**Identification of Caries Risk Determinants in Toddlers: Results of the GUSTO birth cohort study**

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**Declaration of Interests**

K. Godfrey, Y.S. Lee and Y.S. Chong have received reimbursement for speaking at conferences sponsored by companies selling nutritional products. K. Godfrey and Y.S. Chong are part of an academic consortium that has received research funding from Abbot Nutrition, Nestec and Danone. The other authors declare that they have no competing interests.

**Abstract**

The aim of the study was to identify risk determinants leading to early childhood caries (ECC) and visible plaque (VP) in toddlers. Data for mother-child pairs participating in the Growing Up in Singapore Towards healthy Outcomes (GUSTO) birth cohort were collected from pregnancy to toddlerhood. Oral examinations were performed in 543 children during their clinic visit at 24 months to detect ECC and VP. Following logistic regression, ECC and VP were jointly regressed as primary and secondary outcomes, respectively, using bivariate probit model. ECC prevalence was 17.8% at 2 years of age, with 7.3% of children having a VP score >1. ECC was associated with night-time breastfeeding (3 weeks) and biological factors, including Indian ethnicity (lower ECC rate), higher maternal childbearing age and existing health conditions, maternal plasma folate <6 ng/mL, child BMI and plaque index, while VP was associated with psycho-behavioural factors, including frequency of dental visits, brushing frequency, lower parental perceived importance of baby teeth, and weaning onto solids. Interestingly, although higher frequency of dental visits and tooth-brushing were associated with lower plaque accumulation, they were associated with increased ECC risk, suggesting that these established caries-risk factors may be a consequence rather than the cause of ECC. In conclusion, Indian toddlers may be less susceptible to ECC, compared to Chinese and Malay toddlers. The study also highlights a problem-driven utilization pattern of dental services (care sought for treatment) in Singapore, in contrast to the prevention-driven approach (care sought to prevent disease) in Western countries.

**Introduction**

Despite major progress in caries control measures in the last few decades, dental caries in permanent dentition remains the most prevalent global condition [Kassebaum et al., 2015] and early childhood caries (ECC) has been reported to be the most common chronic childhood disease in the US [US Surgeon General report., 2000]. According to NHANES survey data, the prevalence of ECC significantly increased from 24% (1988-1994) to 28% (1999-2004) in 2-5-year-old American children [Dye et al., 2007]. Furthermore, a polarised distribution of ECC has been observed in the US, with 25% of the population accounting for nearly 80% of the disease burden [Kaste et al., 1996]. Identification of the risk factors for ECC may be critical for timely caries prevention in toddlers.

 Studies have reported high levels of cariogenic bacterial species [Zhou et al., 2012], diet [Palmer et al., 2010], oral hygiene status [Tanzer et al., 2001], insufficient maternal Vitamin D levels during pregnancy [Schroth et al., 2014]and low socioeconomic status [Reisine and Psoter, 2001], among others, as risk determinants associated with ECC. Frequently, visible dental plaque has been demonstrated to be an important caries risk indicator in infants and toddlers [Zhou et al., 2012]. However, the contribution of early life risk factors to both visible plaque and ECC concomitantly in infants/toddlers remains uncertain.

 Singapore is a small city-state in South-East Asia, with a multi-ethnic population (namely the Chinese, Malays and Indians) displaying similar distributions in literacy levels and types of residential dwellings. This unique setting may be an ideal community to investigate the ethnic differences in ECC development. In spite of being a 100% fluoridated community in the past six decades, a 2005-2006 community-based study observed 40% caries prevalence and 43.6% incidence rates among 3- to 6-year old children in Singapore [Gao et al., 2009], even worse than the aforementioned data from the US, which currently has water fluoridation coverage of 74.7%, according to the CDC (Centres for Disease Control and Prevention) 2014 data. Previously, a skewed distribution of caries lesions has been demonstrated in Singaporean children, aged 3-5 years, showing 16% of high-risk children carrying 78% of the disease burden [Gao et al., 2009]. To trace the possible reasons for such a polarised disease distribution, the present study aimed to identify biopsychosocial and behavioural risk factors for both ECC (as the primary outcome) and visible plaque (as a secondary outcome), using data collected from pregnancy until 2 years of age.

**Material and Methods**

**Study Design**

Children and their biological mothers were recruited through the Growing Up in Singapore towards Healthy Outcomes (GUSTO) birth cohort study [Soh et al., 2014]. Healthy pregnant women in their first trimester (n=1,237) were enrolled between June 2009 and September 2010, who intended to deliver at KK Women’s and Children’s Hospital (KKH) or National University Hospital (NUH) - the two major public maternity hospitals in Singapore - and who planned to reside in the country for the subsequent 5 years. Expectant mothers receiving chemotherapy or psychotropic drugs or who had type 1 diabetes mellitus were excluded from the study. Ethical approval was obtained from the Centralized Institutional Review Board (CIRB) of SingHealth (reference 2009/280/D) and Domain Specific Review Board (DSRB) of Singapore National Healthcare Group (reference D/09/021). Written informed consent was obtained from all participants upon recruitment.

