogenes* which was similar to the positive control and this in line with the finding with Warnke *et al*., (2008). *N. sativa* showed inhibition zones to S. *pyogenes* at > 20 mg/ml concentration. This was similar to Hasan *et al*., (2013) in which the highest antimicrobial activity was recorded at 100 mg/ml. These plant extracts have considerable activity against Gram-positive bacteria but not Gram-negative (Alhaj *et al*.,2008).

The biological activities of the compounds from the plant extracts depend on the type of solvent that was used during extraction. The most commonly-used solvents were methanol, ethanol, and water (Parekh *et al*., 2009). In this study, the inhibition zones produced by the flaxseed and *N. sativa* extracts were not very high probably because of agro-climate factors, handling of the extracts, as well as the phytochemical ingredients of the extracts (Erdman *et al.*, 2007). Most active antimicrobial compounds were soluble in polar rather than nonpolar solvents (Parekh *et al*., 2009).

We have studied the antimicrobial activities of flaxseed and *N. sativa* extracts of various concentrations against *S. pyogenes*. The results are shown in Table 1. According to Table 1, the diameters of the inhibition zones of *S*. *pyogenes* in *N. sativa* and flaxseed extracts of 100 mg/ml were 6.33 ± 0.33 mm and 6.00 ± 0.0 mm, respectively. At the lowest concentration of the extracts (1 mg/ml), the diameters were 5.67 ± 0.33 mm and 6.00 ± 0.58 mm, respectively. The experiments were done in triplicates and the results expressed in terms of mean ± SE.

Antibacterial effects were demonstrated by the flaxseed extract at concentrations ranging from 5 to 10 mg/ml. From 20 to 100 mg/ml, the antibacterial effects of the flaxseed extract were the same. Evidently, the lignans of flaxseed (secoisolariciresinol) were effective against *S. aureus* and *Vibrio sp.* (Barbary *et al*., 2010). The *N. sativa* extract showed antibacterial effects at concentrations ranging from 1 to 100 mg/ml. In this study, it was effective against *S*. *pyogenes* bacteria. Evidently, a number of plant-derived compounds are more effective against Gram-positive bacteria then Gram-negative bacteria (Morsi, 2000; Ali *et al.*, 2001; Jones *et al.*, 2002).

Table 1. Inhibition zones of *S. pyogenes* in *Nigella sativa* and flaxseed extracts (n=3).

Test extract	**Positive control (mm)**	**Concentration of extract (mg/ml)**					
		1	5	10	20	50	100
Nigella sativa	31.7±1.67	5.67±0.33	5.33±0.33	6.67±1.20	5.67±0.33	6.67±0.33	6.33±0.33
Flaxseed	25.0±2.89	6.00±0.58	6.00±0.00	5.33±0.33	5.33±0.33	5.67±0.33	6.00±0.00

The Minimum Inhibitory Concentration (MIC) of flaxseed, *N. sativa* extracts were determined using resazurin based 96-well plate microdilution method. After the incubation period, columns with no colour changes (blue resazurin colour remain unchanged) were scored as (MIC) value. The result showed that *N. sativa*, flaxseed extract shared the same MIC which was 12.5 mg/ml on *S. pyogenes* (Table 2). Previous studies reported that MIC value for *N. sativa* extract was between <0.25 µg/ml and 1.0 µg/ml of *Staphylococci* species (Ayse *et al*., 2016 & Magdalena *et al.,* 2014). The difference may be due to the presence of various chemical compounds in this type of extract which affect the results of MIC towards *S. pyogenes*, and this may be due to the method of isolation and fractionation that provides a specific target of bioactive compound with antimicrobial properties (Shrivastava *et al.,* 2011). The antibacterial activity of flaxseed extract is associated with its ability to merge with bacterial cell wall thus, combating bacterial growth. Other than that, the existence of long-chain unsaturated fatty acids such as alpha linolenic acid and linoleic acid might contribute to the antimicrobial therapeutic efficacies of flaxseed (Barbary *et al*., 2010).

Table 2. Minimum Inhibitory Concentration (MIC) value (mg/ml) on *S. pyogenes*

Types of Extract		
Nigella sativa	Flaxseed	Penicillin
12.5	12.5	25

Conclusion

In conclusion, flaxseed and *Nigella sativa* extracts have the potential to be developed as antibacterial agents against *S. pyogenes*. However, in this study, the author suggest that these extracts should be explored in vivo to elicit a greater effect to the whole organism systems based on its toxicity, safe dosage as well as its effect on the normal microbiota in the future. Further investigations can be carried out on the synergestic effect since both extracts have good potential to be effective antimicrobial agents in the medical practice.

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Dental treatment needs among patients undergoing screening at a university-based dental institution in Kuantan, Pahang, Malaysia

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Abstract

University-based dental institution in Malaysia receives large number of dental visits, however, dental treatment needs among patients attending this kind of institution is not usually reported. This study aimed to identify the trend of dental treatment needs in a university-based dental institution in Kuantan, Pahang situated in the East Coast region of Peninsular Malaysia. This cross-sectional study utilized secondary data, obtained from list of patients who underwent for screening at Outpatient Clinic, Kulliyyah of Dentistry, International Islamic University Malaysia from 1st January to 31st December 2016. Patient's age, gender, residential area and dental treatment needs were retrieved from the list. All patients were included except those with incomplete data, with old Malaysian identification card or foreign passport or those assigned to receive Orthodontic treatment. Descriptive statistics and Pearson's Chi Square test was run using SPSS® Version 20 software. Conservative care (22.7 %) was the major treatment need among 2,627 patients included in this study. Teenage and adult patients mostly required conservative care while the elder-aged patients have major need for prosthodontics. Females outnumbered males in requiring all types of dental treatment, except for conservative care which was pre-dominantly required by males. Patients residing non-urban area majorly required all types of dental treatment except for endodontics and conservative care which were more frequently necessitated by patients from urban area. Conservative care was the major dental treatment need. The type of dental treatment needs has significant association with patient's age, gender and residential area.

Keywords: treatment need, screening, dental, age, gender

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Introduction

Need is classified into normative, felt, expressed or comparative needs according to Bradshaw (1972) taxonomy. Normative need for dental treatment is a category of need which is usually defined by the experts or professional. However, the process of dental screening usually takes into consideration the felt or

perceived need according to patients' complaints on their oral health conditions. Dental professional or a dental officer then assesses the suitability of dental treatment for the patients.

Dental treatment needs and the oral health status of Malaysians were periodically assessesed every ten years from 1990 to 2010 using National Oral Health Survey of Adults (NOHSA). Since 2010, the oral health status and dental treatment needs in Malaysia have not been assessed at any national scale study. In the meantime, there are local studies that studied on specific dental treatment need, for instance the orthodontic treatment need among various study populations including of school children aged 12 and 16 years old (Zreaqat *et al.*, 2013), adolescents aged 13 to 14 years old (Zamzuri *et al.*, 2014), adults aged 20 to 70 years old (Ravindranath *et al.*, 2017) as well as among the special need people with Down Syndrome (Abdul Rahim *et al.*, 2014). Nevertheless, there are also researches that analysed on general dental treatment needs in certain populations in Malaysia, for instance, among the special need children in Negeri Sembilan, a state which is situated in the centre of Peninsular Malaysia (Mokhtar *et al.*, 2016); among normal (Oo *et al.*, 2011) and hearing-impaired school children (Rahman *et al.*, 2015) as well as among the elderly (Sinor, 2013; Sinor *et al.*, 2018) in Kelantan, a state located in the North East Region of Peninsular Malaysia.

However, there is no specific study that has contextualizes the dental treatment needs based on any university-based Malaysian dental institution. Dental Clinic at Kulliyyah of Dentistry, International Islamic University Malaysia which is established in 2006 is the only university-based dental service provider in the city of Kuantan, Pahang, Malaysia and it is one of the two university-based dental centres that cater for the East Coast region in Malaysia. The number of patients may reach up to 10,000 visits per year indicative for a high demand and need for dental treatment among the surrounding community. In view of this, this study aims to analyse the trend of major dental treatment need in this population.

Materials and Methods

Study Location and Data Collection

Ethical approval (IREC 762) was obtained from IIUM Research Ethics Committee. This study was conducted at IIUM Dental Outpatient Clinic in Kuantan, Pahang. Kuantan is the capital city of Pahang, situated at the East Coast region of Peninsular Malaysia. Our study population was all first-visit patients undergoing screening at IIUM Dental Outpatient Clinic from 1st January 2016 until 31st December 2016. Information on patient's age, gender, residential area and the type of dental treatment needs was retrieved from Outpatient Clinic database. Type of treatment needs were decided by dental officer in-charge at this clinic after carrying out the usual procedure of screening and dental charting for patient's oral health condition. Treatment need was then notified in the patient's folder, and was remarked into the Patient Waiting List of any departments according to their treatment needs. Patients with incomplete data or with old Malaysian identification card or foreign passport and those attended Orthodontic department are excluded.

For demographic profile analysis, patients were classified based on their age group, gender and residential area in Kuantan. Age were classified into six groups of 14 years old and below, 15 to 19 years old, 20 to 34 years old, 35 to 44 years old, 45 to 64 years old, and 65 years old and above. Residential areas were classified into urban and non-urban areas according to Kuantan Municipal

Council. Dental treatment needs was classified as stated in the clinic database; endodontics (anterior), endodontics (posterior), fixed prosthetic (bridge), fixed prosthetic (crown), partial denture, full denture, paediatric dentistry, oral surgery, general dental practice, oral maxillofacial, conservative care and periodontal need.

Statistical Analysis

Descriptive statistics was used to analyze the demographic profiles of this population. Association of demographic background (age, gender and residential areas) with the type of dental treatment needs was analysed using Chi-Square test in SPSS® software Version 20.

Results

Demographic Profiles

Demographic profiles of first-visit patients at IIUM Dental Outpatient Clinic for the year 2016 are tabulated in Table 1. The major age group of patients was from the age of 20 to 34 years old (39.5 %) while the least number of patients was from the age group of 65 and above (4.2 %). The mean age of the patients was 32 ± 17.61 years old. Female patients (55.5 %) outnumbered male patients (44.5 %) with majority of patients residing non-urban areas (37.6 %).

Dental Treatment Needs

Figure 1 shows distribution of dental treatment needs in which the highest treatment need was conservative care (22.7 %) while the lowest treatment need was for oral maxillofacial care (0.6 %).

Association of demographic profiles with types of dental treatment needs

Table 2 shows cross tabulation of age with the type of dental treatment needs. There is significant association between age, X^2 (55, N = 2,627) = 3,466.9, p < .0001 with the type of dental treatment needs. Paediatric patients (age group of ≤ 14) were almost exclusively assigned to receive paediatric dentistry care (97.5 %). Younger-age patients (age group of 15-19, 20-34 and 35-44) mostly required conservative care (38.9 %, 30.9 % and 30.3 %, respectively), followed by periodontal need (17.2 %, 24.5 % and 17.0 %, respectively). Elder-age patients (age group of 45-64 and ≥ 65) have major need for prosthodontics with 30.5 % and 40.5 %, respectively for partial dentures and 9.4 % and 31.5 %, respectively for full dentures. The need for partial dentures increases with increasing age, this is noticeable from the age group 35-44 (8.9 %), 45-64 (30.5 %) and ≥65 (40.5 %). This is also similar to the need for full dentures that begins from the age group of 45-64 (9.4 %) and drastically increased in the age group of ≥65 (31.5 %).

In contrast, the need for oral surgery decreases with increasing age; the need was highest among patients from the age groups of 20-34 (17.9 %), 35-44 (16.7 %), 45-64 (9.6 %) and ≥ 65 (8.1 %). The need for oral maxillofacial care is consistently low compared to other treatment needs across all age groups. This study also found that types of dental treatment needs also significantly differed by gender, X^2 (11, N = 2,627) = 49.8, p < .0001. As cross tabulated in Table 3, females outnumbered males in all types of dental treatment need, except for periodontal need which was pre-dominated by males. The relation between residential areas and the type of dental treatment need was also significant, X^2 (11, N = 2,627) = 112.6, p <.0001.

