NON-TRADITIONAL EXPOSURES AND CHILDHOOD DENTAL CARIES AMONG CHILDREN 1-5 YEARS OLD

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ABSTRACT

Background: Early Childhood Caries (ECC) is one of the most common chronic illnesses among young children, affecting around 23% of children 2-5 years old, leading to pain, discomfort, and poor quality of life. It is a multi-factorial disease that develops through the combined effects of bacteria, tooth morphology, fermentable carbohydrates, time, and various social factors. Several studies have investigated the associations between dental caries and non-traditional factors acquired during the first years of life including; mode of delivery, breastfeeding, and Environmental Tobacco Smoke (ETS). However, the literature on these associations has been inconsistent.

Objective: To investigate the unadjusted and adjusted associations between the presence of Early Childhood Caries (ECC) and Severe-ECC (S-ECC), and three non-traditional factors: breastfeeding, mode of delivery, and ETS, among children 1-5 years old.

Methods: A cross-sectional design was employed, and a sample of 112 caregiver/child dyads was recruited from the ongoing flow of patients at Temple University Maurice H. Kornberg School of Dentistry (TUKSoD). After consent, subjects completed a questionnaire and received a standard intra-oral examination and the American Academy of Pediatric Dentistry (AAPD) Caries-risk Assessment Tool (CAT) by a student doctor. The study was approved by the Temple University Institutional Review Board (Protocol # 23885). Chi-square tests, two-sample t-tests and bivariate logistic regressions were used to assess the unadjusted associations. Two multivariable logistic models were developed for ECC and S-ECC and included demographics, overall CRA, and the three non-traditional risk factors.

Results: The prevalence of ECC and S-ECC were 61% and 30%, respectively. The following variables were significant in the unadjusted analysis for both ECC and S-ECC: child's age, maternal educational attainment, overall AAPD CAT classification, sugary snacks per day, presence of plaque on child's teeth and ETS. Exposure to ETS was associated with an increased adjusted odds ratio for ECC (aOR=5.39 [95% CI: 1.14-25.33], P=0.033), but not for S-ECC. Furthermore, C-section birth was associated with a decreased adjusted odds ratio for both ECC and S-ECC, respectively (ECC: aOR=0.132 [95% CI: 0.02-0.72], P=0.02; S-ECC: aOR=0.141 [95% CI: 0.026-0.748], P=0.021). With inclusion of the AAPD CAT, demographics, and the three non-traditional factors, the overall model accuracy at predicting ECC was 82.2%.

Conclusions and Clinical Relevance: In this study of urban, predominantly African American, and low income children, ECC was found associated with two non-traditional factors, ETS and mode of delivery, suggesting that including them in CRA may improve prediction of future dental caries, and aid in the prevention and treatment of disease. Results from this study support the notion that ECC is a multi-factorial disease, and highlights the importance of adopting oral health education among caregivers.

To my parents, Nabeel and Klood for always believing in me.

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CHAPTER 1.

INTRODUCTION

Dental caries is a disease that starts as subclinical lesions, evolves to various degrees of demineralization (non-cavitated stage) in the tooth surface, may progress over time to large cavitated lesions, and, if left untreated, invades the entire tooth compromising its structure, function and existence. The negative consequences of tooth decay are not limited to the oral cavity; the manifestations of this disease can be visible in aspects of reduced overall health, and diminished emotional well-being of the individual (M. Y. Li, Zhi, Zhou, Qiu, & Lin, 2015). Tooth decay is the most common chronic illness among children, even more than asthma and obesity (Dye, Thornton-Evans, Li, & Iafolla, 2015) . It is postulated that around 40% of children 2 -11 years old in the US have experienced dental caries in some severity (Dye et al., 2015). Dental caries in children is associated with poor oral-health related quality of life, increased pain, and difficulty in biting and chewing (M. Y. Li et al., 2015).

Early Childhood Caries (ECC) is defined as the presence of one or more decayed, missing, or filled tooth surfaces in any primary tooth in a child between birth and 5 years of age (American Dental Association, 2013). In children younger than 3 years of age, any sign of smooth-surface caries is indicative of Severe Early Childhood Caries (S-ECC). From ages 3 through 5, one or more cavitated, missing (due to caries), or filled smooth surfaces in primary maxillary anterior teeth or a decayed, missing, or filled score of ≥4 (age 3), ≥5 (age 4), or ≥6 (age 5) surfaces constitutes S-ECC (American Dental Association, 2013).

ECC is a multi-factorial disease that develops through the combined effects of bacteria, tooth morphology, fermentable carbohydrates, time, and various social factors. The belief that an infant's feeding patterns are the main cause for ECC was reflected in previous terminology referring to the disease as "nursing caries" (Ripa, 1988). However, it has been established that many factors interplay in ECC's etiology including high levels of Streptococcus Mutans (*S. mutans*) in the saliva, consumption of a sugary diet, vertical transmission from an at-risk caregiver, tooth deformities, poor oral hygiene, and socioeconomic disparities (Burt & Pai, 2001; Chaffee, Gansky, Weintraub, Featherstone, & Ramos-Gomez, 2014; Kamińska et al., 2016; Seow, 1991; Thibodeau & O'Sullivan, 1996; Tinanoff & Reisine, 2009). These factors have been combined into several caries risk assessment (CRA) tools, including the American Academy of Pediatric Dentistry (AAPD) caries- risk assessment tool (CAT) used for this study. Conducting CRA has been recommended as part of routine dental care and is valuable for preventing future disease and forming targeted treatment plans (Fontana & Zero, 2006).

Additionally, several non-traditional exposures have been investigated as possible risk factors for ECC and S-ECC including mode of delivery, breastfeeding, and environmental tobacco smoke exposure. The association between these factors and ECC has been inconsistent in the literature due to variations in study methodology and presence of many confounding factors. Moreover, to our knowledge, no studies have attempted to study the improvement in caries prediction from adding such factors to an established CRA tool in a multi-variable model.

