

Abstracts of Theses Approved for the PhD/ MSc at the School of Dental Sciences, Universiti Sains Malaysia, Health Campus, Kubang Kerian, Kelantan, Malaysia

SOCKET PRESERVATION USING BOVINE BONE WITH AND WITHOUT DENTAL IMPLANT PLACEMENT

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Introduction: The alveolar bone is a highly dynamic bone supporting the tooth and its surrounding structures. It resorbs physiologically when the tooth is lost. Every day, thousands of teeth are extracted from the oral cavity leaving a residual defect following the loss of the alveolar bone that led to difficulty in prosthetic rehabilitation. Dental implantology has revolutionised the prosthetic replacement of artificial teeth by providing a high-quality artificial tooth replacement that mimics natural tooth structure and function.

Objectives: The aim of this interventional study was to assess healing, evaluate bone dimension and the resorption rate of the extraction alveolar socket using bovine bone with and without dental implant placement among the treated and non-treated tooth extraction sockets. The goal of these approaches was to preserve or minimise the ridge volume loss following tooth extraction by ridge augmentation procedures. The study also evaluates the degree of osseointegration between the immediate implant surface and the alveolar bone.

Methods: This interventional study was carried out on 30 patients at the University Dental Hospital Sharjah, Sharjah, United Arab Emirates. The patients aged between 18 and 40 years, who needed non-complicated tooth extraction of only one or both mandibular premolar teeth, and being fit and healthy, were included. The project has been approved by UOS and USM ethical committees, and informed consent was obtained. Patients were randomly divided into three groups. In group I, simple extraction was done and the empty extraction socket left untreated and allowed to heal in a conventional way. In group II, extraction sockets were filled with freeze-dried bovine bone xenograft (FDBBX) granules of size 1 mm. A resorbable pericardium membrane was placed to cover the defect to secure the bone granules within the socket and wound closure done with Vicryl suture. In group III, atraumatic extraction was done and an immediate implant placed into the sockets, and the circumferential gap was also filled with FDBBX bone granules and covered with pericardium membrane. This group was additionally subjected to resonance frequency analysis (RFA) by employing Osstell machine for measuring and evaluating

the degree of secondary stability at nine months. The patients were followed-up clinically for healing assessment at 1 week, 3 months and 9 months post-operatively (PO). All groups were subjected to cone beam computed tomography scan (CBCT) for radiological evaluation immediately after the surgical procedure at three months and nine months intervals using Sirona Dental Systems, GALILEOS SIDEXIS. CBCT was performed in three different views; coronal, sagittal and axial which involve linear measurements of the socket alveolar bone. RFA was recorded for group III at nine months.

Results: There were no clinical differences in healing between the groups. Significant difference of bone resorption was evident in alveolar ridge width and height reduction within control group I, 1.84 mm (Confidence Interval (CI) 95%, 0.57 to 3.10) and 1.91 mm (CI 95%, 0.64 to 3.14) respectively at the intervals of day 0 to nine months. No significant alveolar bone resorption was observed within group II and III. Comparison between group I and III showed a highly significant difference of bone resorption in ridge width at three months 2.56 mm (CI 95%, 4.22 to 0.90) $P \leq 0.001$, and at nine months interval 3.2 mm (CI95%, 4.70 to 1.62). Between group II and III, there was a significant difference of bone resorption in ridge width of 1.9 mm (CI95%, 3.43 to 0.34) ($P \leq 0.001$). There was no significant vertical ridge resorption observed among the groups. High RFA values were observed in group III at nine months postoperatively.

Conclusion: The insertion of immediate implants in fresh extraction sockets together with grafting the circumferential gap between the bony socket wall and the implant surface with bovine bone granules were able to preserve a greater amount of alveolar ridge volume when compared to leaving an extraction socket to heal alone in the conventional way or socket preservation with bovine bone graft only. The peri-implant new bone formation developed is of superior quality which led to successful osseointegration between the implant surface and inner surface of the buccal plate. We observed clinically that the USM manufactured FDBBX has completely resorbed and replaced by new bone in the area between the implant and the inner surface of the buccal plate in group III at nine months post-operative.