As shown in Table 4, people residing non-urban areas outnumbered those residing urban areas in getting fixed-prosthetic (bridge) (50.6 %), fixed-prosthetic (crown) (58.5 %), partial denture (60.0 %), full denture (79.1 %), paediatric dentistry care (62.3 %), oral surgery (57.6 %), general dental practice (52.6 %) and periodontal care (51.7 %).

Table 1. Demographic profiles of patients underwent for screening at IIUM Dental Outpatient Clinic for the year 2016.

Demographic Profiles	n (% of total)
Age	
≤14	434(16.5 %)
15 to 19	157(6.0 %)
20 to 34	**1,037 (39.5 %)**
35 to 44	347 (13.2 %)
45 to 64	541 (20.6 %)
≥65	111 (4.2 %)
	Total = 2,627 (100.0 %)
Gender	
Male	1,168 (44.5 %)
Female	**1,459 (55.5 %)**
	Total = 2,627(100.0 %)
Residential Areas	
Urban	1,251 (47.6%)
Non-urban	**1,376 (52.4%)**
	Total=2,627 (100.0%)

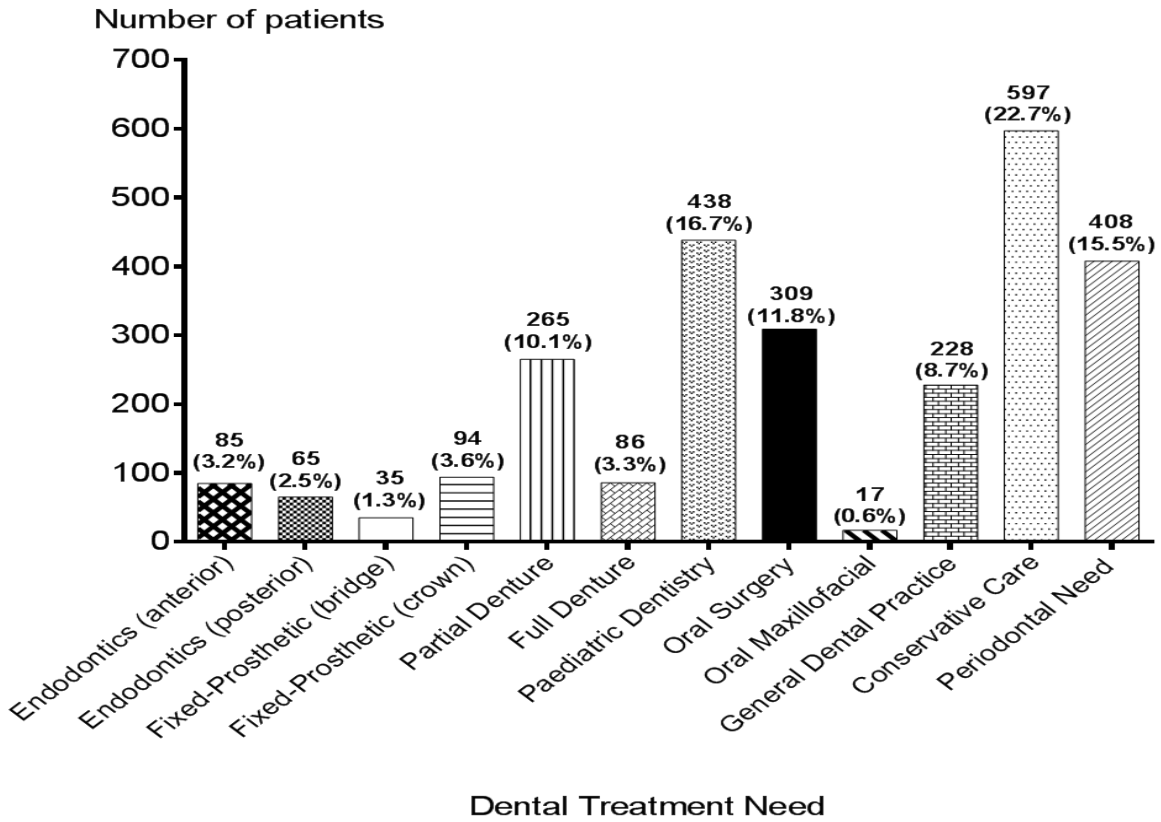

Figure 1. Distribution of dental treatment needs among patients underwent for screening at IIUM Dental Outpatient Clinic for the year 2016.

Table 2. Cross tabulation of dental treatment needs versus age of patients underwent for screening at IIUM Dental Outpatient Clinic for the year 2016

Age group	Dental Treatment Needs													Statistical Analysis
	a	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**	**k**	**l**	**Total**	
≤14														
Count	0	0	0	1	0	0	423	0	0	3	2	5	**434**	
% within age	0.0%	0.0%	0.0%	0.2%	0.0%	0.0%	97.5%	0.0%	0.0%	0.7%	0.5%	1.2%	**100.0%**	
% within treatment	0.0%	0.0%	0.0%	1.1%	0.0%	0.0%	96.6%	0.0%	0.0%	1.3%	0.3%	1.2%	**16.5%**	
15 to 19														X^2 (55, N = 2,627) = 3,466.9**, p <.0001**
Count	13	4	1	4	1	0	15	4	4	23	61	27	**157**	
% within age	8.3%	2.5%	0.6%	2.5%	0.6%	0.0%	9.6%	2.5%	2.5%	14.6%	38.9%	17.2%	**100.0%**	
% within treatment	15.3%	6.2%	2.9%	4.3%	0.4%	0.0%	3.4%	1.3%	23.5%	10.1%	10.2%	6.6%	**6.0%**	
20 to 34														
Count	49	32	10	37	23	0	0	186	11	115	320	254	**1,037**	
% within age	4.7%	3.1%	1.0%	3.6%	2.2%	0.0%	0.00%	17.9%	1.1%	11.1%	30.9%	24.5%	**100.0%**	
% within treatment	57.6%	49.2%	28.6%	39.4%	8.7%	0.0%	0.00%	60.2%	64.7%	50.4%	53.6%	62.3%	**13.2%**	

Note: Treatment a-Endodontics (Anterior), b-Endodontics (Posterior) c-Fixed-prosthetics (Bridge), d-Fixed-Prosthetics (Crown), e- Partial Denture, f-Full Denture, g-Paedodontics, h-Oral Surgery, i-Oral Maxillofacial, j-General Dental Practice, k-Conservative Care and l-Periodontal Need. Chi-square test, X^2 (55, N = 2,627) = 3,466.9, p < .0001

Table 2. (Continued)

Age group	Dental Treatment Needs													Statistical Analysis
	a	b	c	d	e	f	g	h	i	j	k	l	Total	
35 to 44														
Count	13	15	8	21	31	0	0	58	1	36	105	59	**347**	
% within age	3.7%	4.3%	2.3%	6.1%	8.9%	0.0%	0.0%	16.7%	0.3%	10.4%	30.3%	17.0%	**100.0%**	
% within treatment	15.3%	23.1%	22.9%	22.3%	11.17%	0.0%	0.0%	18.8%	5.9%	15.8%	17.6%	14.5%	**39.5%**	
45 to 64														
Count	9	14	15	30	165	51	0	52	0	44	105	56	**541**	
% within age	1.7%	2.6%	2.8%	5.5%	30.5%	9.4%	0.0%	9.6%	0.0%	8.1%	19.4%	10.4%	**100.0%**	
% within treatment	10.6%	21.5%	42.9%	31.9%	62.3%	59.3%	0.0%	16.8%	0.0%	19.3%	17.6%	13.7%	**20.6%**	
≥65														
Count	1	0	1	1	45	35	0	9	1	7	4	7	**111**	
% within age	0.9%	0.0%	0.9%	0.9%	40.5%	31.5%	0.0%	8.1%	0.9%	6.3%	3.6%	6.3%	**100.0%**	
% within treatment	1.2%	0.0%	2.9%	1.1%	17.0%	40.7%	0.0%	2.9%	5.9%	3.1%	0.7%	1.7%	**4.2%**	
Total	**85**	**65**	**35**	**94**	**265**	**86**	**438**	**309**	**17**	**228**	**597**	**408**	**2,627**	

X^2 (55, N = 2,627) = 3,466.9, **p <.0001**

Note: Treatment a-Endodontics (Anterior), b-Endodontics (Posterior) c-Fixed-prosthetics (Bridge), d-Fixed-Prosthetics (Crown), e- Partial Denture, f-Full Denture, g-Paedodontics, h-Oral Surgery, i-Oral Maxillofacial, j-General Dental Practice, k-Conservative Care and l-Periodontal Need. Chi-square test, X^2 (55, N = 2,627) = 3,466.9, p < .0001

Table 3. Cross tabulation of dental treatment needs versus gender of patients underwent for screening at IIUM Dental Outpatient Clinic for the year 2016

Gender	Dental Treatment Needs													Statistical Analysis
	a	b	c	d	e	f	g	h	i	j	k	l	Total	
Male														
Count	32	26	15	32	93	37	178	152	2	93	319	189	**1,168**	
% within gender	2.7%	2.2%	1.3%	2.7%	8.0%	3.2%	15.2%	13.0%	0.2%	8.0%	27.3%	16.2%	**100.0%**	X^2 (11, N = 2,627) = 49.8, **p<.0001**
% within treatment	37.6%	40.0%	42.9%	34.0%	35.1%	43.0%	40.6%	49.2%	11.8%	40.8%	53.4%	46.3%	**44.5%**	
Female														
Count	53	39	20	62	172	49	260	157	15	135	278	219	**1,459**	
% within gender	3.6%	2.7%	1.4%	4.2%	11.8%	3.4%	17.6%	10.8%	1.0%	9.3%	19.1%	15.0%	**100.0%**	
% within treatment	62.4%	60.0%	57.1%	66.0%	64.9%	57.0%	59.4%	50.8%	88.2%	59.2%	46.6%	53.7%	**55.5%**	
Total	**85**	**65**	**35**	**94**	**265**	**86**	**438**	**309**	**17**	**228**	**597**	**408**	**2,627**	

Note: Treatment a-Endodontics (Anterior), b-Endodontics (Posterior) c-Fixed-prosthetics (Bridge), d-Fixed-Prosthetics (Crown), e- Partial Denture, f-Full Denture, g-Paedodontics, h-Oral Surgery, i-Oral Maxillofacial, j-General Dental Practice, k-Conservative Care and l-Periodontal Need. Chi-square test, X^2 (11, N = 2,627) = 49.8, p < .0001

Table 4. Cross tabulation of dental treatment needs versus residential area of patients underwent for screening at IIUM Dental Outpatient Clinic for the year 2016

Residential Areas	**Dental Treatment Needs**													**Statistical Analysis**
	a	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**	**k**	**l**	**a**	
Urban														X^2 (11, N = 2,627) = 112.6, **p<.0001**
Count	43	38	17	42	106	18	165	131	10	108	376	197	**1,251**	
% within gender	3.4%	3.0%	1.4%	3.4%	8.5%	1.4%	13.2%	10.5%	0.8%	8.6%	30.1%	15.7%	**100.0%**	
% within treatment	50.6%	58.5%	48.6%	44.7%	40.0%	20.9%	37.7%	42.4%	58.8%	47.4%	63.0%	48.3%	**47.6%**	
Non-urban														
Count	42	27	18	52	159	68	273	178	7	120	221	211	**1,376**	
% within gender	3.1%	2.0%	1.3%	3.8%	11.6%	4.9%	19.8%	12.9%	0.5%	8.7%	16.1%	15.3%	**100.0%**	
% within treatment	49.4%	41.5%	51.4%	55.3%	60.0%	79.1%	62.3%	57.6%	41.2%	52.6%	37.0%	51.7%	**52.4%**	
Total	**85**	**65**	**35**	**94**	**265**	**86**	**438**	**309**	**17**	**228**	**597**	**408**	**2,627**	

Note: Treatment a-Endodontics (Anterior), b-Endodontics (Posterior) c-Fixed-prosthetics (Bridge), d-Fixed-Prosthetics (Crown), e- Partial Denture, f-Full Denture, g-Paedodontics, h-Oral Surgery, i-Oral Maxillofacial, j-General Dental Practice, k-Conservative Care and l-Periodontal Need. Chi-square test, X^2 (11, N = 2,627) = 112.6, p < .0001.

