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Poster Session P04 – Growth and Development

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## Poster Session P04/Growth and Development

#### P04-50

## Dental status of parenterally fed children – presentation of two cases

A. REMISZEWSKI<sup>1</sup>, D. OLCZAK-KOWALCZYK<sup>1,2</sup>, P. SOBIECH<sup>1</sup>, A. GRZYBOWSKA<sup>1</sup> & K. POPIŃSKA<sup>3</sup>

<sup>1</sup>Department of Paediatric Dentistry, Warsaw Medical University; <sup>2</sup>Department of Oral Pathology, the Children's Memorial Health Institute; <sup>3</sup>Nutrition Clinic, The Children's Memorial Health Institute, Warsaw, Poland

Introduction: Parenteral nutrition is a form of supplementation of nutrients necessary for development of a body that has lost the ability to assimilate nourishment caused by diseases that have been suffered or underdevelopment of the digestive system. Its use comes with a risk of complications, including calcium-phosphate administration and catheter-based infections requiring treatment with antibiotics. They can disturb the course of development processes, including odontogenesis. On the other hand, parenteral nutrition eliminates or limits dental tissue exposure to cariogenic factors. The aim: assessment of the status of dentition and the general development of children fed parenterally.

**Materials and methods:** General development and dentition status of two patients with short intestine syndrome (age 5 and 14 years) were assessed. The 14-year-old boy was also subjected to bone densitometry. The 5-year-old boy had been fed parenterally since birth and the 14-year-old since he was two.

Results: Both patients exhibited height and weight deficiency. The 5-year-old patient endured multiple catheter-based infections requiring treatment with antibiotics. The 14-year-old had an incident of many months of antibiotic treatment at the age of two. Results of bone densitometry of the 14-year-old boy were below standard for his age. Dental examination of both boys showed delay in dentition time, irregularities in teeth anatomy, quantitative and qualitative abnormalities in dental tissues. Decay was not stated, despite unsatisfactory condition of oral hygiene.

Conclusion: Systemic complications of children fed parenterally may have a disadvantageous impact on the course of development processes both of primary and permanent dentition. Research should be continued.

#### P04-51

## Effects of tooth extractions on hippocampus in senescence-accelerated mice

 $\frac{\text{M. IINUMA}}{\text{KUBO}^2}$ , H. HIOKI $^1$ , Y. ICHIHASHI $^1$ , Y. TAMURA $^1$  & K.

<sup>1</sup>Department of Pediatric Dentistry, Asahi University School of Dentistry, Mizuho Gifu; <sup>2</sup>Department of Oral Anatomy, Division of Oral Structure, Function and Development, Asahi University School of Dentistry, Mizuho Gifu, Japan

**Introduction:** The purpose of this study is to evaluate the effects of tooth extractions at young age on the hippocampal function in senescence-accelerated mice P8 (SAM P8).

Materials and methods: 60 male SAM P8 mice were used. The upper molar teeth of both sides of 30 mice were extracted under general anesthesia at 1 month after birth (extraction group). 30 control mice were underwent the same anesthesia without extrac-

tion (control group). Following surgery, solid food was given and bred under conventional conditions. At one week (young), 4 months (mature) and 8 months (old) after surgery, measurement of the plasma corticosterone concentration, the Morris water maze test on spatial memory and the counting of the hippocampal neurons on Nissl staining were performed. The data were statistically processed by analysis of variance and, then, by Tukey's multiple comparison procedure.

**Results:** The plasma corticosterone concentration was significantly lower (P < 0.05), the shortening of the time to reach the platform by the Morris water maze test was significantly slower (P < 0.05) and the number of neurons was significantly lower (P < 0.05) in the mature and old mice of the extraction group than in the agematched controls. In the young mice, no significant difference was noted between the extraction and control groups.