**Data Collection**

Interviewer-administered questionnaires were used to collect general and health-related information during pregnancy: the first visit by 14 weeks of gestation (recruitment) and a second one at 26-28 weeks. During the latter visit, expectant mothers underwent blood tests, including a 75-gram oral glucose tolerance test. The World Health Organization (WHO) criterion of 2-hour post-load plasma glucose level >7.8 mmol/L was used to diagnose gestational diabetes [Alberti and Zimmet, 1998]. The cut-off points for adequate plasma micronutrient levels were selected on the basis of published studies: vitamin B6 (pyridoxal phosphate)=20 nmol/L [Lui et al., 1985]; folate=6 ng/mL [Herbert, 1987]; vitamin B12=300 pg/mL [Allen, 2008]; vitamin D=50 nmol/L [Malabanan et al., 1998]. In addition to anthropometric measurements, information on socio-demographics, lifestyle, and maternal/infant health was collected during these visits. As maternal anthropometry differs across populations, the WHO criterion for Asian populations was used to classify women’s body mass index (BMI) into 4 groups: underweight (<18.5 kg/m2); normal (18.5-22.9 kg/m2); overweight (23-27.5 kg/m2); obese (>27.5 kg/m2) [WHO, 2004].

Prenatal, labour/delivery and birth details, including child’s sex, weight, gestational age and neonatal complications were extracted from hospital case notes. For this study, infants were classified as small for gestational age (SGA) if the birth weight was below the 10th percentile [Mikolajczyk et al., 2011]. Weights were measured using calibrated digital scales for both mothers (SECA 803, SECA Corp, Hamburg, Germany) and their children (SECA 334, SECA Corp, Hamburg, Germany). Breast- and bottle-feeding practices, dietary patterns (including weaning) and child’s oral/general health were ascertained through a detailed interviewer-administered questionnaire during each study visit. Children were followed up postnatally at 3 weeks and subsequently at 3- or 6-month intervals until 18 months of age before their 24-month clinical visit

An interviewer-administered oral health questionnaire was administered by either a registered dentist or trained clinic staff during the 24-month visit, from March 2012 to June 2013, to collect information on children’s oral health practices (feeding patterns, diet habits, oral hygiene measures, and non-nutritive habits) and parental knowledge and attitudes on oral health. Children (n=543) were examined by a single dentist, calibrated against a gold-standard examiner with representative cases (Intraclass Correlation Coefficient >0.80). Children refrained from food, drink, and tooth-brushing for at least 1 hour before any oral examination.

Dental examinations, using knee-to-knee position, were conducted using torchlights. Teeth were assessed mainly by visual inspection, using plane surface mouth mirrors, and aided by tactile inspection when deemed necessary. Autoclaved blunt WHO probes were used to avoid damaging sound enamel surfaces. Prior to the drying/cleaning the tooth surfaces for dental examination (using ISO-2950 notation system), the oral hygiene status was determined using the modified Silness-Löe Plaque Index [Silness and Loe, 1964]. Six index primary teeth (55, 52, 64, 75, 72, and 84) were evaluated based on a four-point scale, with the oral hygiene status considered as “excellent” to “good” for average plaque scores (of 6 teeth) less than 1. When a designated tooth had not yet erupted, an alternative tooth in the same quadrant was taken as a substitute. Teeth were considered present in the oral cavity when any portion of the tooth was visible. A modified International Caries Detection and Assessment System (ICDAS II) was used for ECC detection [Ismail et al., 2007]. Since a certain amount of difficulty and uncertainty has been reported to differentiate between active and inactive lesions in one single appointment previously [Ekstrand et al, 2005], the lesions detected in the present study cannot be accurately ascertained to be active lesions only. However, satisfactory inter-examiner and intra-examiner reliability scores were noted, with scores for codes 1-2 being 0.76 and 0.95 respectively, and for codes 3-6 being 1.00 and 1.00 respectively. Since, visual examination by ICDAS criteria requires cleaning of teeth, for debris/plaque removal, to detect underlying caries lesion, the first examiner recorded the plaque index and hence, inter-/intra-examiner reliability for plaque scores could not be conducted. Sterile gauze was used to remove debris and dry tooth surfaces. No additional detection methods, including radiographs, were used owing to constraints of the field settings and ethical considerations.