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DENTAL ARCH RELATIONSHIPS IN NON-SYNDROMIC UNILATERAL CLEFT LIP AND PALATE (UCLP) CHILDREN OF PAKISTAN

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Introduction: Globally, one out of every 700 live-births are affected by cleft lip and palate. By occurrence rate, it is one of the most common congenital orofacial birth anomaly. Literature indicates that cleft lip and palate has a multifactorial origin, but genetics and environmental studies play a vital role and have been extensively studied individually and in conjunction. A multidisciplinary involvement is absolute to successfully manage and treat cleft lip and palate. Primary surgical repairs are required to restore function and structure. Numerous designs for repair of cleft lip and palate have been devised and practiced but the superiority of outcome of a single surgery over the rest has not been established. It is necessary to assess the treatment outcomes of these primary surgical repairs under the influence of congenital and post-natal factors. Audit can be performed to assess their effect on growth and association of these confounding factors. Dentoalveolar relationships have been extensively used to assess the treatment outcome. Many indices have been developed which are based on different planes of growth. Any type of cleft case has not been studied in Pakistani population previously. There is a severe lack of any knowledge of the treatment outcome and the role of different protocols in Pakistani population. No database of cleft lip and palate has been established.

Objectives: Our aim is to determine the distribution of favourable/unfavourable treatment outcome by using GOSLON Yardstick, modified Huddart/Bodenham system, and EUROCRAN yardstick. And to evaluate the association of the congenital and post-natal treatment factors on the treatment outcome based on these indices.

Materials and Methods: A retrospective study design is considered suitable for desired observations. 101 model pairs of Pakistan children having total unilateral cleft lip and palate with a mean age of 8.05 ± 0.79 were assessed using GOSLON, MHB and EUROCRAN yardsticks.

Results: The mean score for GOSLON index is 3.04 ± 1.25 . The mean score of EUROCRAN based on dental grading is 2.72 ± 0.76 , whereas, based on the palatal surface morphology, the mean score is 2.20 ± 0.73 . The mean score of MHB, based on 5 groups, is 2.85 ± 1.30 . With the help of newly established database, teams providing cleft care can improve and establish protocols based on recent advanced techniques. This will provide base line information and help determine the effectiveness of those protocols.

Conclusion: Mean GOSLON scores, of Pakistani population unravel an intermediate treatment outcome and are comparable with other Asian population studies like Malaysia and Japan. According to modified Huddart/Bodenham scoring system, Pakistani patients have a fair to poor treatment outcome. The results were more sensitive considering transverse planar growth. According to EUROCRAN index, based on dental grading, Pakistani

patients have a higher frequency of poor treatment outcome, which was worse in comparison to the European populations. According to EUROCRAN index, based on palatal surface morphology, Pakistani TUCLP patients have the worse outcome in comparison to previous studies.

Supervisor:
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EVALUATION OF HUMAN AMNIOTIC MEMBRANE AS A SCAFFOLD FOR PERIODONTAL TISSUE ENGINEERING: AN IN VITRO STUDY

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Introduction: Human amniotic membrane (HAM) has many biological properties suitable for periodontal tissue regeneration such as low immunogenicity, anti-fibrosis, anti-inflammation and rich in extracellular matrix component.

Objectives: This study aimed to evaluate the ability of this membrane as a scaffold for the growth of the predominant cells in periodontal tissues, human periodontal ligament fibroblasts (HPDLFs).

Materials and Methods: Commercially available HPDLFs (Lonza, USA) were seeded on glycerol preserved HAM (USM Tissue Bank, Malaysia). HPDLFs attachment and surface morphology were observed through histological analysis and scanning electron microscopy (SEM) respectively. While the cell proliferation was assessed using alamarBlue® proliferation assay and nuclear labeling of DNA using 6-diamidino-2-phenylindole (DAPI) at day 1, 3, 7, 14 and 21. Histologically, HPDLFs showed mono layer to multilayers attachment on HAM from day 1 to day 7. On day 14 and 21, HPDLFs cell layers were reduced to single cell layer with more flattened appearance and longer spindle shaped cells. SEM analysis demonstrated that HPDLFs had attached appropriately on HAM surface at day 1 to day 3 and became overlapping at day 7, while maintaining their flat shape. However, by day 14 and 21 the cells demonstrated alteration in their morphology and later became rounded in shape. Based on statistical analysis (Friedman's Two-Way Analysis of Variance by Ranks followed by pairwise comparison) using SPSS 22.0 proliferation assay showed that HPDLFs viability on HAM had increased significantly from day 1 to day 7 ($P = 0.012$). However, the proliferation of cells showed significant reduction at day 14 ($P = 0.002$) and day 21 ($P = 0.005$). DAPI staining of nuclear DNA showed the presence of HPDLFs up to day 7 only.