Conclusion: The ability of spatial memory declined and the number of hippocampal neurons decreased in mature and old mice, suggesting that the hippocampal function is impaired by the tooth extraction. This study was supported by a Grant-in-Aid for Scientific Research (No.20592420) from the Ministry of Education, Science, Sports and Culture in Japan.

#### P04-52

## The effect of different transfusion on dental development in severe talassemic children

P. HOONCHAREON<sup>1</sup>, V. JIRARATTANASOPA<sup>1</sup>, A. KAWKUNCHON<sup>2</sup> & K. TORCHARUS<sup>3</sup>

<sup>1</sup>Department of Pediatric Dentistry, Mahidol University; <sup>2</sup>Division of Pediatric Hematology, Queen Sirikit National Institute of Child Health; <sup>3</sup>Division of Pediatric Hematology, Phramongkutklao College of Medicine, Bangkok, Thailand

**Introduction:** Thalassemia is a group of inherited defect in synthesis of haemoglobin resulting chronic anemia. Many studies reported craniofacial deformities, growth retardation and delay dentition in severe thalassemia. Nowadays, blood transfusion treatment has been introduced to promote growth and prevent deformities. Dental development is expected to be affected. Therefore, the objective of study was to evaluate dental development in severe thalassemia under different transfusion.

Patients and methods: This research was approved by the Human Subject Committees of Mahidol University, Bangkok, Thailand. All guardians gave written consent. The study conducted on 92 severe thalassemic and 32 healthy children, aged 6–13 years. Thalassemic subjects had history of pre-transfusion hemoglobin less than 7 g/dl and onset of anemia before 2 years. They were divided into 3 groups according to the frequency of transfusion: 1) high transfusion: more than 12 times/year. 2) low transfusion: 6–12 times/year. 3) occasional transfusion: less than 5 times/year. All subjects were taken panoramic radiographs. Seven left lower permanent teeth were evaluated developmental stages employed the method of Demirjian *et al.* The means different between chronological age and dental age were calculated and compared among groups by ANOVA.

**Results:** There were no differences in chronological age and dental age presented in control and high transfusion groups. Low transfusion and occasional groups showed significant delay

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dental development approximately 10 months and 1 year, respectively

Conclusion: Different frequency of transfusion has an effect on dental development. Chronic severe anemia delays dental development and high transfusion may prevent dental retardation.

#### P04-53

#### The effect of BTXA injection on mandibular growth in growing rats

S. Y. KWAK, J. Y. KIM & K. T. PARK

Department of Pediatric Dentistry, The Institute of Oral Health Science, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea

Introduction: This study assessed the effect of unilateral masseter muscle injection of Botulinum toxin A (BTXA) on mandibular growth in growing rats.

Materials and methods: Thirty-six male Sprague-Dawley rats were divided into three groups according to age (Groups 1 to 3, aged 4, 5, and 6 weeks, respectively). Each group was divided randomly into three subgroups (subgroups A, B, and C). Subgroup A served as a control in which the masseter muscles were injected with saline bilaterally. In subgroups B and C, one or both masseter muscles were injected with 3U BTXA, respectively. The experimental animals were sacrificed after 4 weeks. Then, the lengths of the mandibular body, condyle, and coronoid process and the heights of the anterior region, coronoid process, and condyle were measured and compared. Statistical analysis was carried out using the Kruskal-Wallis test with Bonferroni's correction and multiple comparison test.

Results: 1) In Group 1, the mandibular body length and condyle height of subgroups B (BTXA side) and C were shorter than those of subgroup A. 2) In Group 2, the anterior region and condyle heights of subgroups B (BTXA side) and C were shorter than those of subgroup A. 3) In Group 3, the mandibular body length and condyle height of subgroups B (BTXA side) and C were shorter than those of subgroup A.

Conclusion: BTXA injection effectively induces site-specific muscular hypofunction and growth change at local skeletal sites.