**Statistical Analysis**

 The initial sample size calculation was based on the hypothesized odds ratio and base line proportion of positive outcome. We assumed that the minimal detectable effect size (Odds Ratio) would be 2.0, and the proportion of early childhood caries (primary outcome of interest) was set at 20%. Utilizing test for two proportions with two-sided Fisher's Exact Test, a total of 378 subjects were calculated to be required after taking into account 10% attrition rate. This was met with the target recruitment of the GUSTO study set at 1,200 pregnant women (with an ethnic distribution of approximately 60% Chinese, 23% Malay and 17% Indian) and a total of 587 children were recruited for the oral health study during the 24-month clinic visit. The primary outcome variable “caries present” for each child was defined as the presence of white spot/cavitated lesions observed on any tooth surface (ICDAS II codes 1-6) during the 24-month clinic visit. The secondary outcome variable “high plaque index” was defined as score >1 (moderate to abundant accumulation of plaque within the gingival pocket and/or tooth and gingival margin) calculated from the Silness-Löe plaque index conducted during the same visit.

Statistical analysis was performed using STATA software version 14. Potential risk determinants for ECC were screened using bivariate analysis (with a p value of 0.1 as cut-off point) in order to assess each variable individually and not to rule out any clinically important factors. To choose among competing models, the preferred logistic regression model was selected, based on the log likelihood ratio, numbers of significant variables in the model, as well as Hosmer-Lemeshow goodness-of-fit test. The seemingly unrelated bivariate probit model (SUBP) was used to investigate the independent risk determinants for both the primary (ECC) and secondary categorical outcomes (VP), in which the probability of ECC manifestation and high plaque index were modelled jointly using maximum likelihood estimates with robust standard error estimates. Additionally, the statistically significance of the Likelihood-ratio test of rho was assessed to ensure the model was properly estimated. The sociodemographic, prenatal, perinatal, postnatal, and 24-month oral health-related variables were also adjusted in the multivariate SUBP model. Backward step-wise regression procedure was employed.

The Wald Chi-square test, which is reflected by statistical significance of p, was used to determine whether the final fitted model would be best estimated jointly in a recursive manner or not. Additionally, the statistical significance of the Likelihood-ratio test of rho was assessed to ensure the model was properly estimated with the statistical method utilized. The Modified Hosmer-Lemeshow goodness-of-fit test was also applied to check the SUBP model. The final effect size of independent significant risk factors was quantified using the odds ratio (OR) estimate and respective associated 95% intervals (CI). A two-sided *p* value of less than 0.05 was considered statistically significant.

**Results**

**Sample Characteristics**

Out of the 1,237 GUSTO participants, a total of 543 children underwent oral examinations during the 24-month visit. Examination records with incomplete data (for Silness-Löe plaque index and/or ICDAS scoring) were excluded from analysis (n=8). A total of 535 children were included in the analysis (274 boys and 261 girls), of which 54.8% were Chinese, 28.8% Malay and 16.4% Indian. The ethnic distribution was representative of the main GUSTO cohort, including 55.8% Chinese, 26.2% Malay and 18.0% Indians. There were no statistically significant sociodemographic differences between the analyzed and missing/dropout subjects. Initially, we used the full data set available for the statistical modelling, but only 293 data points (n=293) were on the fitted line, as the rest of them (with missing data points) contributed to error terms in the model (not fitted portion). Hence, a total of 296 mother-child pairs were excluded from the final fitted model. As the result of the Hosmer-Lemeshow goodness of fit test was not significant (p=0.719), we accepted the hypothesis that our model is well fitted. The prevalence of ECC in the study population at 2 years was 17.8%, with 3.9% of children exhibiting one or more cavitated lesions. The mean Silness-Löe plaque index was 0.22±0.45, with 7.3% of children having a score above 1. For the non-cavitated lesions (ICDAS), the mean number of affected teeth and surfaces were 0.50±1.47 and 0.63±2.26, respectively. The upper central incisors (tooth #51=10.1% and tooth #61=9.9%) were the most commonly affected (Figure 1a), followed by upper lateral incisors (tooth #52=6% and tooth #62=5.6%) and lower first molars (tooth #74=3.4% and tooth #84=3.9%), with as high as 7.9% of buccal (incisors) and 3.0% of occlusal (molars) surfaces affected (Figure 1b).

For the cavitated lesions, the mean number of affected teeth and surfaces were 0.12±0.84 and 0.19±1.43, respectively. The upper central incisors (tooth 51=3% and tooth 61=2.2%) were the most commonly affected (Figure 2a), followed by upper lateral incisors (tooth 52=0.9% and tooth 62=1.5%) and upper first molars (tooth 54=0.7% and tooth 64=0.9%), with as high as 1.5% of buccal (incisors) and 0.4% of occlusal (molar) surfaces affected (Figure 2b).

**Identification of Caries Risk Determinants**

Several significant caries risk and protective factors were identified via the conventional stepwise logistic regression procedure (Table 2). Some variables in the final model with p>0.05 (e.g. night-time bottle-feeding), were added in the models since they are well-established risk factors/confounders for either or both outcomes. The risk of caries at 2 years of age was associated with Chinese ethnicity, increased maternal age (34 years and above), night-time breast-feeding (within first 3 weeks of life), higher child BMI, child’s dental visits (from 9 to 24 months of age), daily brushing frequency of child’s teeth (at least once per day) and 2-year high plaque index (>1) (all p<0.05). In contrast, higher maternal education level (undergraduate education and above), maternal health condition prior to/during pregnancy (immune system disorders being the most prevalent [34.6%]), and those with normal prenatal plasma folate levels (≥6 ng/mL) were protective factors against ECC (all p<0.05).