Discussion

The predominant need among teenage to adult patients was conservative care, followed closely by periodontal need. The main reason for the high demand for conservative and periodontal care was perhaps due to the high prevalence of caries and gingivitis among Malaysian adults. According to National Oral Health Survey of Adult (NOHSA) in 2010, about 94.0 % of Malaysian adult population had periodontal disease while 88.9 % of Malaysian population had dental caries experience.

This study also shows that majority of the first-visit patients at this clinic were females. This scenario was also observed in Turkish population (Pekiner *et al.*, 2010), and in fact, a previous study in Southern China has also shown that females were more likely to visit dental service provider (Lo *et al.*, 2001). Other than that, NOHSA 2010 also reported a higher proportion of females (53.1 %) that sought after dental treatment at public dental provider compared to males (49.3 %). This female predilection might be due to the difference of oral health between men and women, especially at certain age. The disparity in oral health increases between the genders as a population ages, which may relate to the combination of reproductive hormones influences, pregnancy, diet, as well as morning sickness during pregnancy in women (Lukacs, 2011). Other than pregnancy, menopause is also associated with increased risk of oral health complication, especially in women who developed osteoporosis (Branch-Elliman, 2012). Another plausible reason is that women visit more dental and oral health service providers because they usually are more attentive towards the aesthetics inclusive of the teeth (Akbar *et al.*, 2019).

In addition to gender, age also have significant association with the type of dental treatment need. This study shows that younger-age patients mostly required conservative care while the elder-age patients have major need for prosthodontics, either partial or full dentures. In agreement with this, prosthodontics was the most required treatment need among the elderly patients in Turkish (Pekiner *et al.*, 2010) as well as in Northeast China (Liu *et al.*, 2015) population. This study also shows that the need for partial and full dentures increases with increasing age. This is actually consistent with findings in NOHSA 2010 in which the study has shown that the number of edentulous significantly increases from the age of 35 (1.1 %) to the age 65 (35.6 %) and 75 (53.3 %). When the patient becomes edentulous, then the need for tooth extraction is nullified. The need for oral maxillofacial care which includes treatment for temporomandibular joint symptoms, operculectomy, pericoronitis and bruxism is consistently low compared to other treatment need across all age groups.

Residential area also has significant association with the type of dental treatment need. This study shows that people residing non-urban areas outnumbered those residing urban areas to receive fixed prosthetics of either bridge or crown; partial and full dentures; paedodontic care; periodontal treatment; and being referred to oral surgery and general dental practice departments. In general, people living in rural areas tend to have lower oral health problem, has more caries and fewer teeth compared to the urban residents (Akbar *et al.*, 2019). However, the geographical location of this dental institution and the convenient of transportation made this institution still accessible to both the urban and non-urban residents.

Conclusion

Conservative care was the major dental treatment need identified in this study population. The type of dental treatment

need has significant association with patient's age, gender and residential area. This finding substantially helps in understanding of dental treatment need, especially within the context of a university-based dental care provider in the East Coast region of Peninsular Malaysia.

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Analysis of the anti-cancer effect of ethyl-p-methoxycinnamate extracted cekur (*Kaempferia galanga*) on cancer cell lines with wild-type and null p53

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Abstract

This study aimed to examine the in-vitro anti-cancer potential of ethyl-p-methoxycinnamate (EPMC), the major constituent of *Kaempferia galanga (K. galanga)* in selected human lung adenocarcinoma cells line A549 (p53 wild-type) and H1299 (p53 null). The involvement of p53 pathway in the anti-cancer effect of EPMC on selected cells was determined using MTT assay and Real-time PCR. The MTT results show that EPMC induces cytotoxicity in a dose-dependent manner in A549 cancer cell lines containing the p53 wild-type gene. Meanwhile, our RT-PCR results indicate that the apoptotic activity of EPMC does not involve the p53 pathway. Overall, these results indicate that EPMC compounds of *K. galanga* stimulates in vitro cytotoxic and apoptotic activity unrelated to the p53 pathway.

Keywords: Ethyl-P-Methoxycinnamate, Kaempferia Galanga, p53

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Introduction

Cancer is a major public health problem worldwide. There are many factors that may contribute to the development of cancer. In many cases, cancer involves mutations in protein-encoding genes that regulate cell division. Eventually, more genes become mutated because the genes that normally repair DNA damage become themselves mutated and cease to function. The amplification of mutations in the cell causes further abnormalities in the cell and its daughter cells.

TP53 (tumor suppressor gene p53) plays a significant role in protecting cells from malignancies. It is well-known that p53 suppresses tumor formation and protects against DNA damage by inducing cell cycle arrest, DNA repair or apoptosis (Wang and Sun, 2010). p53 induces cell cycle arrest by trans-activating genes such as p21 (CDK-inhibitor 1, cyclin dependent kinase) (Chiang *et al*., 2013). Furthermore, p53 initiates apoptosis via trans-activating pro-apoptotic proteins such as PUMA (p53 upregulated modulator of apoptosis) (Bai and Wang, 2014), BAX (Bcl-2-associated X protein) or FAS (cell surface death receptor) (Wang and Sun, 2010).

However, the p53 is often mutated in cancer (Klein and Vassilev, 2004).

Evidence suggests that, mutations or deletions in the TP53 gene are present in nearly 50% of human cancers and primarily results in impaired tumor suppression function (Wang *et al*., 2012). Loss of p53 functionality leads to the proliferation of damaged cells that may subsequently transfer the mutations to the next generation. It is believed that it is through this mechanism that deregulation of p53 often leads to the formation of tumors (Khoury and Domling, 2012).

Due to the high occurrence of p53 mutations in human tumors, this tumor suppressor is a key target for novel anticancer therapies. Several research teams have dealt with the possibility of restoring p53 function to treat cancer. Many novel molecules have been identified so far to restore p53 wild-type conformation and thereby recover its tumor suppressive function (Hientz *et al*., 2017).

Thus far, a number of studies has reported that EPMC, a major constituent of volatile oil of *K. galanga* possesses in vitro anti-cancer activities on various cancer cell lines such as human colon cancer SW620, cervical cancer C33A, breast cancer MCF-7 cell lines and oral cancer HSC-3 and Ca922 cell lines (Amuamuta *et al., 2017;* Omar *et al., 2017;* Omar *et al., 2016;* Ichwan *et al., 2019).*

Despite these promising results, the question of the role of *K. galanga* in the p53 pathway remains unknown. Therefore, the objective of this study was to determine the in vitro anti-cancer potential of EPMC extracted from *K.*galanga against human cancer cell lines A549 (lung cancer, p53 wild-type) and H1299 (lung cancer, p53 null). The study also aims to explore the involvement of p53 pathway in anticancer mechanism of EPMC extract on human cancer cell lines.

Materials and Methods

Cell subculturing and maintenance

The human lung adenocarcinoma cells A549 (wild-type p53) and H1299 (null p53) were kindly provided by Prof. Dr. Masa Aki Ikeda, Tokyo Medical and Dental University, Japan. The cells were grown in Dulbecco's modified eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% Penicillin-Streptomycin mixture in a humidified incubator, 5% CO_2 at 37°C.

MTT assay

Cell viability was measured by the ability of the cells to convert soluble MTT into an insoluble formazan crystal. The assay was performed according to the protocol previously described (Ichwan *et al*., 2014) with slight modifications. Exponentially growing cells were subcultured in 96-well plates at an initial density of $2x10^4$/ well. The cells were exposed to pre-defined concentrations of EPMC and doxorubicin for 24 hours.

After 24 hours of incubation, the medium was removed and the cells were washed with PBS. Cell viability was determined by adding 20 µL MTT 3-(4, 5-dimetyhlthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide at a concentration of 5mg/ml (in PBS). Cells were incubated at 37°C in humidified atmosphere for 4 hours. The formazan crystals were dissolved in 100 µL of dimethylsulfoxide (DMSO) for 1 hour. The absorbance was measured in a spectrophotometer at a wavelength of 570nm (reference: 630 nm).

Total RNA extraction

The total RNA Mini Kit was acquired from Geneaid (Taiwan). RNA extraction was performed following the manufacturer's protocol.

Complementary DNA (cDNA) preparation

cDNA synthesis was conducted using ReverTra Ace ® qPCR RT Master Mix Kit (Toyobo, Japan) based on manufacturer's instructions. A total of 8 µl of solution consisting of 4X DN Master Mix, RNA template, and nuclease-free water was prepared. After incubation for 5 min, 5× Master Mix was added to the solution to a total of 10 µl of reverse transcription solution. Then, the solution was incubated at 37 °C for 15min and heated at 98 °C for 5 min to obtain cDNA.

Quantitative PCR (qPCR)

The mRNA expressions of p21 and PUMA were determined by qRT-PCR. The RT-qPCR was performed using the Quantitect SYBR® Green PCR (Qiagen) kit according to manufacturers's instructions. The qRT-PCR reaction mixture consists of 2x QuantiTect SYBR Green PCR master mix, pre-developed gene expression assays, primers mix and H_2O to a final volume of 14 µL were prepared. The relative expression level of the mRNA sample was normalized by the amount of β-actin, a housekeeping gene that was used as endogenous control.

Statistical analysis

Non-parametric Kruskal-wallis was used to test the significant difference of PUMA upregulation between doxorubicin and EPMC.

Results and Discussion

In brief, we used MTT assay to test whether EPMC induced cytotoxicity in human lung adenocarcinoma cell lines A549 (p53 wild-type) and H1299 (p53 null). The cells were exposed to graded concentrations of EPMC (0-20 µg/mL). EPMC was found to decrease cells viability in a dose-dependent manner in the presence of p53 (Figure 1). However, in the absence of p53, EPMC did not induce cytotoxicity and cell death (Figure 2).

The findings of this study would seem to suggest that presence of the p53 gene is a key factor for sensitivity to anticancer agents (El Deiry *et al*., 2003). The H1299 cell line has a recognized p53 gene deletion in both alleles. Thus, p53-null cells succumb to tumorigenesis. Unlike p53-null, p53 wild-type have the ability to suppress malignant growth of transformed cells as well as tumors. Therefore, compounds that activate wild-type p53 would have an application for the treatment of wt-p53 containing human cancer. Nevertheless, mutated p53 confers to the resistance of tumor cells to anticancer drugs by inhibiting p53-dependent pathway (Volgestein *et al*., 2000).

The cytotoxicity of EPMC on human lung cancer cell line was compared against doxorubicin, a well-known chemotherapeutic agent as a positive control. EPMC and doxorubicin both induced the cytotoxic activity of p53 wild-type.

We next sought to determine whether EPMC was involved in the p53 pathway in A549 and H1299 cell lines through the expression of p53 mediated target genes (Beckerman *et al*., 2010). The p53 mediated target genes assessed in the study were p21 and PUMA.

As shown in Figure 3, p21 expression levels were not induced in both cancer cell lines after incubation with EPMC. However, PUMA expression levels in both cancer cell lines increased after treatment with EPMC (Figure 4). Non-parametric Kruskal-Wallis statistical analysis was used to test for significant differences in PUMA upregulation upon treatment with doxorubicin and EPMC. No significant difference in PUMA upregulation between doxorubicin and EPMC was found. This indicates that EPMC is a potent treatment candidate for cancer. Furthermore, from the graph obtained it showed that the increment was more pronounced in A549 (p53 wild-type).