#### P04-54

#### Biomarkers of mineral status in primary teeth in children born pre-maturely

E. MORENO<sup>1</sup>, E. PLANELLS<sup>2</sup>, D. FLOREA<sup>2</sup>, E. MILLÁN<sup>2</sup> & P. PLANELLS<sup>1</sup>

<sup>1</sup>Departament of Estomatologia IV, Universidad Complutense of Madrid; <sup>2</sup>Institute of Nutrition, Biomedical Research Center, University of Granada, Spain

Introduction: Recent studies have demonstrated that children born pre-maturely (BW < 1.5 kg) have lower bone mineral content and density compared with children born at term gestation. The objective is to study the dental mineralization of children born prematurely (PT) by analyzing calcium, phosphorus and magnesium as biomarkers of nutritional status comparing with a control group of children born at term (AT).

Patients and methods: A cohort of 30 healthy children (6–8 vr) PT group was selected and primary dental mineral status was compared with these of 25 AT children (6-9 yr). A food intake questionnaire was asked to obtain (Mataix, 2007 software) %RDA intakes of calcium, phosphorus and magnesium. Calcium and magnesium content of wet mineralized samples were determined by atomic absorption spectrometry, phosphorus was analysed in same samples by colorimetric Fiske Subbarow method. Statistical SPSS 16.0 package was used to comparative study. Permission was obtained from an institutional ethical committee and children's parents gave written informed consent.

Results: No significant differences were obtained in calcium, phosphorus and magnesium intakes between groups. PT children had lower dental calcium, phosphorus and magnesium content than AT children:  $188.5 \pm 10.2 \text{ vs } 254.3 \pm 12.3, 100.4 \pm 5.6 \text{ vs}$  $138.8 \pm 9.2$  and  $5.3 \pm 1.5$  vs  $6.7 \pm 1.3$ , respectively (P < 0.05). No differences were found in phosphorus content.

Conclusions: PT group of children have lower primary teeth calcium and magnesium content, compared with AT children. Mineral supplementation of the early diet may be needed in preterm infants, taking into account that genetic determinants may influence bone mineralization of PT infants.

#### P04-55

#### Emergence of permanent teeth in a Hungarian child population

J. A. NEMES

<sup>1</sup> & Z. PAPP

<sup>2</sup> Faculty of Dentistry, Department of Pediatric Dentistry, University of Debrecen, Debrecen; <sup>2</sup>Private Practice, Nyíregyháza, Hungary

Introduction: Published data for permanent tooth emergence in Hungarian children are more than 40 years old. The aim of the study was to determine the age and sequence of eruption of permanent teeth, as well as gender differences in primary school children

Patients and methods: The emergence of permanent teeth was studied in 609 Hungarian children (305 boys and 304 girls) who had longitudinal records of the emergence of all permanent teeth except 3rd molars. Emergence ages were based on their presence or absence in the oral cavity at clinical examination. We calculated ages at clinical eruption of permanent teeth in months. Counts of permanent teeth corresponding to ages from 6 to 14 are also given. The statistic method for the calculation was the Maximum-Likelihood-Method.

Results: The sequence of tooth eruptions differs significantly in the lower and upper jaw, whereas no significant differences existed when comparing the sides of each jaw. The times of tooth eruption is earlier in females than in males. In case of boys there is a tendency for earlier tooth eruptions in the lower jaw. In respect to the tooth eruption sequence, a change was noted in the upper jaw. In contrast to other reports, the second pre-molar in girls has changed places with the canine and erupts prior to this tooth.

Conclusion: When compared to the results gained from former observations in this region of Hungary, differences were found. Concerning the entire dentition acceleration of the tooth eruption could be noted.