All ECC risk determinants identified via the conventional logistic model remained statistically significant in the bivariate probit model (Table 3), except maternal education level (p>0.05 for both ECC and VP). Additionally, a higher plaque accumulation (plaque index above 1) was demonstrated in children who had a delayed weaning onto solids (9 months), child’s dental visits, child’s brushing frequency and parental belief of importance of baby teeth (all p<0.05). Although daily brushing frequency of child’s teeth (≥once/day) and child’s dental visits from 9 to 24 months of age were associated with lower plaque score, these 2 factors were associated with increased ECC risk.

**Discussion**

This study identified the potential ECC risk determinants, by jointly modelling both ECC and visible plaque, in 2-year-old children using a life-course approach. The rate of ECC (including white-spot lesions) in 2-year old children (17.8%), observed in this study, is lower compared to ECC in Switzerland (25.3%) [Menghini et al., 2008] and China (27.6%) [Zhou et al., 2011]. The low cavitation rate (3.9%) seemingly corroborates with previously reported findings on caries susceptibility, to be low during the first post-eruptive year, but rises rapidly 2 to 3 years following tooth eruption [Carlos and Gittelsohn, 1965]. Moreover, the intraoral caries distribution pattern observed in the present study is parallel with the previously reported data [Gao et al., 2009], confirming the important role of site-specificity in the disease pathogenic pathway.

Interestingly, the findings of our study showed the predominance of biological factors associated with ECC in contrast to the psycho-behavioural factors associated with VP in 2-year old children. These findings, in addition to a previous study, showing association of both biological and psycho-behavioural factors with ECC in 3-6 year Singaporean children [Gao et al., 2010], indicates the major role of biological risk factors early in life.

In the present study, Indian toddlers were shown to be at a lower risk for ECC development when compared to Chinese toddlers, although no significant differences were observed in their plaque index. Pre-schoolers of Indian ethnicity have been reported to have a lower caries rate than Malay pre-schoolers [Gao et al., 2010]. Ethnic differences in maternal dietary practices during pregnancy were observed in another study of the same GUSTO cohort [Chen et al., 2014], in which Indian mothers were observed to consume more cheese and yoghurt, compared to the other two ethnic groups, which may be one of the reasons associated with lower caries experience in Indian children [Tanaka et al, 2012]. . Moreover, ethnic differences in micronutrient levels during pregnancy[Loy et al., 2015], also observed in our GUSTO cohort previously, may potentially play a role in caries susceptibility; nevertheless, future studies to elucidate the specific mechanism(s) are warranted.

 Our findings demonstrated that a pre-existing maternal health condition was protective towards ECC development in 2-year old children. A previous study has reported lower ECC experience in 3-6-year-old Singaporean children with existing health conditions [Gao et al., 2010]. It is speculated that the health conditions of mother or child may heighten the health consciousness and enhance certain health behaviours [Graham et al., 2016]. The present study also showed children, born to mothers with a higher childbearing age (≥34 years), to have higher caries rates when compared to their counterparts. Older mothers have been reported to be associated with infants with low birthweight [Martin et al., 2010] and low birthweight has been shown to be a risk factor for enamel defects in primary dentition [Vello et al., 2010], possibly increasing the caries susceptibility observed in our study.

 Among all the maternal micronutrients investigated, we observed insufficient maternal plasma folate levels (<6 ng/mL) to be a risk factor for ECC in the offspring. It is suggested that lower folate concentrations during pregnancy may perturb DNA methylation by modulating the synthesis of methionine and its derivative, S-adenosyl-methione [Bergen et al., 2012], causing higher homocysteine concentrations and increased salivary oxidative stress, potentially contributing to initiation and progression of dental caries in mother and subsequently in infants/toddlers [Kumar et al., 2011]. It is speculated that a window period may potentially exist during which folate levels may be crucial for optimizing the caries-protective effects of the developing immune system and foetal tissue factors during early life.

Higher BMI (at age 2 years) was associated with caries experience in 2-year old children in our study. This link has been controversial in the literature thus far. While positive correlations between caries risk and higher BMI have been reported [Vazquez-Nava et al., 2010], other studies demonstrated no association [Hong et al., 2008; Wu et al., 2013] or even an inverse relationship [Norberg et al., 2012; Sanchez-Perez et al., 2010]. Additionally, it has been postulated that there may be a U-shaped trend rather than a linear one, as the prevalence of dental caries may be higher among individuals with extremely higher or lower BMI [Hooley et al., 2012]. As the relationship between dental caries and childhood obesity may be age-specific, further studies may be needed to thoroughly characterize this potential association.