In a previous study, PUMA was reported to play an important role in benzyl isothiocyanate (BITC)-induced apoptosis. In the study, treatment with BITC clearly increased the level of PUMA in cells with wild-type p53 (MCF-7) (Anthony *et al*., 2012).

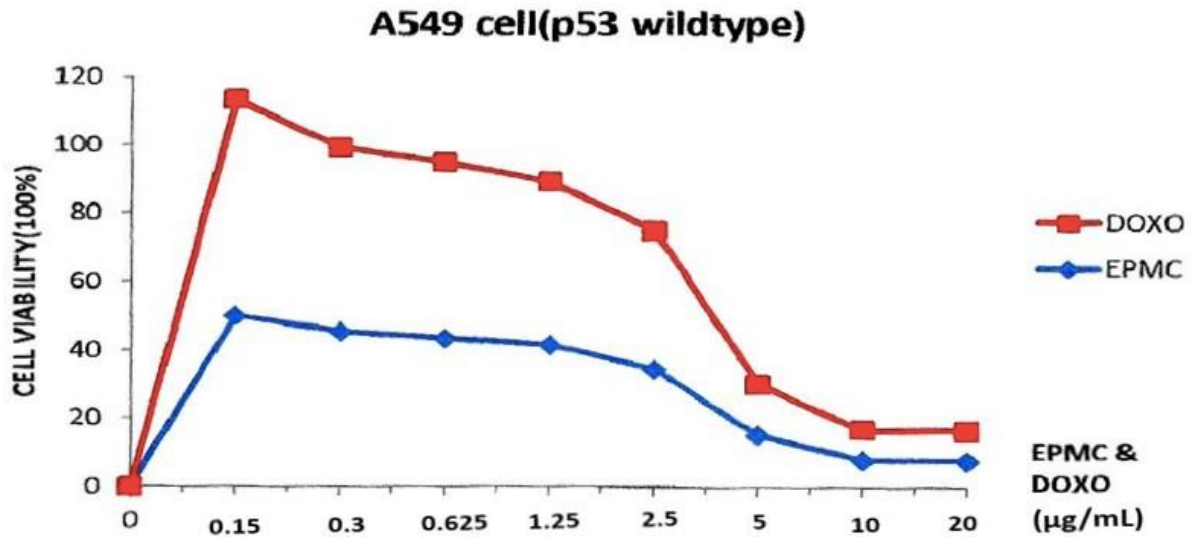

Figure 1. Ethyl-p-methoxycinnamate (EPMC) treatment schedules, EPMC dependent cytotoxicity and induced cell death in the presence of p53.

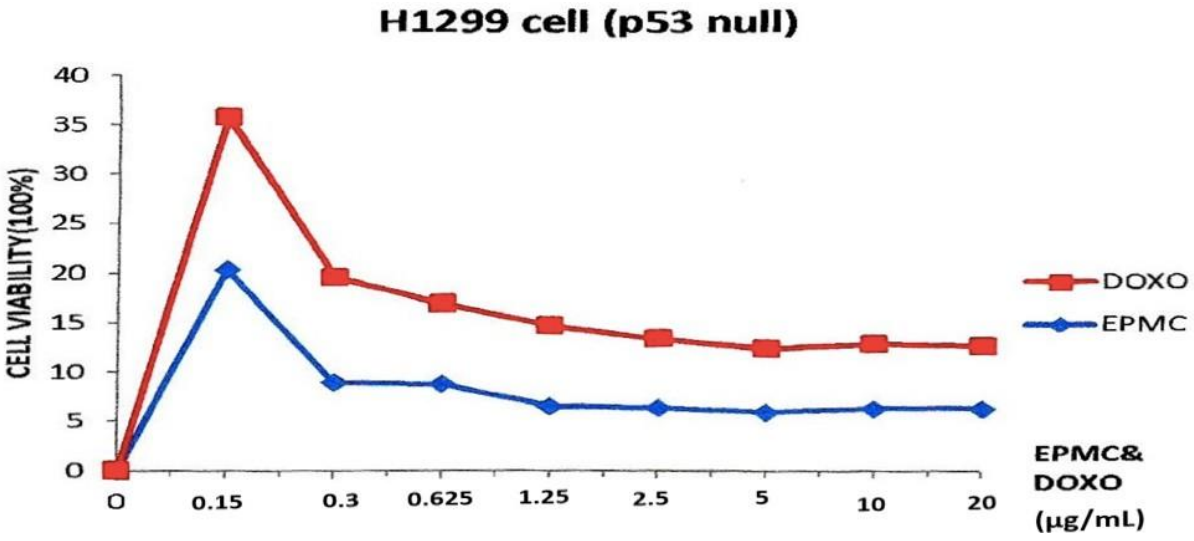

Figure 2. Ethyl-p-methoxycinnamate (EPMC) does not induce cytotoxicity and cell death in dose dependent manner in the absence of p53.

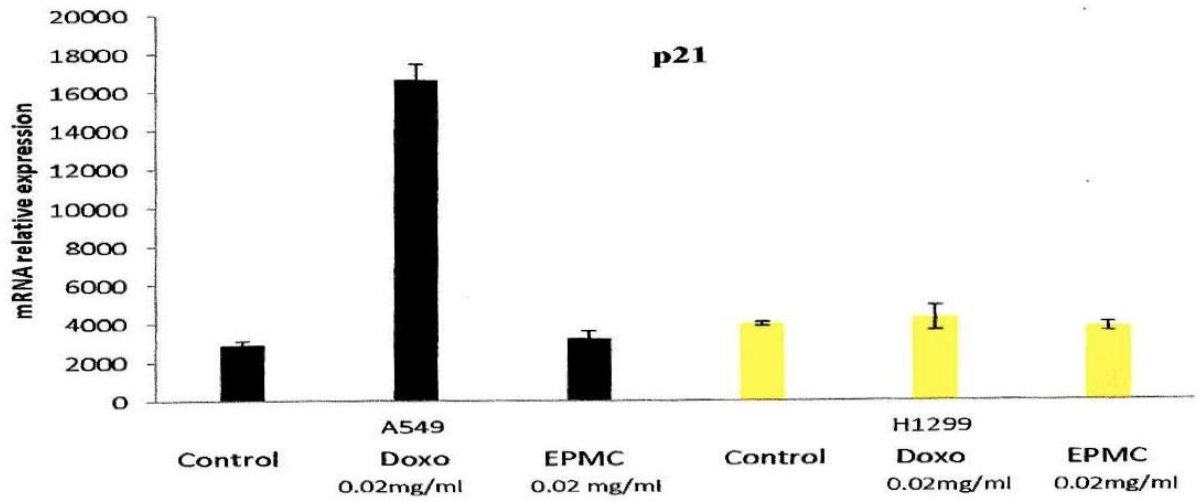

Figure 3. The graph shows that mRNA expression was not induced by EPMC incubation in both; A549 (p53 wild-type) and H1299 (p53 null) cancer cell line.

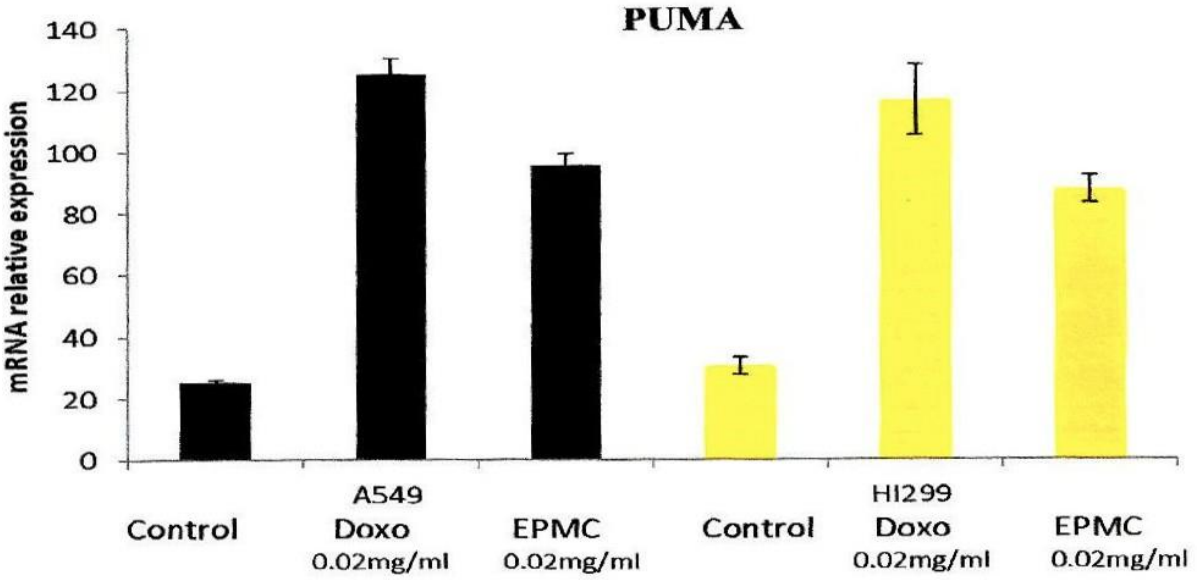

Figure 4. RT-PCR analysis revealed that mRNA expression levels were upregulated in both; A549 (p53 wild-type) and H1299 (p53 null) cancer cell line.

Nevertheless, the upregulation of PUMA mRNA expression does not indicate the involvement of the p53 pathway. This is because PUMA can also be activated independently and thus plays a role in p53-independent apoptosis as in the case of the p53 homolog p73, which is

able to engage the PUMA promoter at the p53 response elements (Li *et al*., 2006).

Thus, in this study EPMC is shown to stimulate cytotoxic and apoptotic effects on human lung cancer cell lines. However, the apoptotic effect of EPMC does not involve the p53 pathway.

Acknowledgement

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Radiographic findings in panoramic radiographs of patients attending Kulliyyah of Dentistry, IIUM

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Abstract

This research was done to study the radiographic finding of the jaws amongst the patients attending at the polyclinic using Orthopanthomograph (OPG). The OPG was taken using the Planmeca Promax 3D and the Planmeca Romexis software (Version 2.1.1.R). The first step was collecting all the OPG images from 1st April 2009 until 31st January 2011. Then, the abnormal radiographs were further divided into 3 groups which were radiolucent, radiopaque and mixed. This classification includes site, size, border, and possible diagnosis as part of the lesion`s appearance. One thousand four hundred and five OPG images were retrieved, 96 images were discarded because of poor quality. The data collected were analyzed statistically by using SPSS Version 16.0. Among 77 abnormal radiographic images, 41 images were radiopaque, 30 images were radiolucent and 6 images were mixed. Out of 77 abnormal images, 34 images that showed bone lesion were from male patients while the rest which was 43 images from female. In conclusion, most of the pathological lesion occur in the mandible.

Keywords: Panoramic, radiograph, jawbone, lesion

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Introduction

Radiograph as a method of investigation plays an important role in making diagnostic decision in determining the diseases. Each individual that need radiographic examination are based on findings from dental history and clinical examination and customized by age and general health of the patient *(Sirisha et al.*, 2013). Thus, radiographs mostly referred to as the clinician's key of diagnostic support (Lee *et al.*, 2013). Besides that radiographs are ordered when dentist sought that the radiographs will provide helpful diagnostic information which will affect the treatment plan (Kapila *et al.*, 2011). However, lesions inside the jaw are sometimes accidentally discovered by the dentist during routine examination.

Oral lesions is always a challenge for clinician and based on the experience, constructing a final diagnosis can be complicated and easily misinterpreted.

Many of the oral pathological lesions cannot be viewed clinically, and the indication for dental radiograph can provide important information on a patient's oral health and general health. (Diz *et al.*, 2013).