Results: This study showed that HAM is able to function well as a scaffold for HPDLFs within 7 days. Retardation of cellular growth after 7 days could be due to possible reasons such as density dependent inhibition of growth or the release

of matrix metalloproteinases by the HPDLFs that might have degraded the membrane.

Conclusion: In conclusion, the findings suggest that HAM could be a promising scaffold for periodontal regeneration. However, cells' behaviour in relation to the membrane over longer culture duration requires further investigations.

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DETERMINATION OF TOOTH SIZE AND ARCH DIMENSION IN A PAKISTANI POPULATION: A NOVEL APPROACH UTILISING DIGITAL MODEL

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Introduction: The prime aim of this thesis is to develop the norms for tooth size, tooth size ratio (Bolton index), arch dimension, arch length and arch perimeter on subjects of Angle's class I (normal) occlusion in Pakistani population. This thesis describes the validity and reliability of digital model measurements, geomorphometrics norms of tooth size and arch dimension analysis by conventional digital caliper and digital stereomicroscope, measurement for Bolton' tooth size ratio (intermaxillary tooth size discrepancy) investigation, tooth size and intermaxillary tooth size discrepancy via circumferential tooth size measurements.

Objectives: To develop the norms for tooth size, tooth size ratio (Bolton index), arch dimension, arch length and arch perimeter on subjects of Angle's class I (normal) occlusion in Pakistani population through novel method utilising 2D HIROX KH7700 stereomicroscope (Japan).

Materials and Methods: In order to establish standard norms for the Pakistani population, we investigated the tooth size and arch dimension using conventional digital caliper (DC) and digital stereomicroscope (SM). The sample consisted of 128 subjects ranging in age from 18 to 24 years. Dental models of each subject for maxillary and mandibular arches were scanned via Hirox digital stereomicroscope for the fabrication of the digital models, and the tooth size and arch dimensions were measured via SM scanned digital models.

Results: Sex differences were assessed, and interrelationships between different variables were explored within the study group. For the data obtained by SM techniques, the men had statistically significant larger arch dimensions and geomorphometrics norms of tooth size than the women ($P < 0.05$). For the Bolton' tooth size ratio (intermaxillary tooth size discrepancy), the sum of anterior tooth size and overall tooth size via SM methods showed

statistically significant result in relation sexual disparities ($P < 0.05$). No significant sexual disparities for Bolton's anterior ratios (BAR) and Bolton's overall ratios (BOR) were observed.

Conclusion: This study has established a new reference database of tooth size and arch dimensions via SM for first time on Pakistani population. These norms for tooth size and tooth size ratio will be helpful for clinical treatment planning in dentistry and forensic dentistry.

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THE EFFECT OF ULTRASOUND THERAPY ON OSSEOINTEGRATION AND MARGINAL BONE LOSS AROUND IMPLANT-SUPPORTED PROSTHESIS

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Introduction: Marginal bone loss is considered to be an inevitable risk factor in implant therapy. The reduction in the height and width of marginal bone level may affect the success rate of implant treatment in terms of aesthetic and function. In addition, primary implant stability during the surgical phase is an important predictor for dental implant success. However, clinicians encounter many occasions where less than the recommended optimum primary stability was achieved during this phase and the further prognosis of the implant become questionable; Low-intensity pulsed ultrasound (LIPUS) has been proposed as a method for promoting and accelerating healing time and accordingly reducing the marginal bone loss.

Objectives: This study was carried out to determine the effect of therapeutic ultrasound on marginal bone loss using CBCT and osseointegration of dental implant using Resonance Frequency Analysis (RFA) techniques.

Materials and Methods: The study sample comprised of 22 patients; which were divided into two groups randomly: ultrasound and non-ultrasound (control) groups, both consisted of 11 patients. The ultrasound therapy was delivered intra-orally on the buccal part of the implant site for a duration of 20 minutes twice a week started 2 weeks following dental implant placement for the subsequent 10 weeks. The same ultrasound therapy protocol was repeated 2 weeks after the delivery of the prosthesis for another 10 weeks. The bone height and width were measured and compared at three different views (coronal, sagittal and axial) using CBCT at day 0, 3 months and 6 months. For statistical analysis, repeated measure ANOVA with significance level of $P < 0.05$ was used for the evaluation and comparison within the same group and between the two different groups based on time.