A higher frequency of night-time breastfeeding (first 3 weeks) was identified as a risk factor in this study. A plausible explanation may be that night-time breast-feeding within the first 3 weeks of life may enhance vertical transmission/ inoculation of cariogenic bacteria during the pre-dentate stage [Plonka et al., 2012], paving the way for cariogenic biofilm to be established at a later stage and thus increasing the child’s future susceptibility to dental caries. Previous studies investigating the overall impact of breast-feeding on dental caries have yielded contradicting results, with some studies showing a significant age-specific association [Tham et al., 2015] while others showing inconsistent results [Valaitis et al., 2000]. Similarly, our data did not identify any association between ECC and nocturnal breast-feeding (>9 months), overall breastfeeding and night-time bottle-feeding. The relationship between breastfeeding and dental caries may be non-linear and age-specific [Tanaka and Miyake, 2012; Valaitis et al., 2000], partially due to the timing of tooth eruption and the calcium-phosphate composition of breast milk [Featherstone, 2008], which may decrease with progressive lactation, thus increasing its cariogenic properties. Moreover, prolonged breastfeeding practices, resulting in delayed weaning onto solids, may result in increased plaque accumulation as shown in our study.

 Interestingly, the frequency and quantity of sweet confectionery or beverages was unrelated to ECC at 2 years of age in the present study. A similar finding has been observed in 2-year old Chinese children previously [Zhou et al, 2012]. We speculate that since ECC develops as a pre-cavitation condition, which takes time to manifest itself in the oral cavity, the snack intake quantity and frequency may not be directly associated with ECC as early as 2 years of age. As illustrated in the Tables, ECC in toddlers is more associated with biological factors, with environmental and/or behavioural factors affecting plaque deposition mainly at this age, which may subsequently lead to development of caries lesions at a later age.

The American Academy of Paediatrics (AAP) advocates oral assessment in high-risk infants to be carried out by a dentist no later than 6 months after eruption of the first tooth, or by 12 months of age, whichever comes first [Hale, 2003]. Early dental visits have been associated with low caries experience in pre-school children in the US [Beil et al., 2014]. However, only 2.6% of the GUSTO toddlers had been to a dentist prior to this study and only 6% of the primary caregivers of children during the 2-year clinic visit reported the period within 1.5 years to be a suitable timing for the first dental visit. It is likely that parents consider infant teeth to be of low importance and would only seek dental treatment for their children upon symptoms of pain, fever and/or suspected conditions. Moreover, history of previous dental visits has been shown to be associated with higher ECC experience in pre-school children in China [Zhou et al., 2012], similar to the findings of our study. Additionally, higher dental visits were shown to significantly reduce the visible plaque accumulation in the present study, indicating that clinical visits were actually a result of child having ECC, rather than a risk factor, and denoting a problem-driven utilization of dental services among Asians, compared to the prevention-based dental visits in other developed countries like the US [Ramos-Gomez et al., 2010].

 A strong correlation has been reported in literature between oral hygiene habits and caries prevalence in primary dentition, where the higher frequency of tooth brushing has been linked to lower caries experience in the child [Gibson and Williams, 1999; Grindefjord et al., 1995]. However, in our study, although brushing frequency of child’s teeth was associated with reduced plaque accumulation (OR=0.62), it was associated with increased caries risk (OR=1.88). It is likely that caregivers reinforced the frequent brushing in children, after seeing their decayed teeth or diagnosis/warning from dentists, parallel to the association between dental visits and ECC experience. In this study, these two established caries risk protective factors turned out to be the consequences and thus, indicators of disease rather than the cause of the disease. Furthermore, presence of dental plaque has been suggested as an indicator of early stages of ECC and its severity [Declerck et al., 2008; Parisotto et al., 2010]. In our study, visible plaque was found to be a strong risk factor for ECC development at 2-years of age. These findings emphasize the need for effective plaque control measures to be in place at an early age to prevent ECC development in young children, which may be influenced by primary caregivers’ perceived importance [Finlayson et al., 2007].

One of the strengths of this study is the utilization of a bivariate probit model to estimate ECC, together with visible plaque accumulation, a known clinical caries risk indicator in young children. Secondly, the disease characteristics and distribution were described in three Asian ethnic groups, which reflect more than 40% of the global population. The study had its limitations as well. As with most of the longitudinal cohort studies with substantial missing data across time points, some subgroup analysis may be underpowered in our study. Our findings in a multi-ethnic Asian cohort sample might not be generalizable to other populations.

In conclusion, our study identified Indian toddlers to have significantly lower caries rate, compared to Chinese and Malays. This study also highlighted the specific problem-driven utilization of dental services in Singapore, in contrast to the prevention-driven approach in Western countries.