This study aims to determine the associations between early life exposures and the presence of dental caries in children 1-5 years old. Specifically, we evaluate the relationships between the presence of ECC and S-ECC and three non-traditional exposures: mode of delivery, breastfeeding, and Environmental Tobacco Smoke (ETS). This assessment will help in better understanding how prediction of dental caries in children may be affected by the inclusion of these non-traditional risk factors. Moreover, given the socio-economic characteristics of this study's urban population, this thesis will shed light on how different factors may contribute to disparities in ECC prevalence.

Specific Aims:

I. To determine the unadjusted association between three non-traditional risk factors; mode of delivery, breastfeeding and ETS exposure and the presence of ECC.

II. To determine the unadjusted association between three non-traditional risk factors: mode of delivery, breastfeeding, and ETS exposure and the presence of S-ECC.

III. To determine the adjusted association between three non-traditional risk factors: mode of delivery, breastfeeding, and tobacco smoke exposure and both ECC and S-ECC. Additionally this study aims to assess the improvement in prediction from adding these factors to a multivariable model with overall CRA and demographics.

CHAPTER 2.

REVIEW OF LITERATURE

Defining ECC and S-ECC

Dental caries is a chronic disease that has affected humans throughout history, and evidence for its presence and treatments have been documented in the writings of ancient Egyptian, Greek, Chinese, and Indian civilizations (Lufkin, 1938). Dental caries presents as carious non-cavitated or cavitated lesions on both the primary and permanent dentition, causing pain, discomfort, and loss of function and aesthetics (Bönecker, Abanto, Tello, & Oliveira, 2012; Filstrup et al., 2003). Early Childhood Caries (ECC) is defined as the presence of one or more decayed, missing or filled tooth surfaces in any primary tooth in a child between birth and 5 years of age (American Dental Associaton, 2013). In children younger than 3 years of age, any sign of smooth-surface caries is indicative of Severe Early Childhood Caries (S-ECC). From ages 3 through 5, one or more cavitated, missing (due to caries), or filled smooth surfaces in primary maxillary anterior teeth or a decayed, missing (due to caries), or filled score of ⩾4 (age 3), ⩾5 (age 4), or ⩾6 (age 5) surfaces constitutes S-ECC (American Dental Associaton, 2013).

This definition has evolved as the understanding of ECC's etiology has changed over time, and some of the previous terms, including nursing caries, bottle mouth, and rampant carious lesions, have been dropped to reflect the multifactorial causes for the development of ECC (Ripa, 1988). ECC develops through the combined effects of bacteria, tooth morphology, fermentable carbohydrates, time, and various social factors.

It is more prevalent among developing countries and disadvantaged populations living in poverty, and many factors contribute to such disparities (Tinanoff, Kanellis, & Vargas, 2002).

Consequences and Impact of ECC

Dental caries is the most common childhood disease affecting around 23% of children 2 -5 years old in the US. (Dye et al., 2015). ECC and S-ECC are linked with a negative impact on the child's overall health and emotional well-being, and have been associated with poor oral health outcomes in adolescent and adulthood (M. Y. Li et al., 2015; Y. Li & Wang, 2002). It has been estimated that 5-7 year old children in the USA will lose approximately 7 million hours of school due to oral health related problem and dental visits, many of which ECC is either a direct component, or an indirect cause (Tinanoff & Reisine, 2009). ECC is associated with lower oral-health-related quality of life, increased pain, and difficulty in biting and chewing for the child (Filstrup et al., 2003). Furthermore, childhood caries experience is a predictor for future caries in adolescents and adulthood, and extensive caries in primary teeth may lead to orthodontic problems and malocclusion later in life (Birkeland et al., 1976).

Treatment of ECC usually involves extensive restorations, tooth extractions and may require general anesthesia, resulting in a high economic burden on communities and health systems. Moreover, many cases of ECC manifest in the Emergency Department (ED) room as severe dentofacial pain, and some of these ED admissions become prolonged and expensive hospitalizations for management of facial cellulitis (Rowley, Sheller, Williams, & Mancl, 2006; Casamassimo, Thikkurissy, Edelstein, & Maiorini, 2009). It is estimated that dental expenditures in the US for children under 5 years old reached $955 million in 2014 (Agency for Healthcare Research and Quality, 2014). Consequently, families dealing with this chronic disease will experience stress and economic burden and, therefore, a diminished quality of life (Filstrup et al., 2003).

Traditional ECC Risk Factors

Although it was previously postulated that deleterious infant feeding patterns, specifically prolonged, frequent, and nocturnal nursing were the main causes of early childhood caries, recent studies emphasize the combined effect of multiple risk factors, of which infant dietary practices play a major role. (Mohebbi, Virtanen, Vahid-Golpayegani, & Vehkalahti, 2008). Frequent exposure to sucrose diet forms a sticky plaque matrix that enables microorganisms to adhere to the teeth and produce an acidic substance responsible for the demineralization of the tooth structure and, over time, the formation of carious lesions (Burt & Pai, 2001). The influence of diet in caries development remains critical as the child grows and solid foods are introduced; high intake of sugary beverages was associated with an estimated (2.02, 95% CI 1.33 to 3.06) increased odds for S-ECC compared to low intake, and frequent snacks between meals was significantly associated with increased prevalence of ECC (Evans et al., 2013; Prakash, Subramaniam, Durgesh, & Konde, 2012a)

The microbiological component, namely the counts of Streptococcus Mutans (*S. mutans*) in the child's saliva, plays an equally important role in ECC development;

children with higher counts of S. mutans were found more likely to develop ECC, and have more severe forms of disease (Thibodeau & O'Sullivan, 1996). ECC and S-ECC are impacted by many oral health factors including oral hygiene, tooth brushing behaviors, and fluoride exposure (Olmez, Uzamis, & Erdem, 2003; Prakash et al., 2012a; Riley, Lennon, & Ellwood, 1999). Due to the high dependency and closeness of young children affected by ECC with their mothers, several maternal factors impact the child's development of disease including maternal levels of S. mutans, maternal tooth decay, and maternal oral hygiene behaviors (Ersin, Eronat, Cogulu, Uzel, & Aksit, 2006; Kim Seow, 2012). Moreover, Edelstein reported in his review that children of caregivers with greater educational attainment had fewer caries incidents than children of parents with lesser educational attainment (Edelstein, 2002).