#### 004-56

#### The development of German versions of paediatric sleep quality assessment instruments

D. SAGHERI<sup>1</sup>, A. WIATER<sup>2</sup>, R. D. CHERVIN<sup>3</sup>, J. A. OWENS<sup>4</sup> & B. BRAUMANN<sup>1</sup>

<sup>1</sup>Department of Orthodontics, Cologne University Hospital, Cologne, Germany; <sup>2</sup>The Children's Hospital Sleep Disorders Laboratory, Porz am Rhein Hospital, Cologne, Germany; <sup>3</sup>Sleep Disorders Center, Department of Neurology, University of Michigan, Ann Arbor, USA; <sup>4</sup>Academic General Pediatrics, Rhode Island Hospital, Brown University, Providence, USA

**Introduction:** The lack of validated German screening instruments for sleep-disordered-breathing in children makes it difficult to identify such children as part of dental examinations and makes it almost impossible to prevent resulting problems such as mouthbreathing, tongue-thrusting and dentofacial deformities. Furthermore, such disorders may well be treated with functional orthodontic appliances. The parent-report 'Pediatric Sleep Questionnaire (PSQ)' and the 'Child's Sleep Habits Questionnaire (CSHQ)' gained widespread use internationally. The aim of this study was to develop German versions of the PSQ and CSHQ.

Materials and methods: WHO translation guidelines were followed in order to assure cultural equivalence. A subsequent two-phase validation study based on focus group testing and standardised pre-testing was carried out in order to evaluate acceptance and usability of the instruments.

Results: Focus-group testing on subgroups of 20 parents of children with proved sleep disorders and healthy children with the 'Think-Aloud'-method highlighted the need for minor adaptations. Subsequent pre-testing on 46 parents of children with sleep disorders and 235 parents of healthy children established their reliability and construct validity. Analysis of concordance between the 2 instruments (corelation coefficient 0.7) showed substantial agreement. The German PSQ contains 77 questions which cover quality of sleep in 2–18-year-old children and the German CSHQ contains 45 questions which quality of sleep in 4–10-year-old children.

**Conclusion:** Confirmatory testing of the PSQ and CSHQ demonstrated their usefulness in identifying both behaviourally and medically based sleep problems. Both instruments can be used to identify sleep-related breathing disorders when plysomnography is not feasible.

#### P04-57

## The central neuronal activity regarding gustatory stimulus in tube-feeding rat

T. OOKA, T. HAINO, S. HIRONAKA & Y. MUKAI Department of Hygiene and Oral Health, School of Dentistry, Showa University, Tokyo

**Introduction:** The purpose of this study was to reveal the changes in activation of central neuronal nucleus involved with feeding and gustatory experience caused by weaning condition when the pups begin to shift from suckling to mastication with the observation using the Fos protein as a marker of immunohistochemistry.

Materials and methods: The Sprague-Dawley rats were separated into following 3 groups (5 pups in each group). I) Tube feeding group including rat pups operated gastrostomy at 9 postnatal day, II) tube feeding group including rat pups operated gastrostomy at 14 postnatal day, III) control group (nursed with dam). The tube feeding rats were infused artificial milk by gastric tube exclusively without oral feeding. At 21 postnatal day (P21) they were fed the pelleted food. Then, all pups were perfused for fixation and extracted the brainstem tissues and prepared to coronal section (50µm in thickness). In the brainstem, we counted the number of Fos-immunoreactive (FI) neuronal cells in nucleus principal sensory nucleus of trigeminal nerve (PrV) and nucleus tractus solitarius (NTS).

**Results:** FI cells were observed in the tube feeding pups obviously, and the statistic comparison showed significant higher count of FI cells in PrV in comparison to control group. Similarly, NTS showed significant increment of FI cells in the tube feeding groups compared with the control rat pups.

**Conclusion:** Consequently, it was considered that differences of ingestion pathway and weaning mode in the development stage influence to the nervous activity in central neuronal nucleus associated with feeding and swallowing function.