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**Legends**

Table 1. Potential Risk Determinants of Early Childhood Caries

Table 2: Identification of Caries Risk Determinants in Toddlers using Multivariate Logistic Regression (Final-Fitted Model)

Table 3: Identification of Caries Risk Determinants in Toddlers using Seemingly Unrelated Bivariate Probit Regression (Final-Fitted Model)

**Table 1: Potential Risk Determinants of Early Childhood Caries**

|  |  |
| --- | --- |
|  | **Independent Variablesa** |
| Sociodemographic Data | 1. Ethnicity: (1) Indian (2) Malayb (3) Chinesec
2. Mother’s age: (1) <28 years (2) 29-33 years (3) ≥34 years
3. Mother’s education level: (1) General Certificate of Education (GCE) and below (2) University (undergraduate education and above)
4. Father’s education level: (1) General Certificate of Education (GCE) and below (2) University (undergraduate education and above)
5. Family household income: (1) SGD$0-$1,999 (2) SGD$2,000-$3,999 (3) SGD$4,000-$5,999 (4) ≥SGD$6,000
6. Child’s gender: (1) Male (2) Female
 |
| Prenatal | 1. Maternal health status: (1) No existing health condition (2) Existing health conditionb
2. Maternal BMI (kg/m2): (1) Underweight (<18.5 kg/m2) (2) Normal (18.5-22.9 kg/m2) (3) Overweight (23-27.5 kg/m2) (4) Obese (>27.5 kg/m2)
3. Maternal intake of medications: (1) No (2) Yesc
4. Maternal intake of supplements: (1) No (2) Yes
5. Plasma levels for vitamin B6: (1) Insufficient (<20 nmol/L) (2) Sufficient (≥20 nmol/L)
6. Plasma levels for folate: (1) Insufficient (<6 ng/mL) (2) Sufficient (≥6 ng/mL)c
7. Plasma levels for vitamin B12: (1) Insufficient (<300 pg/mL) (1) Sufficient (≥300 pg/mL)
8. Plasma levels for vitamin D: (1) Deficient (<30 nmol/L) (2) Insufficient (30-49 nmol/L) (3)Sufficient (≥50 nmol/L)
9. 2-hour oral glucose tolerance test (OGTT) levels: (1) Normal (<7.8 mmol/L) (2) GDM (≥7.8 mmol/L)
10. Alcohol consumption: (1) No (2) Yes
11. Combined tobacco smoke exposure: (1) None (2) Only passive (3) Active and passive
12. Intrauterine growth restriction (IUGR): (1) No (2) Yes
 |
| Perinatal | 1. Mode of delivery: (1) Vaginal (2) Caesarean section
2. Analgesia/sedation during labour: (1) None (2) Pharmacological (3) Inhaled (4) Combined
3. Antibiotics during labour: (1) No (2) Yes
4. Neonatal complications: (1) No (2) Yes
5. Small for gestational age: (1) No (2) Yes
6. Gestational age: (1) <37 weeks (2) ≥37 weeks
7. Birth weight: (1) <2,500 grams (2) ≥2,500 gramsc
8. Birth length: (1) <49 cm (2) ≥39 cm
9. Birth head circumference: (1) <34 cm (2) ≥34 cm
 |
| Postnatal | 1. Maternal intake of medications during confinement period: (1) No (2) Yesc
2. Maternal intake of supplements during confinement period: (1) No (2) Yes
3. Breast-feeding (birth, 3 weeks, 3M, 6M, 9M, 12M, 15M, 18M, 24M): (1) No (2) Yes
4. Exclusive breast-feeding within the first 3 months of life (3M): (1) No (2) Yes
5. Night-time breast-feeding (3 weeksb, 9M, 12M, 15Mb, 18M, 24M): (1) No (2) Yes
6. Night-time bottle use (9M, 12M, 15M, 18M, 24Mc): (1) No (2) Yes
7. Weaning onto solids within the first 9 months of life (9M): Months
8. Child’s BMI (3 weeksb, 3M, 6M, 9M, 12Mc, 15M, 18M, 24Mb): kg/m2
9. Child’s health – infections (3M, 6M, 9M, 12M, 15M, 18M, 24M): (1) None (2) ≥Once
10. Child’s health – allergy-associated symptoms (3 weeksb, 3M, 6Mc, 9M, 12M, 15M, 18M, 24M): (1) None (2) ≥Once
11. Child’s skin prick test at 18M: (1) Negative (2) Positive to at least one allergen
12. Child’s health – intake of medications (cumulative): 18M : (1) None (1) Once (2) ≥Twice / 24M: (1) ≤Once (2) Twice (3) ≥Thrice
13. Child’s health – intake of supplements (cumulative): 18M / 24Mc: (1) None (2) ≥Once
14. Child’s intake of sweet confectionery – daily frequency and grams (18M)
15. Child’s intake of sweet beverages – daily frequency and grams (18M)
16. Household smoking in the presence of the child (24M): (1) No (2) Yes
17. Eruption timing of first tooth (ETFT): Months
18. Number of teeth erupted – 6M, 9M, 12M, 15M, 18M, 24M
19. Child’s dental visits from 9M-24M: (1) None (2) ≥1
 |
| 24-Month Oral Health Questionnaire/Examination | 1. Number of times mother brushes teeth on a daily basis: (1) ≤Once (2) ≥Twice
2. Fluoride tablets taken during pregnancy/lactation: (1) No (2) Yes
3. Dental check-ups on a yearly basis (mother): (1) No (2) Yes
4. Reason for no dental check-ups (mother): (1) Accessibility (2) Dental-related
5. Age of first dental check-up for child: Years
6. Importance of baby teeth: (1) No (2) Yes
7. Reason for tooth decay: (1) Tooth worms/heatiness/ineffective tooth brushing (2) Sugar or bacteria (3) Sugar and bacteria
8. Finger-sucking habit: (1) No (2) Seldom/Occasionally (3) Frequently
9. Duration of pacifier use: Months
10. Sharing feeding/drinking utensils: (1) No (2) Seldom/Occasionally (3) Frequently
11. Frequency of sweet snacks/drinks (daily basis): (1) None (2) Once (3) ≥Twice
12. Frequency of sweet snacks/drinks (before sleeping and without brushing teeth): (1) None (2) Occasionallyc (3) Frequently/almost every night
13. Statement “caregiver’s ability to withhold sugar snacks from child”: (1) Disagree/strongly disagree (2) Neutral (3) Agree/strongly agree
14. Number of times child brushes teeth (daily basis): (1) None (2) ≥Once
15. Statement “caregiver’s efficacy in brushing child’s teeth”: (1) Disagree/strongly disagree (2) Neutral (3) Agree/strongly agree
16. Fluoride-containing toothpaste: (1) No (1) Yes
17. Silness-Löe Plaque Index score: (1) ≤1 (2) >1b
 |