Disparities in ECC

ECC prevalence is highly impacted by socio-economic disparities. It is estimated that that a much higher percentage of 5 year old children from poor families (below 100% poverty level) experience dental caries (55%) compared to children living in non-poor (25%) families (Tinanoff et al., 2002). Additionally, the disease distribution is largely affected by race and ethnicity. Blacks or African Americans had two times the incidence rate of Whites in the US (U.S. Department of Health and Human Services). Despite a recent decrease in ECC prevalence in the US, disease rates remain high in low income populations and racial minorities (Tinanoff & Reisine, 2009).

These socioeconomic factors interact in the development of ECC, and many mechanisms have been proposed to explain such inequalities, including differences in access to care, deleterious oral hygiene behaviors, and psychosocial factors (Patrick et al., 2006). The current study was set in Philadelphia, Pennsylvania, which was ideal for assessing dental caries disparities and their underlying factors; the population is racially diverse with African Americans making up to 42% of the population, and the city's poverty rate is the highest of the ten largest cities in the United States with nearly 2 out of every 5 children living below the federal poverty line (Philadelphia Department of Public Health, 2016). Furthermore Philadelphia has one of the highest rates of adult smoking in the US, a behavior that has been proposed as a mediator for poor oral health in low income populations (Philadelphia Department of Public Health, 2016; Sabbah, Tsakos, Sheiham, & Watt, 2009).

Caries Risk Assessment

> *Caries risk assessment is the determination of the likelihood of the incidence of caries (i.e., the number of new cavitated or incipient lesions) during a certain time period, or the likelihood that there will be a change in the size or activity of lesions already present.*
>
> (American Academy of Pediatric Dentistry, 2013)

The current literature on ECC and its risk factors have enabled the development of different models for caries risk assessment (CRA). These models asses a group of risk factors and protective elements which have been found related to dental caries and its severity from different studies, and assign each patient a level of risk accordingly. CRA is valuable for identifying children at high risk for ECC, informing targeted treatment plans tailored for each patient, and communicating to patients and their caregivers' specific risk factors to prevent future caries. CRA is should become routine practice in pediatric dental clinics, where it is recommended that children undergo CRA at their first dental visit (Twetman, 2016).

Currently the most commonly used caries risk assessments include the American Academy of Pediatric Dentistry Caries-risk Assessment Tool (AAPD CAT), the Caries Management by Risk Assessment (CAMBRA) form, the American Dental Association (ADA) CRA, and the Cariogram. A good CRA needs to combine ease of use and cost effectiveness, with high sensitivity and specificity (Zero, Fontana, & Lennon, 2001). However, a systematic review of studies validating several CRA used in practice concluded that scientific evidence on their validity and accuracy is weak (Tellez, Gomez, Pretty, Ellwood, & Ismail, 2013). The present literature on CRA indicates that multi variable models better predict caries disease than single risk indicators, though more research is needed to develop robust evidence based models, and validate their accuracy in different populations (Twetman, 2016).

This study uses the AAPD CAT, which evaluates a series of biological factors, protective elements, and clinical findings and assigns each patient a level of risk from low, moderate, and high (American Academy of Pediatric Dentistry, 2013). We attempt to assess the accuracy of this CRA cross-sectionally in majority low income population by examining its association with ECC and S-ECC. Additionally, including it in a multi-variable model as a predictor of ECC and S-ECC with demographics and three non-traditional risk factors (breastfeeding, ETS, and mode of delivery) will help reveal the independent association for these factors with ECC and S-ECC.

Non-Traditional Factors and Risk of ECC and S-ECC

Several studies have investigated the associations between dental caries and non-traditional factors including mode of delivery, breastfeeding, and environmental tobacco smoke exposure. However, the literature concerning these factors and their association with dental caries in young children has been inconsistent.

1. Mode of Delivery

The relationship between mode of delivery and differences in the infants gut, oral mucosa and skin microbiota has been the focus of several recent studies (Dominguez-Bello et al., 2010; Huurre et al., 2008; Prince, Antony, Ma, & Aagaard, 2014). In their study, Dominguez-Bello et al. found infants delivered vaginally acquired bacterial communities resembling the mother's vaginal microbiota, whereas the microbiota in infants delivered by C- section was similar to the microbiome on the mother's skin. It has been suggested that due to variations in gut microbiota development, mode of delivery may have significant influences on an infant's immunological function (Huurre et al., 2008). Moreover, infants delivered vaginally had significantly higher Salivary Immunoglobin A levels than those delivered by C-section, indicating that vaginally born children may possess a more mature mucosal immune system (Subramaniam, Dwivedi, & Babu, 2016). Although children born via C-section have been found to have an increased risk of asthma, any associations with oral health conditions remains uncertain (Thavagnanam, Fleming, Bromley, Shields, & Cardwell, 2008).

Concerning the oral flora, mode of delivery was found to significantly affect the composition of the oral microbiota in three and six month old infants; children born vaginally were found to have more diverse oral flora than those born via Cesarean section (Boustedt, Roswall, Dahlan, Dahlgren, & Twetman, 2015; Lif Holgerson et al., 2011). Barfod et al. reported a significantly higher concentration of health-related oral levels of Lactobacilli and Streptococci among vaginally born infants compared to those born via C-section (Nelun Barfod et al., 2011). Interestingly, in a study of 3 months old infants, mode of delivery significantly affected the composition of oral microbiota among breastfed infants only, indicating a possible effect modifier (Holgerson et al., 2013).