Panoramic imaging is a two dimensional radiographic technique for producing a single 2D tomographic image of the facial structures that include both maxillary and mandibular dental arches and their supporting structures (Subbulakshmi, *et al.*, 2016). Besides that, the panoramic imaging produces a

single image of a complete representation of the jaws, teeth, temporomandibular joint, alveolar lobes of the maxillary sinuses. This image forms when two adjacent disks are rotating at the same speed in opposite directions as an X-rays been passed through their centers of rotation.

Lesion is defined as a pathologic disturbance of a tissue, with loss of continuity, enlargement, or function (Regezi, Sciubba, & Jordan, 2015). The most common jaw lesions are cyst and tumours. A cyst is defined as an epithelium lined pathologic cavity (Deepthi *et al.*, 2016). On the other hand, tumour of the jaw can be divided by 2 categories; odontogenic and non-odontogenic tumour. As the name implies, odontogenic tumour is the tumour that derived from the tooth structure related to the tooth while non-odontogenic tumours are the most common neoplasm of the jaw (Zegalie, Speight, & Martin, 2015).

The radiographic jaw lesions can be describe as having either a radiolucent, radiopaque or mixed appearance, relative to density of the adjacent bone which more than 80% of them is radiolucent (Ed, 2017). It can be multilocular or unilocular with well-defined or poor defined border. In general, lesions with well-defined borders are usually benign whereas poor defined borders invariably represent aggressive, inflammatory or neoplastic process (Hall, 2017).

This research is primarily concerned with the radiographic study of bone lesions found in patients coming to the polyclinic of the Kulliyyah of Dentistry, IIUM. The prevalence of bone lesions in patient will be analyzed based on the radiograph that had been taken beginning from the patient registered 1st April 2009 until 31st January 2011.

Thus, the objectives of this study were to find out the prevalence of bone lesions in patients attending polyclinic, Kulliyyah of Dentistry clinics through OPG, its radiographic findings and to assess the most common radiographic lesions and location.

In addition the study was done in order to set a potential data base on bone lesions in patients attending KOD, IIUM.

Material and Methods

A retrospective cross-sectional study was done to assess the prevalence of radiographic bone lesion in the patient coming to the Kulliyyah of Dentistry, IIUM. The approach of the study is quantitative, by collecting data (radiographic images) taken in this Kulliyyah from 1st April 2009 until 31st January 2011. It is appropriate to use cross sectional study because this study involve different group of people coming to the polyclinic, Kulliyyah of Dentistry.

The total number of collected radiographs (OPG) in the polyclinic is 1405. The images collected had been and screened for any abnormalities by two specialists in Oral maxillofacial pathology and Oral maxillofacial surgery due to the absence of maxillofacial radiologist. The data was collected by using the Planmeca Promax 3D and the Planmeca Romexis software version (2.1.1.R). The radiographs that has artifact, ghost image, distorted and blurred will be excluded from the study. After data has been collected and was visually checked for correction, the data was analyzed using SPSS Version 16.0.

Results

Out of the 1,405 OPG collected, 96 OPGs were excluded from the study due to technical defects and 1,309 were included. The number of radiographs with radiographic lesions were 77 and the rest, 1,232 were presented with normal radiographic findings. Out of the 77

images, 34 (44.2%) images showed bone lesion were from male patients and 43 (55.8%) were female. Regarding the race, the data showed that Malay patients were the highest 67 (87%), followed by 9 (11.7%) of Chinese and only 1 (1.3%) Indian respectively)

The prevalence of radiographic lesions of the jawbone in this study was 5.9%.

Table 1 shows the radiographic presentation radiolucent lesions were 30 (39%), (Figure 1) and radiopaque lesions were 41 (53.2%), (Figure 2). The remaining 6 (7.8%) were mixed lesions (Figure 3).

Sixty five radiographic lesions were found in the mandible, 37 on the right body and seven on the left body. However, less lesion occurred in the maxilla as shown in Table 2.

Most of the radiographic bone lesions found in this study had a well-defined margin which was 63 (81.8%) and the rest 14 (18.2%) were not clearly defined. Most of bone lesions 71 (92.2%) appeared as unilocular, whereas six (7.8%) were presented as multilocular.

Size of lesions were divided into 4 groups, small (2.5-7.5mm), (62%), moderate (7.6-10mm), (21%0 and large (above 10 mm). (17). Minimum size observed was 2.5 mm, and the maximum size was 19 mm, mean value was 7.344 and standard deviation was 3.19.

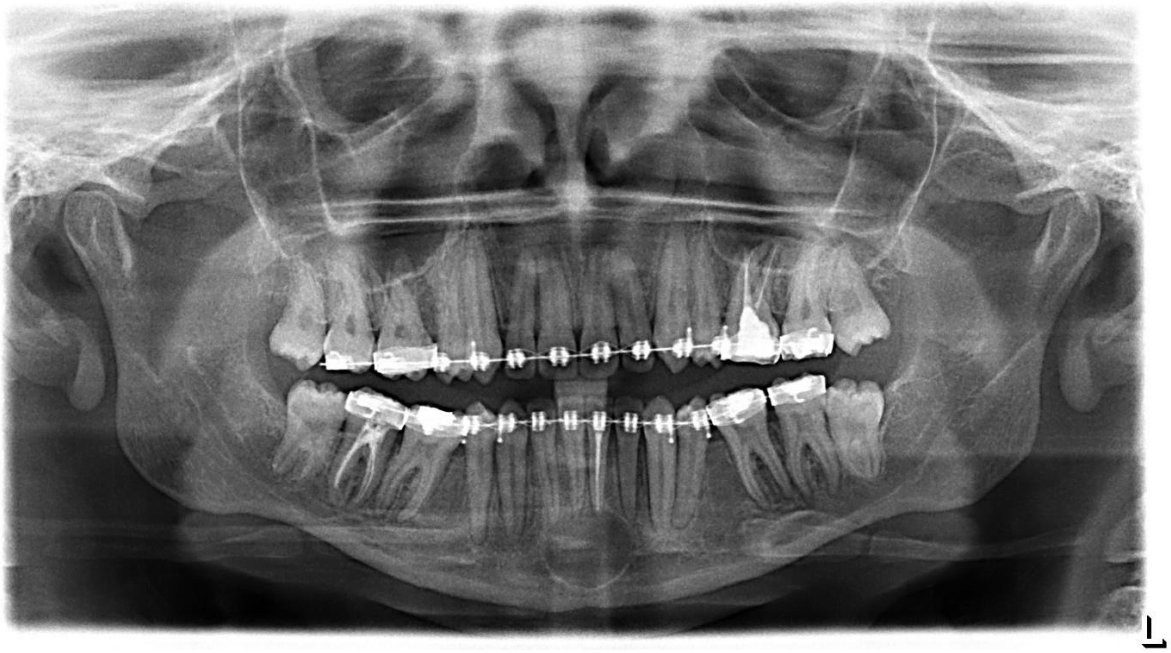

Figure 1. Panoramic radiograph showing a radiolucent lesion in the lower anterior region of the mandible.

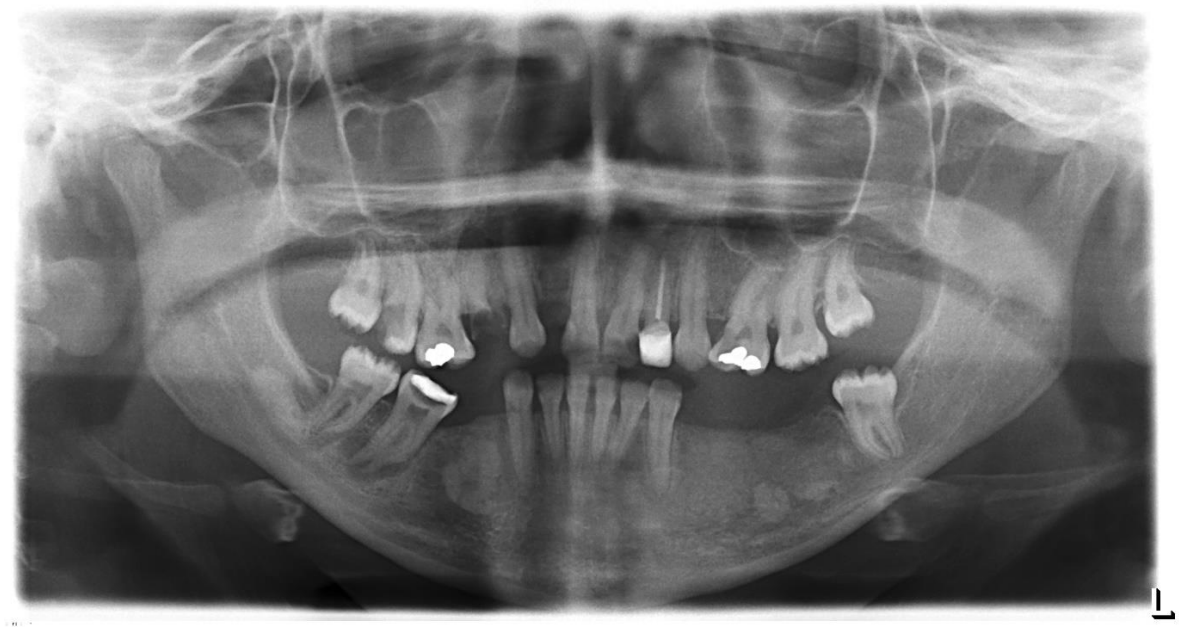

Figure 2. Panoramic radiograph showing radiopaque lesion in the lower left and right body of the mandible.

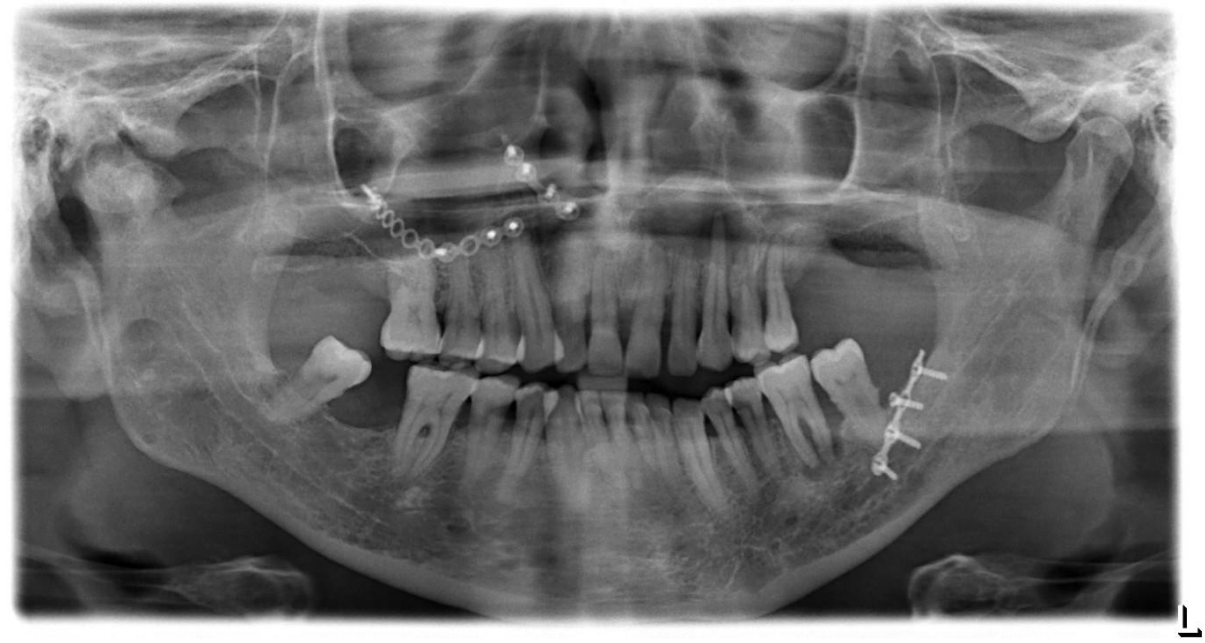

Figure 3. Panoramic radiograph showing a mixed radiolucent- radiopaque lesion in the lower posterior right angle of the mandible.