#### P04-58

## Morphological and chemical aspects of primary teeth from pre-term infants

M. RYTHÉN<sup>1,2</sup>, J. G. NORÉN<sup>1</sup>, F. STEINIGER<sup>3</sup>, W. DIETZ<sup>3</sup> & A. ROBERTSON<sup>1</sup>

<sup>1</sup>Department of Pedodontics, Institute of Odontology at the Sahlgrenska Academy, University of Gothenburg, Gothtenborg, Sweden; <sup>2</sup>Department of Pedodontics, Public Health Service, Region of Western Götaland, Gothenburg and Borås, Sweden; <sup>3</sup>Centre of Electron Microscopy, Friedrich-Schiller-University Jena, Germany

**Introduction:** Clinical and morphological studies have shown enamel aberrations in teeth from pre-term children. This study describes histo-morphology of primary enamel and compares the chemical composition in enamel and dentin in primary teeth from pre-term children with normal children born at term.

Patients and methods: Enamel in 44 exfoliated primary teeth, from 14 children with a gestational age below 29 weeks, were examined, using polarized light microscopy (POLMI) and scanning electron microscopy (SEM). Chemical analyses of 17 of the examined teeth and of 36 exfoliated primary teeth from healthy children born at term were performed, using X-ray micro analysis (XRMA). Statistical analysis, using Mann–Whitney *U*-test. Written informed consent from the parents and ethical consent by the Ethical Research Committee at the University of Gothenburg, was given. Results: In POLMI the neonatal line was found in 1/3 of the sections. In the postnatal enamel 31 teeth showed a high degree of porosity. The SEM analysis confirmed these findings. In agreement with the morphological study, the concentration of carbon was higher, the value of calcium and the ratio Ca/P was lower in the outer part of the enamel compared with enamel from healthy children.

Conclusion: Enamel mineralization disturbances in primary teeth from pre-term children showed disturbancescompared with teeth from healthy children. These irregularities were localised in the outer enamel layers. This study was supported by grants from the Research & Development Council in the Region of Western Götaland, Sweden and the Research & Development Council in Southern Älvsborg County, Sweden.

#### P04-59

## Combined effect of TCDD and fluoride on dental hard tissue formation *in vitro*

E. SALMELA<sup>1</sup>, A. M. PARTANEN<sup>1</sup>, C. SAHLBERG<sup>1</sup>, P. L. LUKINMAA<sup>2,3</sup> & S. ALALUUSUA<sup>1,4</sup>

<sup>1</sup>Department of Pediatric and Preventive Dentistry, Institute of Dentistry, University of Helsinki; <sup>2</sup>Department of Oral Pathology, Institute of Dentistry, University of Helsinki; <sup>3</sup>Department of Pathology, Helsinki University Central Hospital; <sup>4</sup>Department of Oral and Maxillofacial Diseases, Helsinki University Central Hospital, Helsinki, Finland

**Introduction:** The most toxic dioxin congener, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), impairs dental hard tissue formation and mineralization *in vitro* and *in vivo*. A further, well-established cause of enamel hypomineralization is excess fluoride intake. In a previous *in vitro* study fluoride disturbed or prevented enamel matrix secretion and mineralization. Dentin mineralization was nonhomogeneous. Our aim was to investigate if simultaneous exposure to TCDD and sodium fluoride (NaF) can elicit additive effects on dental hard tissue formation *in vitro*.

**Materials and methods:** Mandibular first and second molar tooth germs (n=152) of E18 mouse embryos were cultured for 12 days with TCDD (5, 10, 15nM) or NaF (2.5, 5, 10, 15,  $20\mu$ M) alone or the two agents in combinations of several concentrations. The

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agents were added to the medium from the start of culture. Control teeth were cultured without TCDD/NaF. The explants were photographed, fixed, processed to paraffin sections and stained with HE. Morphological changes were studied visually from the photographs and tissue sections.

**Results:** TCDD alone impaired dentin mineralization and enamel formation and NaF reduced enamel matrix secretion. The severity of the effects increased with the concentrations. While the effects of 10 nM TCDD and 15  $\mu$ M NaF alone were barely detectable but consistent, their combined effect on dental hard tissue formation was clear.