However, the association between mode of delivery and S. mutans, the most common cariogenic microbe has been inconsistent across the literature. While some found mode of delivery to influence time of S. mutans acquisition and its concentration, others reported no significant relation (Y. Li, Caufield, Dasanayake, Wiener, & Vermund, 2005; Merglova & Polenik, 2016; Reed, Cunningham, Latham, Shirer, & Wagner, 2014; Saraithong et al., 2015; Thakur, Singh, Chaudhary, & Manuja, 2012; Zhou, Lin, Lo, & Wong, 2011). Li et al. postulated that the early exposure of vaginally born children to a variety of bacteria in the perineum, compared to aseptic conditions in cesarean born children, is responsible for these differences, resulting in an oral flora more susceptible to S. mutans colonization in the cesarean born children (Y. Li et al., 2005). In their prospective cohort study, Li et al found children born via C-section were colonized by S. mutans up to a year earlier than those born vaginally (Y. Li et al., 2005).

Since early colonization with S. mutans is linked to more severe ECC, it is consequently expected that children born via C-section are more likely to develop ECC

(Alaluusua & Renkonen, 1983). Studies on the mode of delivery's relationship with dental caries have resulted in conflicted findings. While some research found no significant differences in ECC prevalence or time of first dental caries across different modes of delivery, (Kuthy et al., 2014; Poureslami, Bafti, Hashemi, & Salari, 2012), a prospective study on 3 year old Danish girls reported that among those with ECC, mode of delivery affected the mean value of the decayed-filled-surface index (dfs), an indicator of disease severity. However, these differences did not reach statistical significance (Barfod, Christensen, Twetman, & Lexner, 2012). Additionally, Yepes et al. reported that among African American children, those delivered by C-section experienced restorative and emergency claims 25% more compared with those born vaginally (Yepes, Bush, Li, Talbert, & Nash, 2014).

Opposite results were described by Fontana et al., where vaginal birth was a significant predictor for caries progression toward cavitation in young children (Fontana et al., 2011). Moreover, a cross sectional study of 180 preschoolers reported that children born vaginally showed an increased prevalence of ECC and higher levels of S. mutans (Pattanaporn et al., 2013). A study conducted among Polish children reported that children born via C-section were at a lower risk for dental caries in the first permanent molar, a finding that was attributed to higher educational and socioeconomic status among mothers who deliver by C-section in Poland (Borowska-Strugińska et al., 2016). This variation in results across the current literature could be a consequence of differences in study populations, methodological issues, and presence of many confounders, and it indicates the need for further research in this area.

2. *Breastfeeding*

The overall health benefits of breastfeeding to both mother and child are well documented in the literature. However, the effect this practice may have on young children's caries development is inconsistent across studies. Some studies reported no significant association for breastfeeding and dental caries, while others varied on whether breastfeeding offers a protective effect or if it acts as a potential risk factor for ECC, especially in cases of ad libitum (on demand), prolonged and nocturnal breastfeeding (Bissar, Schiller, Wolff, Niekusch, & Schulte, 2014; Chaffee, Feldens, & Vitolo, 2014; Kato et al., 2015; Prakash, Subramaniam, Durgesh, & Konde, 2012b; Thitasomakul et al., 2009; Zhou et al., 2011). It is very important to understand the relationship between breastfeeding and dental caries given the large percentages of breastfeeding mothers, the recent increase in breastfeeding uptake, and the impact this information may have on new recommendations and policies (Hunt, 2014).

In a study involving low income families, nocturnal breastfeeding was a significant risk for ECC in children 18 -38 months old where those who were breast fed at night were 3.6 times more likely to develop ECC compared to those who did not (Nakayama & Mori, 2015). Several studies support this notion and the American Dental Association identified unrestricted, at will, nocturnal breastfeeding as risk factors for ECC (American Dental Associaton, 2000). The negative impact of nocturnal breastfeeding has been explained by reduced salivary flow during sleep time, resulting in reduction of the cleansing and buffering properties of the saliva, therefore lowering the PH of oral fluids and prolonging the teeth's exposure to cariogenic carbohydrates in milk (Weber-Gasparoni, Kanellis, Levy, & Stock, 2007). However, human breast milk, although higher in sugar content than bovine milk, has shown similar or reduced carcinogenicity to formula milk from in-vitro studies (Bowen & Lawrence, 2005;

Erickson & Mazhari, 1999) . Additionally, sleeping with a bottle of formula milk was reported as a risk factor for ECC (Mohebbi et al., 2008). Therefore, it is more likely that nocturnal feeding in general increases the risk of ECC, rather than breastfeeding per se.

An additional factor to consider is ad libitum breastfeeding, or on-demand breastfeeding. A large cross sectional study involving 1500 children reported an increased prevalence of caries in children accustomed to ad libitum breastfeeding (Prakash et al., 2012b). Ad libitum breastfeeding may increase risk of ECC by increasing frequency of exposure to milk carbohydrates. Subsequently, longer periods of high saliva acidity result in higher rates of enamel decalcification. It is critical to note that most studies that have reported this association are cross-sectional, or case-control studies. A cause-effect relation cannot be established, and it is unclear if underlying confounders are responsible for the differences observed.

Additionally prolonged breastfeeding has been reported in many studies as a risk factor for ECC (Bissar et al., 2014; Chaffee et al., 2014; Y. Li, Navia, & Bian, 1996; Vachirarojpisan et al., 2004; Zhou et al., 2011) . Consequently, dental practitioners have encouraged mothers to stop breastfeeding after the child's first birthday, contradicting current WHO recommendations of breastfeeding for up to two years of the child's life (Valaitis, Hesch, Passarelli, Sheehan, & Sinton, 2000). The cutoff for breastfeeding as a risk for ECC is unclear across the literature, ranging from 12 months to 24 months of breastfeeding. In a study among a low-income population, breastfeeding for more than 24 months was associated with the highest S-ECC prevalence (Chaffee et al., 2014). Additionally, prolonged breastfeeding was associated with increased colonization with S. mutans, and a recent study reported that a mother's S. mutans levels predicted S. mutans levels of her child in the subset of breastfed children only (Reed et al., 2014). A recent study from Japan that used longitudinal survey data from over 43,000 infants concluded that children who were breastfed for 6 to 7 months were at higher risk for dental caries at

30 months of age compared to children who were exclusively bottle-fed (Kato et al., 2015).