Results: Results showed that there was an increase in marginal bone level in the ultrasound group from day 0 to 6 months and marginal bone loss in the control group

within the same time interval. The marginal bone increase was more statistically significant at the buccal bone plate of $P < 0.05$. In both groups, ultrasound and control, there was an increase in RFA values but the increase was more pronounced in the ultrasound group compared to control group from day 0 to 6 months.

Conclusion: Overall, LIPUS presented valuable addition to the bone healing around dental implant and can be utilized as a treatment modality to save implant with questionable stability and to enhance bone regeneration around dental implants.

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EFFECTS OF LOW LEVEL LASER THERAPY ON THE GINGIVAL AND PERIODONTAL TISSUES IN ORTHODONTIC PATIENTS

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Introduction: Periodontal problems were stated to be one of the most acquainted adverse effects associated with fixed orthodontics. The primary aim of this research was to study the effect of LLLT as an adjunct with oral hygiene instructions, routine scaling and polishing in orthodontic patients to control the possibility of orthodontic treatment induced gingivitis and periodontitis in initial phase of orthodontic treatment.

Objectives: The primary objective of this research was to study the effect of LLLT on Plaque Index (PI), Gingival Index (GI), Bleeding on Probing (BOP) and Probing Depth (PD) as an adjunct with oral hygiene instructions routine scaling and polishing in orthodontic patients to control the possibility of orthodontic treatment induced gingivitis and periodontitis in initial phase of orthodontic treatment.

Materials and Methods: This was an experimental; randomized clinical trial study, our sample comprised 40 pre orthodontic patients, with an age range of 16 to 30 years. The duration of the study was six months; LLLT was applied at every visit i.e. 1, 2, 3, 4, 5 and 6 months on one side of each jaw, while the other half of the each arch was control. The clinical parameters, PI, GI, BOP and PD for the research were recorded at zero, first, third and sixth visit.

Results: Repeated measure showed that there is statistically significant inter-group and intra-group differences (< 0.05). The scores increased more in the control group. Moreover, there was no significant difference in T1 stage when compared between LLLT and control group. However, both groups were showed significant differences in T2, T3 and T4 stage of evaluation of the all clinical parameters.

Conclusion: The current study was able to unveil that an additional treatment with LLLT reduced the periodontal indices, as evaluated by the PI, GI, BOP and PD. All periodontal indices scores generally increased along the evaluation period in control groups.

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CYTOTOXICITY AND GENOTOXICITY EVALUATION OF LOCALLY PRODUCED BIPHASIC CALCIUM PHOSPHATE

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Introduction: Locally produced Biphasic Calcium Phosphate (BCP) with ratio of 20/80 Hydroxyapatite (HA)/Beta-tricalcium phosphate (β -TCP) promotes the differentiation of human dental pulp cells (HDPCs). It is imperative that the genotoxicity of this biomaterial has to be evaluated for any health risk to the patient prior to the commercialisation of the product.

Objectives: The objectives of this study are to evaluate the cytotoxicity and genotoxicity of locally produced BCP scaffold synthesised in School of Dental Sciences, Universiti Sains Malaysia, Malaysia by using MTT assay, Ames Salmonella/microsome mutagenicity assay and alkaline single-cell gel electrophoresis (Comet) assay.

Materials and Methods: For Ames assay, five tester strains of *Salmonella typhimurium* TA98, TA100, TA102, TA1537 and TA1538 were treated with the extract of locally produced BCP (0.3125, 0.625, 1.25, 2.5 and 5 mg/ml) with concurrent negative (distilled water) and positive (2-Aminoanthracene, 4-Nitro-o-phenylenediamine, sodium azide, mitomycin, and 9-aminoacridine) controls both in the presence and absence of metabolic activation system (S9) mix. Three different inhibitory concentrations of locally produced BCP [IC₅₀ (67.57 mg/ml), IC₂₅ (33.78 mg/ml)], and IC₁₀ (13.51 mg/ml)] obtained from MTT assay were used to treat the HDPCs in Comet assay. Negative (complete growth medium) and positive control [50 μ M hydrogen peroxide (H₂O₂)] were also concomitantly included. DNA damage of the cells was observed by analysing the tail moment and tail intensity produced by the cells.