**Conclusion:** Both TCDD and NaF impair dental hard tissue formation, depending on the concentrations. The combined effect of the two agents at concentrations, which alone cause no or barely detectable effects, is clear.

#### P04-60

## Dental development in children with mild-to-moderate hypodontia

E. S. TUNC, S. BAYRAK & A. E. KOYUTURK

Faculty of Dentistry, Department of Pediatric Dentistry, University of Ondokuz Mayıs, Samsun, Turkey

**Introduction:** To compare dental development in a group of children with mild-to-moderate hypodontia and a matched group. **Patients and methods:** A study group of 70 children (43 females, 27 males) with hypodontia aged 5.3–12.5 years was race-, age-, and sex-matched with 140 healthy, normal controls. Dental ages of children were calculated using a modified dental-age estimation method. Differences between dental and chronological ages were analyzed by *t*-test, and the correlation between difference in dental and chronological age and number of missing teeth was analyzed by Spearman's correlation test.

**Results:** Dental development in children with mild-to-moderate hypodontia was significantly delayed in comparison to the matched group (P < 0.05); however, the mean difference did not exceed 0.3 years in either sex. There was no correlation observed between the difference in dental and chronological age and the severity of hypodontia.

**Conclusion:** Children with mild-to-moderate hypodontia were found to have a delay in dental development of a few months, which was statistically significant. Due to individual variations, each patient should be individually examined.

#### P04-61

## Age estimation of Amelogenesis Imperfecta patients using three different methods: a retrospective study

Z. KIRZIOĞLU, <u>K. G. ULU</u> & A. C. ALTUN

Department of Pediatric Dentistry, University of Süleyman Demirel, Isparta, Turkey

**Introduction:** Developing teeth are used to assess maturity and estimate age in forensic dentistry, anthropologic studies. The aim of this study is to evaluate age estimation methods for Amelogenesis Imperfecta (AI) patients and refer the appropriate one.

Materials and methods: 38 patients with type I, II and III of AI (22 female and 16 male) aged 4–15 years (average age  $9.06\pm2.36$  years) were recruited between the years of 2002-2008. Informed consents from all participants and/or their parents were provided. The diagnosis of AI was made by three skilled examiners. All patients were evaluated for malocclusion, eruption pattern, dental age, pulpal pathologies, missing and malformed teeth. Dental development was measured on panoramic radiographs in 29 patients aged between 6–13 years by using methods of Nolla (1960), Demirjian (1973) and Haavikko (1974). Dental age

for each method was compared with chronological age for each subject.

**Results:** Focal calcifications were observed in AI patients. No statistically significant differences were observed between three age estimation methods. In boys (presenting with a mean age of  $8.08 \pm 2.7$ ) the mean age estimated using the methods of Haavikko, Demirjian, Nolla was  $9.5 \pm 1.6$ ;  $10.5 \pm 2.3$ ;  $9.9 \pm 2.5$ , respectively. The mean age calculated with Haavikko, Demirjian, Nolla for girls was  $8.78 \pm 2.20$ ;  $9.48 \pm 2.74$ ;  $8.86 \pm 2.54$  and the actual chronological age was  $9.08 \pm 2.09$  for girls.

**Conclusion:** In this study including 38 patients with differents types of AI, the method for age estimation of Haavikko was the most accurate, followed by Nolla and Demirjian.