Despite a large body of literature investigating the relationship between breastfeeding and ECC, a systematic review concluded that due to differences in breastfeeding definitions and study methodologies, prolonged breastfeeding is not a risk factors for ECC (Lavigne, 2013). Moreover, a study using a nationally representative sample in the United States found that breastfeeding and breastfeeding duration were not associated with an increased risk for ECC (Iida, Auinger, Billings, & Weitzman, 2007). A cross sectional study from Iran, where breastfeeding is the norm, concluded that breastfeeding per se, its duration, and nocturnal breastfeeding were not related to ECC (Mohebbi et al., 2008).

Some studies have suggested that breastfeeding provides a protective effect against dental caries. In a large cross-sectional study on 2 year old toddlers, formula-fed babies had significantly higher prevalence of dental caries and more disease severity compared to exclusively breastfed babies (Majorana et al., 2014) . Milk-bottle feeding at night was associated with an odds ratio of 5.5 (95% CI 2.1–14.1) in a study involving around 500 1- 3 year old children from Iran (Mohebbi et al., 2008). Furthermore, a cohort study from children in Thailand concluded that breastfeeding for 6 to 11 months reduced the risk of ECC, and that frequency of nocturnal feeding whether from bottle or breast increased caries risk in a dose response effect (Nirunsittirat et al., 2016). A recent meta-analysis that compared the effects of breastfeeding to bottle feeding on dental caries in children, concluded that breastfed babies were less likely to develop dental caries, and reported that breastfeeding per se may be preventative against dental caries (Avila, Pordeus, Paiva, & Martins, 2015)

In light of this conflicted body of research, this study attempts to investigate through a cross-sectional design the association between overall breastfeeding duration and nocturnal breastfeeding duration and both ECC and S-ECC, in a low income population of children from North Philadelphia.

3. Environmental Tobacco Smoke

Environmental tobacco smoke (ETS) also known as second hand smoke includes smoke exhaled from a smoker and that from the burning end of a pipe, cigarette or cigar. The health risks associated with ETS are well known and comparable to that of first hand smoking, and are even more dangerous for young children (Law & Hackshaw, 1996). In relation to oral health, ETS is a known cause to inflammation of oral membranes, it alters salivary glands function and affects oral flora composition (Avsar, Darka, Bodrumlu, & Bek, 2009; Hanioka, Ojima, Tanaka, & Yamamoto, 2011). Adult smokers have significantly higher number of decayed and filled tooth surfaces (DFS) compared to non-smokers of the same age group (Axelsson, Paulartder, & Lindhe, 1998). Second hand smoke has also been associated with approximately 30 % increased odds for dental caries in permanent teeth of adolescents (Ditmyer, Dounis, Mobley, & Schwarz, 2010).

In relation to ECC and Severe-ECC, the literature concerning ETS has been inconsistent. Since formation of primary teeth takes place during gestation, it is important to consider the effects of both prenatal and postnatal ETS exposure. Studies involving live rats showed second hand smoke affected the mineralization of enamel in the offspring teeth (Dong et al., 2011). Children whose mother smoked five or more times a day during pregnancy had significantly higher risks of developing dental caries compared to those who were not exposed (Majorana et al., 2014). In research from nationally representative data of US children, prenatal maternal smoking was independently associated with increased odds for ECC with an adjusted odds ratio of 1.68 (95%CI [1.01–2.79]) (Iida et al., 2007).

Postnatal ETS at 4 months of age increased children's risk for dental caries by two fold as reported by a large, retrospective cohort study from Japan (S. Tanaka et al., 2015). However, prenatal smoking was not associated with an increased risk of ECC in this study. Three year old children who lived in a household with at least one smoker were at 50% increased odds for dental caries (OR＝1.5, 95％ CI: 1.04—2.0) (Nakayama & Mori, 2008). Additionally, children with high levels of cotinine, an indicator for passive smoke exposure after birth, were found at an increased risk for having filled and decayed teeth (Aligne C, Moss ME, Auinger P,Weitzman M., 2003). The association with dental caries was stronger for maternal smoking exposure compared to paternal only exposure. (Hanioka, Nakamura, Ojima, Tanaka, & Aoyama, 2008). Moreover, a dose-response relationship was found between cumulative postnatal exposure to ETS at home and dental caries (K. Tanaka, Miyake, & Sasaki, 2009). Still, some studies found no significant association between ETS and dental caries in young children (Kuthy et al., 2014).

It has been proposed that second-hand smoke exposure increases risk for ECC by several developmental, immunological, and socio-behavioral mechanisms. Tobacco and nicotine exposure prenatally may alter the development and mineralization of teeth structure, and it has been shown that children born to smoking mothers were more likely to develop enamel hypoplasia, a developmental condition that results in weaker enamel and higher susceptibility to dental caries (Leviton et al., 1992). Frequent passive smoke exposure alters salivary functions and immunological responses, resulting in higher susceptibility to infection by cariogenic microorganisms, consequently increasing rates of dental caries (Avsar et al., 2009). Furthermore, parental smoking status may be associated with differences in socioeconomic status, child rearing practices, and attitudes towards oral hygiene behaviors. Therefore, the effect of parental smoking may be confounded by other factors including tooth brushing and diet (Hanioka et al., 2011). In light of this body of literature, we attempt to investigate the role of prenatal and postnatal ETS as non-traditional risk factors for ECC and S-ECC. This investigation will provide valuable information on ETS among children living in urban low income backgrounds, and potentially describe an underlying cause for the socioeconomic disparities in ECC.