a Sample size for each independent variable is different.

b Significant in bivariate analysis p<0.05.

c Significant in bivariate analysis p<0.1.

**Table 2: Identification of Caries Risk Determinants in Toddlers using Multivariate Logistic Regression (Final-Fitted Model)**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Factors** |  | **Na=293** | **Caries %**  | **P-value** | **Adjusted OR** **(95 % CI)** |
| Ethnicity | Chinese | 161 | 19.3 | 0.040 | 2.85 (1.05-7.75) |
| Malay | 84 | 19.0 | 0.964 | 1.03 (0.31-3.41) |
| Indian | 48 | 10.4 |  | 1 |
| Mother’s Age | ≥34 years | 101 | 22.8 | 0.025 | 3.07 (1.15-8.20) |
| 29-33 years | 90 | 16.7 | 0.195 | 1.90 (0.72-5.00) |
| <28 years | 102 | 13.7 |  | 1 |
| Mother’s Education Level | University | 160 | 15.0 | 0.026 | 0.40 (0.18-0.90) |
| ≤GCE | 133 | 21.1 |  | 1 |
| Maternal Health Status | Existing Condition | 127 | 10.2 | 0.040 | 0.41 (0.17-0.96) |
| No Existing Condition | 166 | 23.5 |  | 1 |
| Prenatal Plasma Folate Level | ≥6 ng/mL | 264 | 17.0 | 0.033 | 0.28 (0.08-0.90) |
| <6 ng/mL | 29 | 24.1 |  | 1 |
| Medications during Pregnancy | Yes | 43 | 7.0 | 0.153 | 0.27 (0.05-1.63) |
| No | 250 | 19.6 |  | 1 |
| Medications during Confinement Period (Mother) | Yes | 48 | 12.5 | 0.324 | 0.60 (0.22-1.65) |
| No | 245 | 18.8 |  | 1 |
| Night-time Breast-feeding (3 Weeks) | Yes | 179 | 22.9 | 0.003 | 4.07 (1.60-10.36) |
| No | 114 | 9.6 |  | 1 |
| Night-time Bottle-feeding (24M) | Yes | 190 | 19.5 | 0.195 | 1.69 (0.76-3.76) |
| No | 103 | 14.6 |  | 1 |
| Child’s Skin Prick Test (18M) | Positive | 36 | 27.8 | 0.071 | 2.5 (0.92-6.85) |
| Negative | 257 | 16.3 |  | 1 |
| Child’s BMI (24M) | kg/m2 | 293 | 17.7 | 0.004 | 1.37 (1.10-1.71) |
| Child’s Dental Visits (9M-24M) | ≥Once | 9 | 44.4 | 0.015 | 6.03 (1.42-25.72) |
| None | 284 | 16.9 |  | 1 |
| Parental Belief (Reason for Tooth Decay) | Sugar or Bacteria | 177 | 15.8 | 0.127 | 0.41 (0.13-1.29) |
| Sugar and Bacteria | 72 | 18.1 | 0.071 | 0.41 (0.15-1.08) |
| Beliefs/Ineffective Tooth Brushing | 44 | 25.0 |  | 1 |
| Child’s Intake of Sweet Snacks at Night | Frequently | 81 | 14.8 | 0.942 | 0.97 (0.39-2.38) |
| Occasionally | 75 | 25.3 | 0.098 | 2.03 (0.88-4.69) |
| Never | 137 | 15.3 |  | 1 |
| Child’s Daily Brushing Frequency (24M) | ≥Once | 241 | 19.5 | 0.010 | 4.20 (1.40-12.59) |
| None | 52 | 9.6 |  | 1 |
| Child’s Plaque Index Score (24M) | >1 | 24 | 37.5 | <0.001 | 9.16 (2.71-30.99) |
| ≤1 | 269 | 16.0 |  | 1 |