Table 1. Distribution of different types of radiographic lesions according to gender, loculation and appearance.

Total	Gender		Loculation		Appearance		
	Male	Female	Uni-locular	Multi-locular	Radio-lucent	Radio-paque	Mixed
25 (36.4%)	17 (22%)	11 (14.3%)	28	0	28 (93%)	0	0
5 (6.5%)	1 (1.3%)	4 (5.2%)	5	0	0	5 (12.2%)	0
25 (32.5%)	9 (11.7%)	16 (20.8%)	23	2	0	25 (61%)	0
3 (3.9%)	1 (1.3%)	2 (2.6%)	1	2	1 (16.7%)	2 (4.9%)	0
3 (3.9%)	2 (2.6%)	1 (1.3%)	3	0	0	3 (7.3%)	0
13 (16.9%)	4 (5.2%)	9 (11.7%)	11	2	2 (6.7%)	6 (14.6%)	5 (53.3%)
77 (100%)	34 (44.2%)	43 (58.2%)	71	6	30 (100%)	41 (100%)	6 (100%)

Table 2. Site of radiographic lesions seen in OPG

Site	Lesions	Percentage (%)
RT mandible ramus	1	1.3
RT mandible body	36	46.8
LT mandible ramus	3	3.9
LT mandible body	25	32.5
RT maxilla posterior	3	3.9
RT maxilla anterior	4	5.1
LT maxilla posterior	2	2.6
LT maxilla anterior	3	3.9
Total	77	100

Discussion

Based on the result, it showed that 77 (6.78 %) among 1232 patients attending the dental clinic of Kulliyyah of Dentistry, IIUM, have jaw lesion. From all the patients having jaw lesion, 44.2 % were male and 55.8 % were female. The result may not show the exact number of the lesions affecting each gender since certain diseases are related to gender. For instance, in odontogenic kerato cyst, the ratio of male to female is 2.1 to 1.2 (Al-Moraissi et al., 2017).

The characteristic appearance of each lesion varies, and it was categorized into three; radiolucent, radiopaque and mixed radiolucent and radiopaque. From the collected results, the radiopaque lesion was the highest among the collected sample with a percentage of 53.2 %, however, Eldaya in 2017 stated that more

than 80 % of the lesions should be of radiolucent (Eldaya et al., 2017).

In this study, the mandible was found to be affected by (84.5 %) of the jaw lesions. Similarly, Araki *et al.* (2011) found that radiopaque lesions were mostly found in molar and premolar region of mandible.

Conclusion

This study showed that 6.25 % of the radiographs exhibited pathological lesions. Moreover, most of the lesions were discovered accidentally in the radiograph. More studies are recommended with a larger sample size. In conclusion, most of the pathological lesion occur in the mandible, and less in the maxilla.

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Isolation of *Candida* species in children and their biofilm-forming ability on nano-composite surfaces

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Abstract

Candida species including *Candida albicans*, *Candida krusei* and *Candida glabrata* are opportunistic microorganisms that inhabit oral cavity. The objective of this study is to determine the effect of dental caries on *Candida* spp. biofilm-forming ability on nano-composite with the hypothesis that dental caries enhances the colonization of *Candida* spp. To assess *Candida* spp. colonisation in the oral cavity of the paediatric patient, samples were obtained from 30 subjects aged five to six years old from Kuantan, Pahang, Malaysia. The samples were collected from buccal mucosa, palate and tooth surfaces using sterile swabs. 10 mL of patient's saliva suspension was also collected. Following that, the samples were inoculated on CHROMagar and incubated for 24 h at 37 ºC. *Candida* biofilm of caries isolate *C. albicans* (HNFC2), and *C. albicans* ATCC 32354 were developed on three different types of nano-composites. The study showed that no *C. albicans* was isolated from the caries-free oral cavity while 76% of children with caries possessed *Candida* spp. 65% of the yeasts were isolated from the tooth surface. Only 35% of the total isolates were obtained from soft tissues, including palatal and buccal mucosa. *C. albicans* is the most isolated *Candida* spp. with 82% and 67% of the yeast were obtained from the tooth surface and buccal mucosa, respectively. Besides, HNFC2 significantly colonised the nano-composites more than the ATCC ($P < 0.05$). In the comparison of the three types of nano-composites, nano-hybrid-based containing pre-polymerised filler (cB) exhibited the least *C. albicans* HNFC2 cells colonisation with 7.7 x 10^3 cells mL^{-1}. In contrast, the nano-composite that contained bulk-filled nanohybrid (cC) was the most colonised with 14.3 x 10^3 cells mL^{-1}. In conclusion, dental caries enhances the colonization of *Candida* spp. in children's oral cavity, and that caries isolate form more biofilm on nano-composites compared to the lab strain *C. albicans*.

Keywords: Paediatric, dental caries, Candida species, biofilm formation, nano-composite

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Introduction

Oral microbiome exists in both planktonic and biofilm (plaque) forms (*Kolenbrander et al., 2010*). More than 2000 groups of pathogens present in the oral cavity with opportunistic pathogens encompass a substantial number of them. These opportunistic pathogens have been shown to involve in the establishment of several oral or systemic disease (Dewhirst *et al.,* 2010; Warinner *et al.,* 2014). Currently, the categorization of this microbiome is based on shotgun metagenomics and comparative 16S rRNA gene sequencing. Formerly, they were divided according to simple sugar fermentation, their morphology and chemical contents (Chaffin, 2008; Donovan *et al.,* 2018).

Candida is a yeast that belongs to the kingdom fungi. It is known as imperfect fungi within the family of *Cryptococcaceae* (Rybalkin *et al.,* 2014). It has sizes ranged from two to five micrometre, and it also can be presented in hyphae and yeast form (Samarayanake, 2002; Rós Ásmundsdóttir *et al.,* 2008). A few types of *Candida* spp. for instance, *C. albicans* and *Candida dubliniensis* have aptitude in septate hyphae formation. This ability is essential during pathophysiology of disease as *Candida* spp. in hyphae form is more active in tissue invasion and more capable of injuring the invaded tissue (Samaranayake, 2006; Sudbery, 2011).

Candida spp. are part of a healthy microbiome. There are 200 recognized species in the genus of *Candida* with seven species are reported to play an essential role in pathogenesis of human diseases including *C. albicans, Candida kefyr, C. glabrata, C. krusei, Candida parapsilosis, C. dubliniensis and Candida stellatoidea* (Samarayanake, 2002*;* Rós Ásmundsdóttir *et al.,* 2008; Sida *et al.,* 2016). Among all these *Candida* spp., *C. albicans* has been testified to be the most predominant in the oral cavity (Akdeniz *et al.,* 2002; Nejad *et al.,* 2013). Virulence factors of *Candida* vary between species, and they include the phenotypic switching ability, hydrophobic cell surface, biofilm formation as well as hydrolytic enzymes, candidalysin and quorum sensing molecules production (Haynes, 2001; Williams *et al.,* 2011; Arzmi *et al.,* 2012; Arzmi *et al.,* 2014; Kragelund *et al.,* 2016; Sida *et al.,* 2016).

Candida spp. mainly *C. albicans* is one of the causes of many harmful diseases, including oral candidiasis and oral squamous cell carcinoma (Arzmi *et al., 2018*). *C. albicans* is a normal commensal of the human body and causes no damage. However, when the host defences are weakened, it is capable of becoming pathogenic and causing severe problems (Ramirez-Garcia *et al.,* 2013). *C. albicans* is an innocuous dimorphic fungus. However, it can become pathogenic and harmful when the balance of microbial flora has been disturbed, or immune system of the host has been debilitated (Zunt, 2000; Byadarahally Raju *et al., 2011*).

A study conducted in Singapore showed that *C. albicans* was the most prevalent species isolated from the blood of disease-ridden patients. Aside from *C. albicans*, *C. tropicali*s, *C. glabrata C. parapsilosis*, and *C. krusei* were also reported to be isolated from the subjects. Besides that, *C. albicans* was frequently found in the paediatric patient, too (Yang *et al.,* 2003; Pereira *et al.,* 2010). Besides, a retrospective study conducted in Lyon, France from 1998 to 2001 revealed that candidal infection was mainly caused by *C. albicans* (49.5%) and followed by *C. glabrata* with 12.6% and *C. parapsilosis* with 12.1%. The same study also showed that in onco-haematology patients, candidemia was majority caused by *C. krusei* and *C. albicans* (Martin *et al.,* 2005). A study in Malaysia has discovered that the most dominant *Candida* spp. isolated from the bloodstream was *C. albicans* (44.2%). They were also able to isolate other *Candida* spp. aside from *C.*

albicans such as *C. parapsilosis* (26.0%) and *C. tropicalis* (17.7%) (Ng *et al.*,1999).

Nano-composite has become the choice of dental practitioners in modern dentistry to replace other restorative materials such as amalgam due to a better aesthetics quality and abrasion resistance (Cramer *et al.,* 2011). Furthermore, in combination with good bonding, nano-composite presents adhesive properties which can preserve the tooth structure during cavity preparation which is not possible with amalgam restoration (Correa *et al.,* 2012). In order to improve its mechanical and physical properties, conventional composite has been improved by incorporating different compositions that possess different properties (Burgers *et al.,* 2009).

The objective of this study is to determine the effect of dental caries on *Candida* spp. biofilm-forming ability on nano-composites with the hypothesis that dental caries enhances the colonization of *Candida* species.

Materials and Methods

Sample isolation

Before the commencement of sample collection from children at International Islamic University Malaysia (IIUM) Dental Polyclinic and Adik Arif Kindergarten in Kuantan, Pahang, Malaysia, ethical approval (IREC 2018-172) was obtained from the IIUM Research Ethics Committee (IREC) on the 8th May 2018.

Briefing regarding this study was given to the parents, and written consent was obtained before sample taking. The data was recorded on clinical examination sheet. A total of 30 healthy pre-schooled children (15 caries-free patients and 15 patients with caries) aged five to six years old consented by parents were included in the study. The exclusion criteria were children with co-morbidity and not consented by parents. The samples were collected in the presence of dental clinicians. The oral rinse technique was conducted to isolate microbial samples from the subject. In brief, patients were requested to have their mouth rinsed with 10 mL of sterile saline for one minute and spit in a sterile container. Following that, the surface of the teeth, palate and buccal mucosa were swabbed with a sterile swab, and the samples were transported immediately to the laboratory for identification of *Candida* spp.

Identification of *Candida* species

The samples that were previously collected from paediatric patients were inoculated onto *Candida* spp. selective CHROMagar (BD, USA) aseptically which was prepared beforehand for identification of *Candida* spp. Prior inoculation, each petri dish was divided into four parts and labelled with saliva suspension (S), tooth (T), palate (P) and buccal mucosa (B). To identify *Candida* spp. from saliva suspension, the transport mediums which contain microbial isolates were vortexed vigorously using a vortex mixer (Biologix, Singapore) followed by inoculation on CHROMagar using sterile swabs.

Meanwhile, to identify *Candida* spp. from other oral sites, the collection swab which was used to collect the sample from the oral surfaces of children was swabbed on CHROMagar aseptically. The plates were incubated at 37 ºC for 24 h to 48 h, aerobically. The colour of the colony grown was recorded. The colony which exhibited green, dark pink and white were identified as *C. albicans*, *C. krusei* and *C. glabrata*, respectively. The species of *Candida* spp. were finally confirmed using API 20C AUX (Biomerieux, USA).

Enumeration of *Candida* species from saline suspension

The method by Alnuaimi et al. (2013) was conducted to enumerate the number of *Candida* spp. In brief, saliva suspension collected from the patient was vortexed vigorously. Later, 100 µL of the

suspension was serially diluted in 900 µL sterile saline. Following that, 10 µL of the diluted suspension was pipetted onto haemocytometer, and a clean glass coverslip was placed to secure the sample. Finally, the number of *Candida* spp. was enumerated by observing the haemocytometer under a light microscope (Olympus, Japan). The morphology of *Candida* spp. was also recorded based on the observation under the microscope.