In conclusion, the current literature on ECC and S-ECC has vastly improved the understanding of this complex disease and this has been reflected in the increased uptake of multi-factorial caries risk assessments in pediatric dental clinics. However, the disease remains largely affected by socioeconomic and racial disparities, indicating a need for further research in low income groups and racial minorities. Furthermore a consensus has not been reached on the role of non-traditional factors, including mode of delivery, breastfeeding and ETS, in relation to dental caries in the primary dentition. Therefore, this cross-sectional study aims to extend the current research on these three factors from an urban, racially diverse, and low-income community in the US. Furthermore, to our knowledge no study in the US has assessed the improvement in caries prediction from adding such factors to a validated CRA tool, through a multivariable model.

CHAPTER 3.

METHODS

Research Methods

Sample and Setting

All research procedures were performed in the Temple University Kornberg School of Dentistry (TUKSoD) Pediatric Dentistry Clinic. Eligibility was determined from the electronic patient scheduling program used by various dental clinics at TUKSoD. Potential subjects were recruited through the ongoing flow of patients to TUKSoD Pediatric Dentistry Clinic by phone or in person. Additionally, advertising flyers were posted in designated areas in TUKSoD. Patients were informed and recruited at the pediatric clinic lobby during patients scheduled visits. All subjects were seeking dental treatment at the TUKSoD. About 112 caregiver/child 1-5 years old dyads were recruited out of a total of 184 eligible patients approached by research personnel during a period of 4 months from October 2016 to February 2017 resulting in a 60.5% participation rate. Patients deemed to be ineligible or who did not wish to participate in the research were referred for clinical services as appropriate

The study was approved by the Temple University Institutional Review Board (Protocol # 23885) and the Research Committee at TUKSoD. The IRB approved protocol is attached in Appendix A. Participation in the research took several hours in total, and included completion of the survey (Appendix B), review of medical records, and AAPD CAT. All procedures were conducted after informed consent had been obtained in person

(Appendix C). The consent to participate in the study was provided by the caregiver of the children. Participants were entered into a contest to receive a $250 dollar gift card once they completed all required assessments. Only one of the participants received this payment.

Inclusion and Exclusion Criteria

There were no restrictions on enrollment in terms of gender, economic status, or ethnic group. Inclusion criteria for the patients was: children 1-5 years of age, arriving at the TUKoSD for regular or emergency treatment, and inclusion criteria for the caregiver was caregiver 18-65 years old, able to speak and read English, and willing to provide consent to participate in the study for himself/herself and his/her child. Children six years and older, and those whose parents refused to provide consent were excluded from the study.

Study Procedures

Pilot Testing

Before start of the study, pilot testing of the survey questionnaire (Appendix B) was conducted among 7 caregivers, and data obtained from these caregivers was not used for the actual study. Pilot testing required approximately 2 weeks, and was conducted in the same setting as the actual study at the TUKSoD. pediatric dental clinic. The pilot testing included only administering the survey questionnaire used for the study. This was done to assess any difficulties among caregivers in comprehending the language used in the survey, or issues in following the instructions of the survey.

Consent and Survey

After obtaining informed consent from the child's caregivers, the questionnaire survey (Appendix B) was given to the caregiver. The survey is in a written format and was self-administered by the caregiver, with a member of research staff available to answer any questions. It required approximately 10 -15 minutes to complete the survey questions. The survey questions were majorly developed by research personnel, but also included a series of questions on ETS adopted from the Global Adult Tobacco Survey (Center for Disease Control and Prevention, 2011).

Intra Oral Exam, Caries-risk Assessment Tool (CAT)

Each child received a standard intra-oral examination by a student doctor and classical risk assessment for dental caries was conducted using the American Academy of Pediatric Dentistry Caries-risk Assessment Tool (AAPD CAT) (American Academy of Pediatric Dentistry, 2013). The dental records for participants were later reviewed and data related to the main caries outcomes were extracted.

Clinical calibration exercises were conducted to standardize the criteria for detecting dental caries. Likewise, student investigators were calibrated for objectively collecting information on risk factors. All assistants and others working on the project were educated about the importance of strictly respecting patients' rights to confidentiality and had completed training concerning proper practice in accordance with HIPAA regulations.

Study Design

Primary Predictors

Three main predictors were assessed:

1) Mode of delivery, defined as either cesarean birth or vaginal birth and obtained from a single question in the survey.

2) Breastfeeding duration; defined by two variables; Overall breastfeeding duration defined as never breastfed, breastfed for less than 6 months or breastfed for more than 6 months; Nocturnal breastfeeding duration defined as never breastfed, breastfed at night for less than 6 months or breastfed at night for more than 6 months, and both were obtained from two separate questions from the survey.

3) Environmental Tobacco Smoke (ETS) Exposure, defined in a dichotomous measurement as never exposed to any ETS (prenatal or postnatal), or exposed to either prenatal, postnatal smoke or both. This predictor was derived from a series of five questions from the survey on the following: prenatal maternal smoking, prenatal maternal second hand smoke exposure from workplace and from household, postnatal maternal smoke exposure, and postnatal smoke exposure from the household. These questions were combined due to the observed high correlations between them, and to enable more robust statistical estimates given the relatively small sample size.

Primary Outcome Measures:

Two main outcomes measures were extracted from the dental records and were defined as a dichotomous variable:

1) Early Childhood Caries (ECC): defined as the presence of one or more decayed, or filled tooth surfaces in any primary tooth in a child between birth and 5 years of age.

2) Severe-Early Childhood Caries (S-ECC): defined in children younger than 3 years of age as any sign of smooth-surface caries, and in ages 3 through 5 as one or more cavitated, or filled smooth surfaces in primary maxillary anterior teeth, or a decayed filled score of ≥4 (age 3), ≥5 (age 4), or ≥6 (age 5) surfaces.