a Initial Sample Size=535

bVariables in the final model with *p* value >0.05 were added in the model since they are well-established risk factors/confounders for the outcome.

**Table 3: Identification of Caries Risk Determinants in Toddlers using Seemingly Unrelated Bivariate Probit Regression (Final-Fitted Model)**a,b,c

|  |  |  |  |
| --- | --- | --- | --- |
| **Factors** |  | **Dental Caries** | **Dental Plaque Accumulation** |
| **P-value** | **Adjusted OR** **(95 % CI)** | **P-value** | **Adjusted OR** **(95 % CI)** |
| ***Biological characteristics*** |  |  |  |  |  |
| Ethnicity | Chinese | 0.006 | 2.13 (1.24-3.64) | 0.668 | 1.18 (0.55-2.52) |
| Malay | 0.441 | 1.28 (0.68-2.42) | 0.338 | 0.65 (0.27-1.58) |
| Indian |  | 1 |  | 1 |
| Mother’s Age | ≥34 years | 0.007 | 1.90 (1.20-3.03) | 0.808 | 0.93 (0.52-1.66) |
| 29-33 years | 0.087 | 1.50 (0.94-2.39) | 0.617 | 0.88 (0.53-1.45) |
| <28 years |  | 1 |  | 1 |
| Mother’s Education Level | University | 0.132 | 0.75 (0.52-1.09) | 0.401 | 0.83 (0.54-1.28) |
| ≤GCE |  | 1 |  | 1 |
| Maternal Health Status | Existing Condition | 0.011 | 0.61 (0.42-0.89) | 0.075 | 1.50 (0.96-2.34) |
| No Existing Condition |  | 1 |  | 1 |
| Prenatal Plasma Folate Level | ≥6 ng/mL | 0.002 | 0.39 (0.21-0.71) | 0.733 | 1.14 (0.54-2.42) |
| <6 ng/mL |  | 1 |  | 1 |
| Night-time Breast-feeding (3 Weeks) | Yes | 0.003 | 1.87 (1.24-2.83) | 0.307 | 1.27 (0.80-2.00) |
| No |  | 1 |  | 1 |
| Child’s BMI (24M) | kg/m2 | 0.002 | 1.18 (1.06-1.32) | 0.141 | 1.10 (0.97-1.24) |
| Eruption Timing of First Tooth | Age in Months | - | - | 0.052 | 0.88 (0.78-1.00) |
| ***Behavioural characteristics*** |  |  |  |  |  |
| Weaning onto Solids (9M) | Age in Months | - | - | 0.014 | 1.32 (1.06-1.64) |
| Night-time Bottle-feeding (24M) | Yes | 0.136 | 1.35 (0.91-1.99) | 0.324 | 0.79 (0.50-1.26) |
| No |  | 1 |  | 1 |
| Child’s Dental Visits (9M-24M) | ≥Once | 0.049 | 2.31 (1.00-5.34) | <0.001 | 0.01 (0.01-0.02) |
| None |  | 1 |  | 1 |
| Parental Belief (Reason for Tooth Decay) (24M) | Sugar or Bacteria | 0.128 | 0.65 (0.38-1.13) | 0.728 | 1.13 (0.58-2.20) |
| Sugar and Bacteria | 0.067 | 0.65 (0.41-1.03) | 0.650 | 1.16 (0.61-2.22) |
| Beliefs/Ineffective Tooth Brushing |  | 1 |  | 1 |
| Parental Belief (Importance of Baby Teeth) | Yes | 0.078 | 0.58 (0.31-1.06) | <0.001 | 0.29 (0.16-0.54) |
| No |  | 1 |  | 1 |
| Child’s Daily Brushing Frequency (24M) | ≥Once | 0.012 | 1.88 (1.15-3.07) | 0.040 | 0.62 (0.39-0.98) |
| None |  | 1 |  | 1 |

a Sample Size=327

bVariables in final model with *p* value >0.05 were added in the model since they are well-established risk factors/confounders for the outcome.

c Modified Hosmer-Lemeshow goodness-of-fit test for biprobit (p=0.67) and Murphy’s score test for biprobit (p=0.64)