Results: Based on MTT assay, the locally produced BCP was not toxic when treated with concentrations of 200, 100, 50, 25, 12.5, 6.25 and 3.125 mg/ml on HDPCs. The results are expressed based on the average number of revertant colonies per plate. The number of revertant colonies treated with locally produced BCP extract was less than double as compared to the number of revertant colonies in negative control. The tail moment and tail intensity of HDPCs treated with BCP extract with all the three inhibitory concentrations showed no

significant difference compared to negative control ($P > 0.05$), indicating that locally produced BCP did not induce DNA damage to HDPCs.

Conclusion: The results based on Ames assay and Comet assay indicate that the locally produced BCP is non-genotoxic under the present test conditions.

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DETERMINATION OF TOOTH SIZE AND DENTAL ARCH DIMENSION IN A TRANSGENDER BANGLADESHI POPULATION

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Introduction: Research related to tooth size, tooth size discrepancy, arch size in transgender population is yet to be explored in dentistry.

Objectives: The purpose of this study was to establish normative data on mesiodistal, buccolingual, diagonal dimension, arch width and tooth size ratio in transgender population.

Materials and Methods: The data were derived from dental casts of 150 transgender individuals. Data were analysed using descriptive, independent *t*-test, paired *t*-test and one sample *t*-test. The mean and standard deviation were calculated for individual tooth size, arch size, arch width (Pont's index), Bolton's overall and anterior ratios, separately for transgender males and females.

Results: Result showed that the mesiodistal, buccolingual and diagonal widths of the maxillary teeth showed higher variability than the mandibular teeth and the mean value was higher in transgender females than in males and revealed statistically significant differences between right and left sides of maxilla and mandible. Bolton's anterior ratios were found to be 78.50 (± 3.92) and Bolton's overall tooth ratio 91.27 (± 3.79). Statistically significant was showed in Bolton anterior ratio. The result showed that the means of the maxillary arch width shows the greater variability than mandible. The arch widths were larger in transgender females than that of transgender males. Also, the results showed statistically significant difference was observed in maxillary and mandibular arch width as well as arch perimeter.

Conclusion: These findings indicate that population-specific standards are necessary for clinical assessments and for several dental treatment purposes. Moreover, it is appropriate to use transgender norms in a regular dental practice for transgender individuals.

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ADHESION OF STREPTOCOCCUS MUTANS ON TOOTH COLOURED RESTORATIVE MATERIALS

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Introduction: The initial adhesion of *Streptococcus mutans* on the surface of materials contributed to the biofilm formation and may cause dental caries. In order to restore a carious tooth, the use of composite resin and resin-modified glass ionomer cement (RMGIC) in the restoration field has been increased due to the demand for aesthetic value.

Objectives: To study the effect of tooth coloured restorative materials on the accumulation of *S. mutans* on it surfaces by evaluating the fluoride release, surface roughness and biofilm thickness after *S. mutans* incubation, *S. mutans* growth on the materials and gene expression of the genes associated with adherence of *S. mutans* biofilm.

Materials and Methods: Four materials were used in this study which were RMGICs; KetacTM N100 and Fuji IITM LC and composites resins; FiltekTM Z350 and FiltekTM Z250. A microscopy study was performed which include atomic force microscopy (AFM) for evaluation of surface roughness of the incubation materials, confocal laser scanning microscopy (CLSM) for evaluation of biofilm thickness and scanning electron microscopy (SEM) for distribution observation of *S. mutans* on materials. Fluoride release measurement was carried out to analyse the fluoride release of RMGICs. Bacteria growth was done to assess the growth activity of *S. mutans* on the tested materials. Gene expression was also performed to determine the gene expression levels of *gtfB* and *gbpB* genes.

Results: Fuji II LC gave a significantly higher of fluoride release compared to Ketac in both storage media ($P \leq 0.001$). Both nanofilled materials gave a lower value of surface roughness while no significant difference of biofilm thickness between nanofilled and microfilled materials was shown except on day 7. RMGIC groups gave a lower *S. mutans* growth compared to composite resin group at all the incubation times. Nanofilled RMGIC gave significantly lower of expression levels of *gtfB* and *gbpB* gene compared to other materials $P < 0.05$.