These outcomes are usually recorded by all the TUKSoD students. For the study purposes the data were extracted from the electronic odontograms by research team after completion of study procedures. The missing tooth surface due to caries component of the two definitions was not assessed for both ECC and S-ECC, due to difficulties in distinguishing through the extracted odontograms teeth lost to caries from those missing due to physiological tooth exfoliation or trauma. Moreover, only frank carious lesions were considered for both definitions, and in calculating the dft index (decayed filled tooth) and dfs index (decayed filled surface) as early non cavitated lesions were not included.

Covariates:

The following demographics were assessed in the survey and included in the final analysis: child's age in years; child's race defined as a categorical variable (African

American, White, Mexican or other race); child's gender (Male versus Female); low family economic status defined as presence of at least one of the following: annual household income of less than 20,000$, presence of someone in household who receive WIC benefits (Women, Infants, and Children federal nutritional program), receives SNAP benefits (Supplemental Nutrition Assistance Program), or receives food stamps; and maternal education (high school graduate or less versus having some college education, college graduate or more). The overall AAPD CAT was included as a measure for traditional risk factors and was defined as low, moderate, or high (American Academy of Pediatric Dentistry, 2013). This was extracted from the participants' dental records, and was measured by a trained dental student. The assessment includes the following factors: biological factors, including mother/primary caregiver has active caries, parent/caregiver has low socioeconomic status, child has >3 between meal sugar-containing snacks or beverages per day, child is put to bed with a bottle containing natural or added sugar, child has special healthcare needs, and child is a recent immigrant, protective factors, including child receives optimally-fluoridated drinking water or fluoride supplements, child has teeth brushed daily with fluoridated toothpaste, child receives topical fluoride from health professional, and child has dental home/regular dental care. Clinical findings include the following: child has >1 decayed/missing/filled surfaces, child has active white spot lesions or enamel defects, and child has plaque on teeth. The categorization of low, moderate, or high is based on presence of several factors for each patient, but clinical judgment by the examining dentist may justify the use of just one factor in determining overall risk.

The following covariates were derived either from the survey or the risk assessment's individual items and were included in the bivariate analysis: Nocturnal feeding with bottle of formula (never bottle-fed, bottle-fed for less than 6 months, or bottle fed for more than 6 months), child receives beverages other than water at night (never vs. rarely, sometimes, usually or always), times sugary snacks are offered between meals (never or less than once a day vs. to daily or more than once a day), preterm birth (yes/no), caregiver, mother or father share utensils with child (never or rarely vs. sometimes, usually or always), dental visit in last 12 months (yes/no variable); maternal tooth decay in last 12 months (yes/no), and child has plaque on teeth (yes/no).

Data management

All demographic information and questionnaires were administered in person. Data was entered into an excel spreadsheet. Data was downloaded to an IBM-compatible computer, stored on a password-protected account, and was exported to a computer data analysis program (SPSS.24). Data was cleaned and checked for errors prior to analysis. Participant names and corresponding identification numbers were stored separately in a locked file cabinet or password-protected file.

Protected Health Information that was collected includes the patient's name, date they were seen for the study, and phone number. The electronic PHI data was downloaded to a computer using encrypted, password-protected storage of electronic data. All participants received a unique random identification number, which would correspond to that person's data. Participant names and corresponding identification numbers were stored separately in a locked file cabinet or encrypted, password-protected storage of electronic data.

A separate file with identifiers was in receipt of the principal investigator. All data with identifying information was stored in locked files or encrypted, password-protected electronic data storage. Data being analyzed was identified by subject codes (using the subject identification number), and identifying information was removed. All assistants and others working on the project were educated about the importance of strictly respecting patients' rights to privacy and confidentiality, and have completed training concerning proper practice in accordance with HIPAA regulations.

Ethical Considerations

The study was approved by the Temple University Institutional Review Board and the research committee at Temple University Kornberg School of Dentistry Protocol# 23885. The study involves minors between 1-5 years old. Written consent was obtained from their accompanying guardian. The permission of one parent was sufficient even if both parents were alive, known, competent, reasonably available, and share legal responsibility for the care and custody of the child. The consent script was provided to the parent/guardian (Appendix C). Assent was not obtained because the capacity of the children is so limited that they cannot reasonably be consulted.

It was emphasized that participation in the study is completely voluntary. The subject's directed guardian accompanied the subject during the evaluation. Minimal risk was presented to the children. Study subjects were entered into a raffle to receive a $250 gift card once they complete their participation in the study. This information was included into the consent form respectfully.

Analytic methods

Initial exploratory analyses were conducted to determine distributional issues of study variables. Any necessary transformations were then conducted. Statistical significance was set at $\alpha=0.05$ for all tests. All analyses were conducted in SPSS 24.

Specific Aim I

To determine the unadjusted association between the presence of ECC and three non-traditional risk factors: mode of delivery, breastfeeding and tobacco smoke exposure.

Simple differences between groups (ECC yes/no) were evaluated using chi-square tests for categorical variables, and two-sample means for continuous variables. Bivariate logistic regression was executed for each of the risk factors and the outcome of ECC.

Specific Aim II

To determine the unadjusted association between the presence of S-ECC and three non-traditional risk factors: mode of delivery, breastfeeding and tobacco smoke exposure.

Simple differences between groups (S-ECC yes/no) were evaluated using chi-square tests for categorical variables, and two-sample means for continuous variables. Bivariate logistic regression was executed for each of the risk factors and the outcome of S-ECC.

Specific Aim III

To determine the adjusted association between either ECC or S-ECC three non-traditional risk factors: mode of delivery, breastfeeding and tobacco smoke exposure,

in a multivariable model including main socio-demographic and overall AAPD caries-risk assessment tool.

Two separate multivariable logistic models for caries prediction were developed based on the two outcomes of the study (ECC and S-ECC). These two models were developed by entering the predictors and confounders in three separate blocks. The first step demographics were entered, including child age, race, gender, and maternal education. The second block included the overall AAPD CAT as a combined measure for traditional risk factors. The third block included the three non- traditional exposures of interest (mode of delivery, overall breastfeeding duration, and pre and post- natal ETs). Other confounders that were measured in the bivariate analysis were not included in this model to avoid multi-collinearity and because they were embedded in the overall AAPD CAT. Differences in pseudo R-squared, overall model significance, sensitivity and specificity of the model were used to assess the improvements in ECC and S-ECC prediction from addition of each step.