#### P04-62

## Influence of feeding methods on the development of the mandibular dental arch

 $\frac{\text{T. YONEZU}^1}{\text{MATSUBARA}^2}$ , M. YAKUSHIJI $^1$ , S. SHINTANI $^1$ , N.  $\frac{\text{MATSUBARA}^2}{\text{MATSUBARA}^2}$  & H. SIRAI $^2$ 

<sup>1</sup>Department of Pediatric Dentistry, Tokyo Dental College, Chiba; <sup>2</sup>Combi Co, Tokyo, Japan

Introduction: The positive impact of breast-feeding on health has been the subject of extensive study and review. However, there is a difference of opinion regarding the influence of feeding methods on the growth of the jaw of infants. The purpose of this study was to examine various mandibular pre-dental arch parameters in infants. Materials and methods: Infant feeding data were collected and then at age 3 months, these infants were examined and study models obtained. The feeding methods were categorized as 'breast-fed' if the infants were ever breast-fed, and as 'bottle-fed' for those never breast-fed. The samples evaluated consisted of 32 sets of dental casts (breast-fed: 21 infants, bottle-fed: 11 infants) obtained from infants. Pre-dental arch shapes were measured using noncontact, high speed, 3-dimensional shape measuring system (UNISN INC). Digital computerized measurements of the anterior arch length and arch widths at several regions of the dental arch were performed. Comparisons between the breast-feeding and bottle-feeding were performed with the paired Student's t-test.

**Results:** Mean anterior arch length (6.4 vs 5.9 mm) was greater in those breast-fed infants. Mean anterior arch width (24.4 vs 23.5 mm) was also greater in those breast-fed infants. However, these parameters were not significantly different between feeding methods. These results indicated that the arch width of breast-fed infants were, in general, greater than in those of bottle-fed infants. **Conclusion:** We conclude that the method of feeding may have some influence on dental arch development during the 3-months period. However, further research is needed to clarify such differences with a larger sample size and also a longer observation period.

#### P04-63

## Establishment of ameloblasts derived from induced pluripotent stem cells

M. ARAKAKI, A. YAMADA & S. FUKUMOTO

Department of Oral Health and Development Sciences, Division of Pediatric Dentistry, University Graduate School of Dentistry, Sendai, Japan

**Introduction:** Recently, it is possible to regenerate neuronal cells, osteoblasts and adipocytes from stem cells in tooth germ and dental pulp. However, it is difficult to regenerate tooth cells, including ameloblasts, from those kinds of stem cells. Establishment of ameloblasts from stem cells may be important to form regenerated tooth and understand the molecular mechanism of

ameloblast differentiation. The purpose of this study is to differentiate ameloblast and odontoblast from induced pluripotent stem (iPS) cells have been generated from mouse somatic cells by introducing transcription factors.

Materials and methods: We performed to culture mouse iPS cells with rat dental epithelium cell line SF2-GFP cells, established in our group. Ameloblast differentiation was analyzed by RT-PCR using LNA primers for ameloblastin gene.

Results: The AMBN mRNA of mouse tooth germ, but not those of rat was detected by LNA primers for rat AMBN. These primers are useful to analyse the expression of AMBN in mouse iPS cells. When iPS cells were co-cultured with rat dental epithelium cells, iPS cells showed similar morphology of dental epithelium cell. RT-PCR showed iPS cells expressed AMBN indicating that iPS differentiated into ameloblasts.

**Conclusion:** We found that iPS cells derived from mesenchymal cells could differentiate into ameloblasts by co-culture with dental epithelial cells. This induction method may be useful to form regenerated tooth.

#### P04-64

## TMJ internal derangement following condylar fractures: impact on facial growth

P. DEFABIANIS

Department of Biomedical Sciences and Human Oncology, Section of Pedodontics, Traumatology and Oro-Facial Malformations in the Growing Patients, Dental School, University of Torino, Italy

**Introduction:** Many clinical studies have shown how jaw injuries are the single most important cause of subsequent TMJ internal derangement. Proper function of the masticatory system is certainly the most influential variable in facial growth. The aim of the study was to evaluate relationships between post-traumatic internal derangement and disturbed facial growth in children.