Identification of *Candida* species colony morphology

To identify the colony morphology of *Candida* spp., a loopful of *Candida* spp. that was previously cultured on CHROMagar was inoculated onto a fresh CHROMagar using single dilution streaking method to obtain a single colony of *Candida* spp. Later, the plate was incubated for 24 h to 72 h at 37 ºC, aerobically. Finally, the colony morphology, including margin, elevation and form, were observed and recorded.

Preparation of nano-composite beads

Three different types of nano-composites (cA, cB and cC) as described in Table 1 were prepared using a round plastic mould (6 mm diameter x 5 mm height). Each bead was then polished beforehand. Following that, all nano-composites were sterilized using ultra-violet (UV) light radiation technique.

Growth of *Candida albicans*

C. albicans American Type Culture Collection (ATCC) 32354 was sub-cultured on yeast peptone dextrose (YPD) agar and incubated at 37 °C for 24 h. Following that, a single colony of *C. albicans* was inoculated in YPD broth and standardized using a spectrophotometer to obtain an OD_{620nm} 0.1 that was equivalent to 10^6 cells mL^{-1}. Finally, the 1.5 mL of the suspension was aliquoted into 2 mL sterile Eppendorf tube and stored at -20 °C (Figure 4). A similar protocol was repeated to grow *C. albicans* isolated from the oral cavity of children with caries (HNFC2).

Static biofilm formation

The study of static biofilm was conducted according to the modified protocol by Arzmi et al. (2016). Initially, 750 µL of YPD broth was pipetted into each well of 12-well plate. Following that 750 µL of *C. albicans* suspension standardized at 10^6 cells mL^{-1} in YPD broth was added in the same well. The suspension was mixed using a sterile pipette. Wells that contained only YPD broth were representing as the negative control. Finally, the sterile nano-composite bead was placed into each well aseptically, and the plate was incubated at 37 °C for 24 h.

Enumeration of *Candida albicans*

Following incubation, the growth medium was discarded, and each well was rinsed twice with 1 mL of sterile distilled water. Later, the nano-composite bead was transferred into a sterile 15-mL tube containing 3 mL of sterile distilled water. The tube was sonicated using ultra-sonicator (Amsonic, USA) for 60 s. Finally, 10 µl of the suspension was pipetted onto haemocytometer to measure the cell number (Alnuaimi *et al.*, 2014). All protocols were repeated in three biological replicates to confirm reproducibility.

Data analysis

All data were statistically analysed using SPSS Statistic software version 25.0. Independent T-test was used to compare lab strain, and caries isolates *C. albicans* and analysis of variance (ANOVA) associated with *post hoc* Tukey test was used to compare three different nano-composites. The data were considered statistically significant when $p < 0.05$.

Results

Colonization of *Candida* species in paediatric patients

Our data showed no *Candida* spp. was isolated from caries-free children.

However, 76% of the total samples from caries patients contained *Candida* spp. isolates (Figure 1). Of these, 65% of *Candida* spp. were isolated from the teeth while 18% were isolated from saliva suspension. Besides, 18% of the samples were also isolated from buccal mucosa (Figure 2). No *C. albicans* was isolated from the palate.

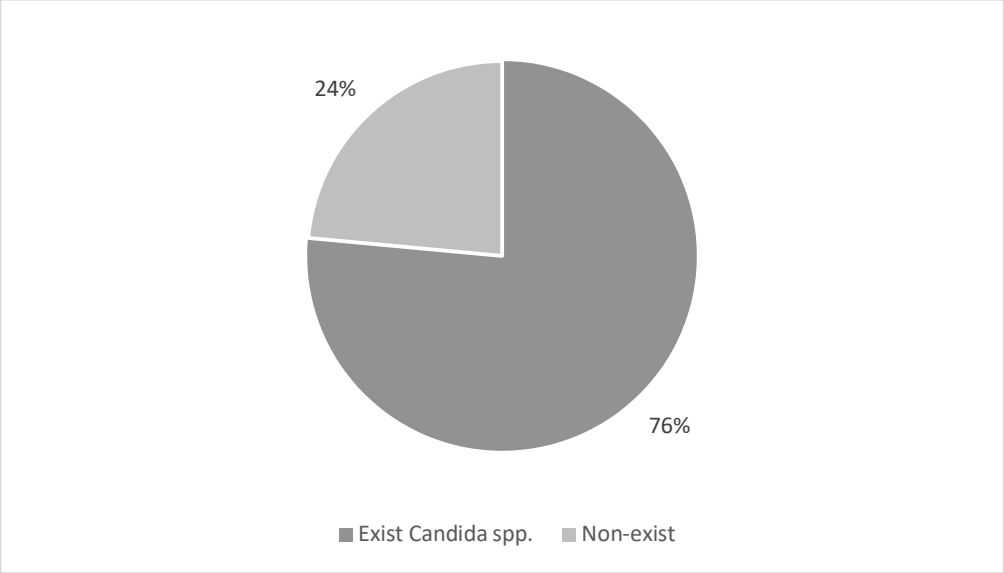

Figure 1. Percentage of caries children with *Candida* spp. (N=15).

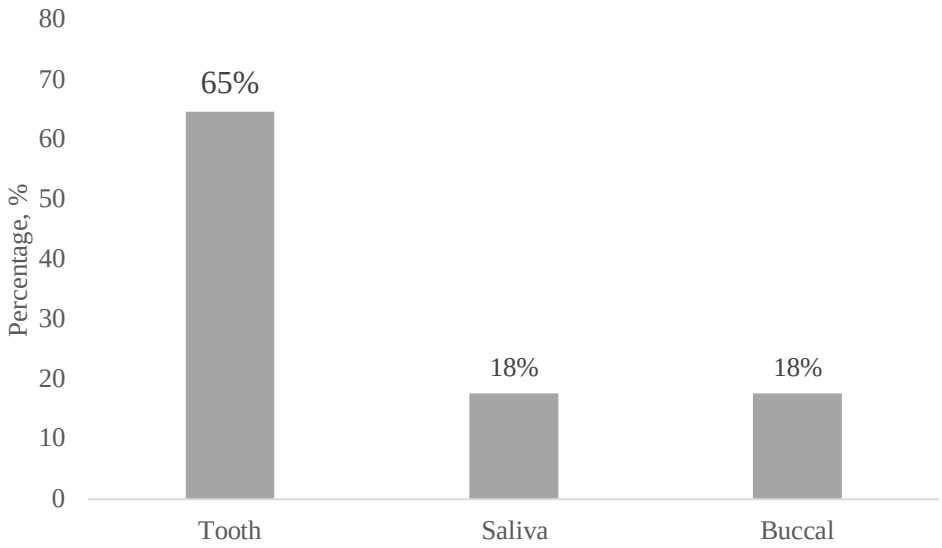

Figure 2. Percentage distribution of *Candida* spp. in children based on location; tooth, saliva and buccal mucosa of children with dental caries (N=15). No *Candida* spp. was isolated from palate.

Distribution of *C. albicans* and non-*C. albicans* in the oral cavity of children with dental caries

Candida spp. were observed to colonise the tooth surface, saliva suspension and buccal mucosa of paediatric patients with dental caries. There were 82% of the samples isolated from tooth surface of caries patients exhibited as mono-species *C. albicans*, while 9% was non-*C. albicans* (Figure 3). There were only 9% of patients with dental caries possessed both *C. albicans* and non-*C. albicans* in the tooth surface.

In addition, 67% of the paediatric patients with dental caries had mono-species *C. albicans* isolated on the buccal mucosa, whereas 33% of the isolates had non-*Candida albicans* (Figure 4). However, in saliva isolates, all samples that were isolated from patients with caries exhibited as mono-species *C. albicans* with the cell morphology was predominantly by the yeast form (Figure 5).

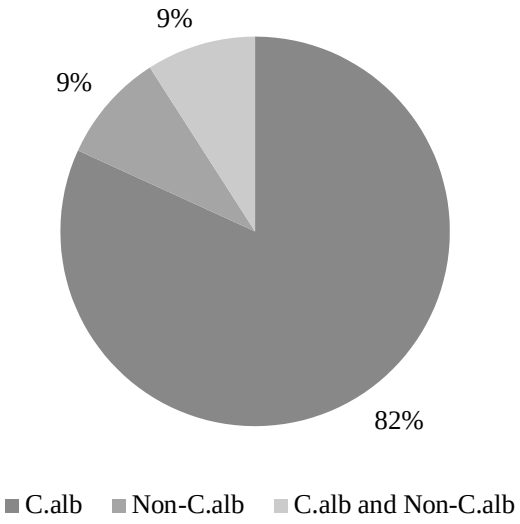

Figure 3. Percentage of caries children possess *C. albicans* only (C.alb), non-*C. albicans* only (Non-C.alb) or both *C. albicans* and non-*C. albicans* (C.alb and Non-C.alb) on the caries tooth surfaces (N=15).

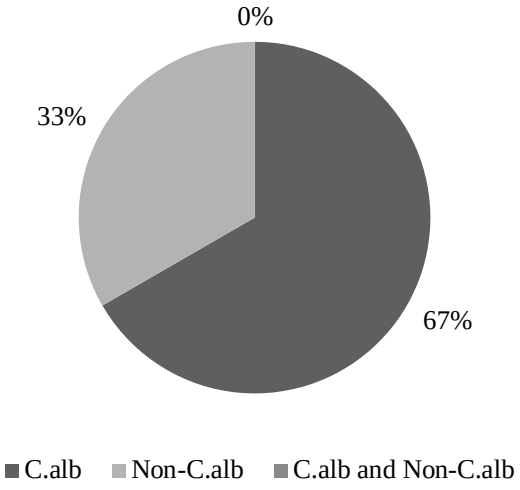

Figure 4. Percentage of caries children with *C. albicans* only (C.alb), non-*C. albicans* only (non-C.alb) or both *C. albicans* and non-*C. albicans* (C.alb and Non-C.alb) isolated from the buccal mucosa of caries patients (N=15).

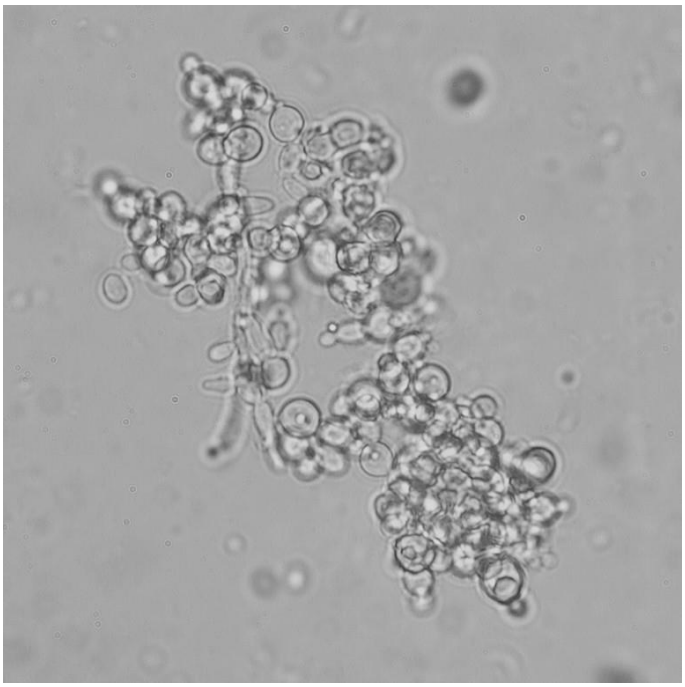

Figure 5. *C. albicans* isolated from saliva of caries children as observed under light microscope at 1000x magnification.

Colony morphology of *Candida* species

All *C. albicans* isolated from paediatric patients with dental caries exhibited as a circular shape, entire margin and convex elevation. Meanwhile, *C. krusei* that was isolated exhibited irregular shape, undulate margin and raised elevation. Finally, *C. glabrata* that were isolated from caries patients exhibited circular shape, entire margin and convex elevation.