Conclusion: These finding suggested a nanofilled RMGIC as the ideal material in reducing the accumulation of *S. mutans*, which could inhibit the adhesion of *S. mutans* on the surface materials.

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DENTAL ARCH RELATIONSHIP IN BANGLADESHI CHILDREN WITH NON-SYNDROMIC UNILATERAL CLEFT LIP AND PALATE (UCLP)

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Introduction: Cleft lip and palate (CLP) is one of the most common birth defects. Multiple factors are believed to be responsible for an unfavorable dental arch relationship (DAR) in CLP. Facial growth (maxillary) retardation, which results in class III malocclusion, is the primary challenge that CLP patients face. Congenital factors (UCLP type, UCLP side, family history of cleft, family history of class III) and postnatal treatment factors (cheiloplasty, palatoplasty) may influence treatment outcomes in unilateral cleft lip and palate (UCLP) children, which has led to a great diversity in protocols and surgical techniques by various cleft groups worldwide.

Objectives: The aim of this retrospective study was to evaluate DAR of non syndromic Bangladeshi UCLP children and to explore the various congenital and postnatal treatment factors that are responsible for unfavorable DAR.

Materials and Methods: Eighty four dental models were taken before orthodontic treatment and alveolar bone grafting. The mean age was 7.69 ± 2.46 (mean \pm SD). All the subjects had primary surgery (cheiloplasty and palatoplasty) at the same hospital. DAR was assessed blindly by five raters using GOSLON Yardstick (GY) and EUROCRAN index (EI) and by two raters using modified Huddart Bodenham (mHB) scoring system. Furthermore, all the subjects were divided into two groups; favorable and unfavorable groups. This grouping was carried out because patients in the favorable groups may not need further treatment after palatoplasty or cheiloplasty or they could be treated with conventional orthodontics, whereas patients in the unfavorable groups sometimes required surgical correction. Kappa statistics was used to evaluate the intra- and inter-examiner agreements, chi square was used to assess the associations and logistic regression analysis was used to explore the responsible factors that affect DAR.

Results: Total 37 subjects (44% of all subjects) were categorised into unfavorable group (category rating 4 and 5) using GY. The mean GOSLON score was 3.238. Intra- and inter-examiner agreements were very good. Using crude and stepwise backward regression analysis, significant association was found between family history of skeletal class III malocclusion ($P = 0.015$ and $P = 0.014$ respectively) and unfavorable DAR. Complete UCLP ($P = 0.054$) and left sided UCLP ($P = 0.053$) also seemed to be correlated with unfavorable DAR using crude and stepwise backward regression analysis respectively but no significant associations was found. Total 47 subjects (56% of all subjects) were categorised into unfavorable group (category rating 3 and 4) using EI. The mean EUROCRAN scores were 2.44 and 1.93 for DAR and palatal morphology (PM) respectively. Intra- and inter-examiner agreement was good to very good. Using crude and stepwise backward regression analyses, significant associations were found between the modified Millard

technique ($P = 0.047$, $P = 0.034$ respectively) of cheiloplasty and unfavorable DAR. Complete UCLP ($P = 0.017$) was also significantly correlated with unfavorable DAR. The PM showed a significant association with the type of cleft, type of cheiloplasty and type of palatoplasty. Total 39 subjects (46% of all subjects) were categorised into unfavorable group (category ratings poor and very poor) using mHB scoring system. The total mHB score was -8.26. Intra- and inter-agreement was very good. Using crude and stepwise backward regression analysis, significant association was found between positive history of class III ($P = 0.025$, $P = 0.030$ respectively) and unfavorable DAR. Using chi square test, complete UCLP ($P = 0.003$) and V-Y pushback palatoplasty ($P = 0.005$) were also significantly correlated with unfavorable DAR.

Conclusion: This multivariate study suggested that DAR of non syndromic Bangladeshi UCLP children was significantly correlated with some of congenital and postnatal treatment factors by using different indices.