Patients and methods: 25 children, 16 boys, 9 girls, 14 year of age or younger – (mean age: 5.7; range: 3–9 at the time of injury) were selected out of a group of 74. They all referred a history of major face injury. None of them referred a history of TMJ pain, mechanical dysfunction, orthognatic treatment or TMJ surgery before the trauma. They all had been treated by physiotherapy following a specific treatment protocol and underwent combined clinical and radiographic standardized evaluation for 5 years. None of them was treated orthodontically after the trauma.

Results: 19 patients were found to have at least one abnormal and internally deranged TMJ on imaging studies; symptoms included – either individually or in various combination – pain and/or mechanical TMJ dysfunction. Out of this group, 11 patients showed a mandibular asymmetry with chin deviation to the smaller or more degenerated TMJ. Three patients showed an evident mandibular retrognathia with marked bilateral TMJ remodelling; three patients showed normal TMJ(s) with normal facial structure. Conclusion: These data suggest that in children TMJ derangement may potentially alter mandibular growth.

#### P04-65

## Body weight of Australian children undergoing treatment of caries under general anaesthesia

H. FUNG<sup>1</sup>, N. PRABHU<sup>1</sup>, A. CAMERON<sup>1</sup> & A. BLINKHORN<sup>2</sup>

<sup>1</sup>Department of Paediatric Dentistry, Westmead Hospital; <sup>2</sup>Faculty of Dentistry, University of Sydney, Sydney, Australia

**Introduction:** Over 90% of dental caries in pre-school children remains untreated in most countries. Previous studies have found that children with early childhood caries (ECC) show a

significantly reduced height and/or weight when compared with controls. However, there has been a great increase in childhood obesity over the last decade. The aim of this pilot study was to investigate the body weight of Australian children requiring general anaesthesia (GA) for management of dental caries.

Materials and methods: Ethics was obtained from the Human Research Ethics Committee, Sydney West Area Health Service. A retrospective audit was conducted of all children (1113) who required dental treatment under general anaesthesia at Westmead Hospital between January 2007 and June 2008. Records of 447 children (age < 72 months) were examined. Basic demographic information, a detailed dental and medical history, dmft, treatment provided and the weight on the day of the surgery were recorded for each patient. Percentiles for weight were calculated using CDC/NCHS data files that are currently used for Australian norms.

**Results:** The average dmft for this cohort was 9.2. The weight of 74% of boys and 51% of girls was  $\geq$ 75<sup>th</sup> percentile, almost 50% of boys were above the 90th percentile. Less than 5% of children weighed less than the 10th percentile. No relationship was found between rates of caries and weight.

**Conclusion:** The majority of children requiring treatment under GA for EEC presented with a weight above average.

#### P04-66

## Acoustic characteristics of children of the Japanese consonants s[j]

T. SUGIYAMA, J. ASARI, M. SATO & M. INOUE

Department of Pediatric Dentistry, Showa University School of

Dentistry, Japan

**Introduction:** The articulation of children occurs in association with the developments. We conducted acoustic analysis of these articulations in order to investigate organic effects of the oral cavity, one of the articulation organs.

Patients and methods: The subjects were composed of five healthy children (primary dentition completion) and five children with cleft lip and palate. The purpose and methods of this study were explained to all subjects, and the articulation was recorded upon obtaining consent. The test sounds were selected as connecting preceding and following vowels of [a] to consonants of [s] and [ʃ] as in VCV (vowel-consonant-vowel) syllable [asa] and [aʃa]. Recording was conducted in a sound insulation room.

**Results:** Regarding children with cleft lip and palate, palatalized articulation was the most frequently identified by the diagnosis based on auditory impression. Results of measurements demonstrated that the maximum sound pressure of children with cleft lip and cleft palate tended lower than that of healthy children.

Conclusion: As a diagnosis of speech and language disorders, auditory impression is currently used in clinical setting. On the contrary, acoustic analysis allows the visualization of articulation characteristics of patients based on figures, independent of clinical experience of the user. The study suggested that the utilization of acoustic analysis has a potential to actualize a diagnosis based more on quantitative evidence.

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