Comparison of biofilm formation of *C. albicans* between nano-composites

C. albicans ATCC 32354 showed adhesion on cA, cB and cC nano-composites with $4.3 \pm 0.58 \times 10^3$ cells mL^{-1}, $4.3 \pm 0.82 \times 10^3$ cells mL^{-1} and $3.8 \pm 1.14 \times 10^3$ cells mL^{-1}, respectively. However, there was no significant difference observed between the three nano-composites ($p > 0.05$).

C. albicans HNFC2 exhibited $14.3 \pm 4.98 \times 10^3$ cells mL^{-1} adhered on cC nano-composite followed by cA nano-composite, which had $9.9 \pm 4.13 \times 10^3$ cells mL^{-1}. Besides, there were $7.7 \pm 0.5 \times 10^3$ cells mL^{-1} adhered on cB nano-composite with a significant difference was observed between cC and cB nano-composites ($P < 0.05$).

Comparison of biofilm formation of *C. albicans* between lab and clinical strains

C. albicans HNFC2 had significantly more cell adhered on the cA nano-composite compared to *C. albicans* ATCC 32354 ($p < 0.05$). This similar trend was also observed in both cB and cC nano-composites. There were $9.9 \pm 4.13 \times 10^3$ cells mL^{-1} of *C. albicans* HNFC2 were adhered on cA nano-composite, which was significantly higher than *C. albicans* ATCC 32354, which exhibited $4.3 \pm 0.58 \times 10^3$ cells mL^{-1}. There were $7.7 \pm 0.5 \times 10^3$ cells mL^{-1} of *C. albicans* HNFC2 cells adhered on cB nano-composite, which significantly more than the *C. albicans* ATCC 32354 cells that adhered on the same nano-composite which exhibited $4.3 \pm 0.82 \times 10^3$ cells mL^{-1} ($p < 0.05$). The

adhesion of *C. albicans* HNFC2 cells on cC exhibited 14.30 $\pm$ 4.98 x 10^3 cells mL^{-1} which was significantly higher than *C. albicans* ATCC 32354 which exhibited 3.80 $\pm$ 1.14 x10^3 cells mL^{-1} ($p < 0.05$).

Discussion

Candida spp. was observed to be isolated only from caries patients. This data supported the hypothesis of the present study, which stated that dental caries enhances the colonization of *Candida* spp. in children with dental caries. Most of the caries research were reported that a high number of *Streptococcus mutans, Lactobacillus* species, and *Scardovia* species in children diagnosed with severe early childhood caries (S-ECC) as its presence intensify the presence of these bacteria. Previous *in vitro* and *in vivo* studies have shown that the existence of *C. albicans* can lead to a complex bacterial-fungal interaction and result in the growth of a cariogenic biofilm environment (O'Donnell *et al.,* 2015; Bowen *et al.,* 2017). For example, induction of glucosyltransferase B by *C. albicans* in addition to bacterial accumulation via chemical-metabolic interactions able to promote the growth of *S. mutans* (Sztajer *et al.,* 2014*;* Kim *et al.,* 2017). Cross-feeding interaction between *S. mutans* and *C. albicans* heightens their growth further while creating a Gtf activation loop and promotes the development of highly acidified microenvironment which is a suitable environment for acidogenic-aciduric bacteria (Bowen *et al.,* 2017).

Candida spp. was observed to be isolated mostly on the tooth surface and less on the buccal mucosa and saliva suspension. A study has shown that a hard surface in constant colonization of early colonizer of oral biofilm such *as S. mutans*. *S. salivarius* dominates the microbiota in the oral cavity during the early neonate life until the appearance of the teeth (Sachdeo *et al.,* 2008). The eruption of the teeth during the first year of life leads to colonization by *S. mutans* and *S. sanguis* (Cortelli *et al.,* 2008). Oral biofilm is a structured community of microbes which adheres to oral surfaces and is compressed within extracellular polymeric substances (EPS), formed from multi-microorganisms and environment of the oral cavity (Filoche *et al.,* 2010). The ability to form biofilm on the oral surfaces is one of important virulence factor of *Candida* spp. in the oral cavity. Colonization of microorganism on hard and soft tissue surfaces is initiated by adhesion of *Candida* spp, which lead to the formation of an organized microbial community known as biofilm (Hofer, 2016). Formation of biofilm is the first step in the establishment of dental caries. *Candida* spp. have the ability to co-aggregate with other microorganisms. This ability assists the yeast in attaching on the oral surface which is pre-colonised by the early coloniser bacteria such as *S. mitis, S. oralis* and *S. sanguinis* attached on the acquired pellicle that can form easily on hard tissue surface (Kiyora *et al.,* 2000). *Candida* spp. are found to colonize the tooth surface due to formation of the salivary pellicle. Deposition of pellicle on the tooth surface is the start of the formation of the initial plaque layer (Zijnge *et al.,* 2010). This evidence supports the hypothesis of the present study that dental caries enhances the colonization of *Candida* spp. in children.

Our results also showed that *Candida* spp. were isolated from buccal mucosa. *Candida* spp. have been shown to colonize mucosal surfaces (Salerno *et al.,* 2011; Hofer, 2016). The oral cavity presents abundant surfaces for microbial colonization. Colonization of these surface was started by biofilms of divergent microbial complexity inimitable to each species. While oral biofilm can arise on dental surfaces and mucosal surfaces inside the mouth, the constitution of the microbiomes varies greatly depending on the type of surface (Marsh *et al.,* 2011). The previous study has suggested that

microbes such as *S. mutans* required hard surfaces for continuous colonization. However, they can also be detected on the soft tissues in low levels (Sachdeo *et al., 2008*). It has also been shown that *S. mutans* fundamentally disappeared from the oral cavity when all teeth were extracted and reappeared again when the denture was worn. The denture provides a hard surface for colonization (Sachdeo *et al.,* 2008). However, this does not negate the fact that microbes such as *S. mutans* present on the mucosal surface. *Candida* spp. is known to have the ability to co-aggregate with other microorganisms and adhere to the oral surface colonised by the early coloniser bacteria such as *S. mutans* (Kiyora *et al.,* 2000), which can justify the presence of *Candida* spp. on buccal mucosa.

Candida spp. were also isolated from the saliva suspension. This result was supported by the previous research, which also found *Candida* spp. such as *C. albicans* aside from other species of microbes in saliva obtained from children (Xiao *et al.,* 2018). Saliva contains proteins such as mucins and statherins, which act as adhesion receptors used by the mannoproteins exist in the *Candida* spp. Imbalance of normal microbial communities was evident in the condition of decrease or complete absence of saliva in a patient with xerostomia (Salerno *et al., 2011*). Saliva contains numerous, different proteins and peptides with different molecular mass. The proteomic study revealed over 1050 diverse kinds of proteins in saliva. One of the constituents of the saliva, which is mucins consist of highly glycosylated particles (Silletti *et al.,* 2008). More than a few protein organizations such as MUC5B networks increase the complexity of the saliva structures.

Despite the presence of *Candida* spp. on several oral surfaces sampled, none were found on the palatal surface. Williams *et al.* (2011) mentioned that *Candid*a spp. must present in sufficient amount with an adequate rate of progress to permit their sustained attachment in order for them to colonize a mucosal surface. Thus, *Candida* biofilms are not typically seen on the palatal mucosa of healthy individuals. However, in cases of commensal carriage, colonization can be detected (Williams *et al.,* 2011).

Our data also showed that the majority of the isolated *Candida* spp. comprised of *C. Albicans.* Previous clinical studies revealed that compared to other *Candida* spp. such as *C. tropicalis, C. krusei,* and *C. glabrata, C. albicans* was frequently detected in high numbers in plaque-biofilms from toddlers with early childhood caries (ECC) (de Carvalho *et al.,* 2006; Raja *et al.,* 2010; Yang *et al.,* 2012; Koo *et al.,* 2014). Other *Candida* spp. such as *C. glabrata, C. krusei* and *C. tropicalis* were also detected but not as frequent or as abundant as *C. albicans* (de Carvalho *et al.,* 2006). Harriott and his colleagues also pointed out the capability of *C. Albicans* as the most dominant fungal pathogen that can cause superficial and systemic infections (Harriott *et al.,* 2011).

Our results have also shown that *C. albicans* attached directly to the surfaces of the restorative materials. Furthermore, *C. albicans* biofilm was also observed regardless of the type of nano-composite used for both lab and clinical strains. Besides, dental restoration materials have also been shown to induce biofilm formation. The biofilm accumulation of *C. albicans* on nano-composites may cause material surface deterioration, which will further help in the progression of the biofilm formation of different strains of *C. albicans*. *C. albicans* biofilm was observed to adhere firmly onto the nano-composite beads suggested that none of the nano-composites exhibited antifungal properties. These findings were similar to the previous study which indicated the

ability of *C. Albicans* to adhere on various abiotic surfaces including prosthesis, denture base, relining materials and some dental restorative materials (Segal *et al.,* 1988, Waltimo *et al.,* 1999; Maza *et al.,* 2002; Pereira *et al., 2007;* Lawaf & Azizi, 2009; Belduz *et al.,* 2017). *C. albicans* has been reported to form biofilm on various oral surfaces including prosthesis that can lead to oral pathogenesis including oral candidiasis (Akdeniz *et al.,* 2002; Blankenship *et al.,* 2006; Nejad *et al.,* 2013; Sida *et al.,* 2016). The present study showed a significant difference of cell adhesion on the same nano-composite between lab strain *C. albicans* ATCC 32354 and caries isolate *C. albicans* HNFC2, thus supported the hypothesis of the present study that caries isolate forms more biofilms compared to the lab strain. The previous study has shown that caries-free children had no *C. albicans*. Meanwhile, 82% of children with caries teeth presented *C. albicans* inside their oral cavity (unpublished data). Furthermore, caries prevalence has been reported to be correlated with the presence of *C. Albicans,* especially in children, adolescents and young adults (Klinke *et al.,* 2011). Furthermore, the acidic environment can also contribute to the growth of *C. albicans* suggesting that children with caries may possess more *C. albicans* compared to children with a healthy oral condition (Thaweboon *et al.,* 2008).

C. albicans has been shown to adhere on all nano-composites with cB exhibited the least adhesion by caries isolate *C. albicans* HNFC2. In contrast, cC exhibited the most adhered by the yeast strain. These results supported the hypothesis of the present study which *C. albicans* form different cell number in biofilm grown on different nano-composite and that *C. albicans* biofilm formation is nano-composite surface dependent. Each type of dental restorative material and its specific chemistry and their configuration including matrix and fillers arrangement suggested contributing to the different number of *C. albicans* cells adhered on different nano-composites (Beldûz *et al.,* 2016). The adhesion ability of *C. albicans* on dental nano-composite resin materials seems to vary depending on the type of matrix of the nano-composite. Even though all nano-composites that were used in this study were nano-hybrid resin composite type, however, the specific composition has been shown to differ thus affecting the level of *C. albicans* adhesion on the surface of the beads (Table 1).

Table 1. Compositions of dental materials

Manufacturer	Type	Compositions
cA	a nano-hybrid composite with pre-polymerized fillers	• SphereTEC fillers • Non-agglomerated barium glass fillers • Ytterbium fluoride Eethacrylic polysiloxane nano-particles
cB	Bulk fill nano-hybrid composite	• Fluoro-alumino-silicate glass • Bis-GMA • UDMA Bis-MPEPP • TEGDMA • Reaction initiator
cC	a good blend of both nanotechnology and hybrid technology	• Strontium glass filler type and high filler loading • Fluorescent agent

Conclusion

Dental caries enhances the colonization of *Candida* spp. in the oral cavity of children and that that caries isolate forms more biofilm on nano-composite compared to the lab strain *C. albicans* thus supported the hypothesis of the study.

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