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DETERMINATION OF DUSP6 GENE MUTATION AND ITS EFFECT ON CRANIOFACIAL MORPHOLOGY AMONG MALAYSIAN MALAY WITH CLASS III MALOCCLUSION PATIENTS ATTENDING AT HOSPITAL UNIVERSITI SAINS MALAYSIA

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Introduction: Class III malocclusion is a dominant inherited, slowly progressive dento-skeletal disharmony. It is characterised by over growth of mandible, stunted growth of maxilla, or a combination of both. The etiology of class III malocclusion and the role of genes in this phenotype remain indistinct. Recently, dual specificity protein phosphatases 6 (DUSP6) gene mutations have been reported to cause autosomal dominant form of class III malocclusion.

Objectives: The main objective of this study was to determine the DUSP6 gene mutation in three generations of Malaysian Malay subjects having class III malocclusion and to conduct their cephalometric analyses.

Materials and Methods: Genetic analyses of DUSP6 gene were carried out in 30 subjects by selecting three individuals representing three generations, respectively, from ten Malaysian Malay families having Class III malocclusion and 30 healthy controls. Cephalometric radiographs were obtained only from class III malocclusion subjects and pre-determined cephalometric linear and angular measurements were performed using Romexis software. *t*-Test and analysis of variance (ANOVA) were used to analyse the cephalometric

measurements from both mutation and non-mutation groups of class III malocclusion subjects.

Results: In the current study, a heterozygous missense mutation c.1094C > T (p. Thr 365 Ile) was identified in DUSP6 gene in three members of one family with class III malocclusion, whereas no mutation was found in the control group. *t*-Tests showed significant differences in angular measurements Co-Gn-B and SN-MP variables in mutation group compared to the non-mutation group. Moreover, ANOVA showed no significant differences for all variables except in *yen* angle of 1st versus 2nd generation.

Conclusion: In conclusion, current study successfully identified a missense mutation in DUSP6 gene among one Malaysian Malay family affected by class III malocclusion and cephalometrically found mandible was more prognathic from cranial base in mutation group compared to non-mutation group. The outcome of this study broadened the mutation spectrum of class III malocclusion and the importance of DUSP6 gene in craniofacial morphology.

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CYTOTOXICITY AND GENOTOXICITY EVALUATIONS OF WHITE MINERAL TRIOXIDE AGGREGATE AND MALAYSIAN WHITE PORTLAND CEMENT

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Introduction: Advances in dental biomaterials have helped in the development of endodontic research.

Objectives: To determine the genotoxic effect of Malaysian white Portland cement (MWPC) and white mineral trioxide aggregate (WMTA) using Ames test and Comet assay.

Materials and Methods: The cytotoxicity of WMTA and MWPC was evaluated using 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay after 72 hours. The mutagenicity of both materials were assessed using Ames test both in the absence and presence of metabolic activation system (S9 mix) on mutated Salmonella strains (TA98, TA102, TA1535, TA1537 and TA1538) and the DNA damage using Comet assay on human periodontal ligament fibroblasts (HPLF) cell line. Concurrently, negative and positive controls were also included. In Ames test, the data are presented as mean of number of revertant colonies compared to the negative control. For Comet assay, one-way ANOVA followed by post-hoc comparison was used for statistical analysis ($P < 0.05$).

Results: WMTA and MWPC exhibited favourable biological profiles on viability of HPLF cell line based on

MTT assay and the IC₅₀ for WMTA and MWPC was 18.71 and 19.91mg/ml respectively, IC₂₅ was 3.33 and 3.55mg/ml respectively and IC₁₀ was 0.59 and 0.63mg/ml for WMTA and MWPC respectively. These three inhibitory concentrations for both materials were used in Comet assay. The number of revertant colonies of TA98, TA102, TA1535, TA1537 and TA1538 for both the tested materials and conditions displayed low and consistent reversion rates. This showed that, WMTA and MWPC did not cause any potential mutation as the number of revertant colonies was less than the number of revertant colonies of the negative control. In the Comet assay, no significant comet formation was found in all groups of WMTA, MWPC and negative control except in the positive control. There was no significant difference in the mean tail moment and % DNA in tail between all the groups of WMTA, MWPC and negative control ($P > 0.05$). However, the mean tail moment and % DNA in tail between all the groups of WMTA, MWPC and negative control were significantly different from that of the positive control ($P < 0.05$).

Conclusion: MWPC is non-genotoxic and comparable to WMTA as a potential and economical substitute material in endodontic field under the present test conditions.

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MORPHOMETRIC ANALYSIS AND NASAL RECONSTRUCTION USING COMPUTER AIDED DESIGN AND MANUFACTURING (CAD/CAM)

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Introduction: Prosthetic rehabilitation of nasal reconstruction is the common option for nasal deformity case. Currently, conventional method was used to produce nasal prosthesis. It involves laborious steps, complicated procedure and time consuming. Therefore, an alternative method applying the computer technology known as Computer Aided Design and Computer Aided Manufacturing (CAD/CAM) was introduced.

Objectives: In general, the aim of the study was to produce nasal prosthesis generated from Computed Tomography (CT) data selected from database and mould printing using 3D printer. Other than that, morphometric analysis of nose was conducted to obtain the data of normal nose measurements.

Materials and Methods: A cross sectional study was carried out on 88 CT data from 20 to 70 years old involved seven parameters. MIMICS software was used to measure the 3D image. The CT data were retrieved from Radiology Department, Hospital Universiti Sains Malaysia (HUSM). The Nasal Database (NasalBase) was developed to store the nasal

measurements and its images. It will be used by the clinician in selecting the best shape of nose model for specific patients to produce the nasal prosthesis. A clinical study was done on a patient in HUSM. The patient needs a maxillofacial prosthesis to cover the defect part involving right eye, nose and cheek. The comparison of time taken to produce the prosthesis was carried out using conventional method and CAD/CAM.

Results: Based on the study, the morphometric analysis showed that males have higher mean values compared to females. There were significant differences between males and females for all parameters. The strong correlations were identified between the nasal and facial parameters while others parameters show weak correlations. The database of measurement and its images could be as reference for clinician. The nasal prosthesis fabricated from the 3D printed mould showed the good result. It was good in shape and texture.

Conclusion: The study illustrated that there were different between conventional and computer aided method in fabrication of maxillofacial prosthesis. It could be concluded, computer aided method can simplify the procedure of prosthesis fabrication by reducing the processing time. Hence, the production time, patient waiting time and number of appointments can be reduced. In addition, the quality of the prosthesis was also improved and did not fully depend on the technologist skill. Thus, this study revealed that the computer aided method has the good potential and the result was acceptable to be implemented in prosthesis fabrication specifically in HUSM.

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AN ALTERNATIVE METHOD FOR THE LOGISTICS REGRESSION MODEL AND ITS APPLICATION IN BIostatISTICS: A BENTHIC ECOSYSTEM FUNCTIONING IN PULAU BIDONG

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Introduction: Logistic regression model is a method that is frequently used by researchers, especially in the field of science and dentistry. However the usage of this method in other field is to limit. This logistic regression method is likely to be use because it gives a result through odd ratio. Generally, this research is more emphasis on building a methodology analysis in the field of aquatics. This is because the statistics in these areas are limited and less practised despite its importance cannot be denied. Thereby, the modeling presented in this thesis can provide a space and change of improvement that is useful and helpful to the research.

Objectives: The specific objective of this study is to build an algorithm of alternative logistic regression model

that focused on benthos feeding habits. The finding of this research also make a comparison between original multiple logistic regression model and alternative method and also make inference to the both of the model to get the information on efficiency of the model.

Materials and Methods: This thesis shows one alternative logistic regression method with the combination of bootstrap and weighted method than being applied to the benthic ecosystem data using Statistics Analysis System (SAS). Basically each algorithm is built that based on alternative method detailed one by one to get the better image on the uniqueness compare to existing methods.

Results: The usage of alternative method found that there are four main factors that significant ($P < 0.05$) which affect the benthic feeding patterns compared with ordinary method. The first factor is the size factor [$b_1 = -0.3001$, $P < 0.05$], the second factor is the flattened body factor [$b_2 = 0.7480$, $P < 0.05$], the third factor is the body shape factor [$b_3 = 0.3607$, $P < 0.05$], and the last one is the distribution of benthos factor [$\beta_4 = 0.0897$, $P < 0.05$].

Conclusion: As a conclusion, alternative method of logistic regression shows the result that better than ordinary method and the result is parallel to the previous study that have been made. Through the ordinary method, almost all of the factor that is supposed to be significant do not shows the true characteristics but through the alternative method, the characteristic can be exactly highlighted. This is show that alternative method showing the better modeling characteristic to the data set that is studied and can be applied successfully.

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