CHAPTER 4.

RESULTS

Study Sample

The final sample analyzed included n=112 child/caregiver dyads, and the majority of study participants lived in North Philadelphia neighborhoods. The great majority of caregivers who filled out the survey were the child's mother (83%), and the mean caregiver age was 31 years. No differences on the main outcome or the three predictors of interest were found by relation of caregiver, albeit more missing cases were present for a caregiver other than a mother, hence caregiver's relation to the child was not included for analysis. Furthermore, the following variables were measured but not included in the analysis either due to their lack of variability in the study sample: child's birth weight, where 91% of children were at normal birth weight (between 5.5 lbs and 8.8 lbs); child's dental insurance status, where 94% reported being insured; and overall child health assessment by caregiver, where 99 % of children were rated as having good, very good, or excellent health.

Summary Statistics

Participants included in the analysis were 50% male, 70% African American, 78.6% living in homes with low family economic status (annual income less than $20,000 or with a person who receives WIC benefits , SNAP benefits or food stamps), and 46% having a mother with educational attainment of high school graduate or less (shown in Table 1). Moreover, 51% of the children were classified as high risk by the AAPD CAT, 93% of children were reported to brush teeth daily, 61% had plaque on

Table 1
Summary Statistics

		ECC			S-ECC		
	Total	**No**	**Yes**	**P-value**	**No**	**Yes**	**P-value**
Total	110	67 (60.9%)	43 (39%)		75(70%)	32(30%)	
Gender ***(n,%)***							
Female	56 (50%)	30 (53%)	26 (46%)	0.082	35 (62%)	21(37%)	0.09
Male	53 (49%)	37 (70%)	16 (30%)		41(77%)	12(22%)	
Age in Yrs. ***mean, sd***	3.66 (sd=1.2)	3.17 (sd=1.2)	4.28 (sd=0.85)	.007*	3.31 (sd=1.2)	4.3 (sd=0.8)	.014 *
Race ***(n,%)***							
African American	77 (70%)	45 (58%)	32 (41%)	0.418	53 (69%)	24(31%)	0.68
White, Mexican or other race	33 (30%)	22 (66%)	11(33%)		24 (73%)	9 (27%)	
Receives WIC, STAMPS, or income under 20,000$) ***(n,%)***							
No	24 (22%)	14 (58%)	10 (42%)	0.77	16 (67%)	8 (33%)	0.69
Yes	86 (78%)	53 (61%)	33 (38%)		61 (71%)	25(30%)	
Maternal Education ***(n,%)***							
High school graduate, GED or less	51 (47%)	26 (51%)	25 (49%)	.041*	31 (61%)	20(40%)	.039 *
Some college, college graduate or professional school	57 (53%)	40 (70%)	17(30%)		45 (79%)	12(21%)	
Overall AAPD Caries-risk Assessment Tool ***(n,%)***							
Low	33 (30%)	30 (91%)	3 (9%)	.001*	32 (97%)	1 (30%)	0.00 1*
Moderate	20 (18%)	16 (80%)	4 (20%)		18 (90%)	2 (10%)	
High	57 (52%)	21 (37%)	36 (63%)		27 (47%)	30(52%)	
Mode of Delivery ***(n,%)***							
C-section	27 (25%)	19 (70%)	8 (30%)	0.254	22 (81%)	5 (18%)	0.14
Vaginal birth	81 (75%)	47 (58%)	34 (42%)		54 (67%)	27 (33%)	
Environmental Tobacco Smoke Exposure, ***(n,%)***							
No	69 (65%)	47 (68%)	22 (32%)	.026*	52 (75%)	17 (24%)	0.09
Yes	37 (35%)	17 (46%)	20 (54%)		22(60%)	15 (40%)	
Ever Breastfed ***(n, %)***							
Yes	72 (67%)	43 (60%)	29 (40%)	0.97	50 (70%)	22 (30%)	0.92
No	35 (33%)	21 (60%)	14 (40)		24 (67%)	11 (31%)	
Duration of Overall Breastfeeding ***(n, %)***							
Never breastfed	34 (31%)	20 (59%)	14 (41%)	0.29	23 (67%)	11 (32%)	0.58
Less than 6 months	51 (47%)	28 (55%)	23 (45%)		34 (67%)	17 (33%)	
More than 6 months	23 (21%)	17 (74%)	6 (26%)		18 (78%)	5 (21%)	

Table 1 *(continued)*							
Duration of Nocturnal Breastfeeding ***(n,%)***							
Never breastfed	40 (37%)	26 (65%)	14 (35%)	0.669	29 (72%)	11 (27%)	0.77
Less than 6 months	38 (3%)	21(55%)	17 (44%)		25 (66%)	13 (34%)	
More than 6 months	29 (27%)	18 (62%)	11 (38%)		21 (72%)	8 (27%)	
Nocturnal feeding with bottle of formula ***(n,%)***							
Never bottle fed	17 (15%)	10 (59%)	7 (41%)	0.791	12 (70%)	5(29%)	0.99
Less than 6 months	16 (15%)	11 (69%)	5 (31%)		11 (69%)	5 (31%)	
More than 6 months	75 (69%)	45 (60%)	30 (40%)		53 (71%)	22 (29.3%)	
Receives Beverages other than water at night ***(n,%)***							
Never	78 (73%)	45 (58%)	33 (42%)	0.288	52 (67%)	26 (33%)	0.20
Rarely, sometimes, usually or always	29 (27%)	20 (69%)	9(31%)		23 (79%)	6 (21%)	
Times sugary snacks are offered between meals ***(n,%)***							
Never or less than once a day	51 (48%)	37 (72%)	14 (27%)	.017*	41 (80%)	10(20%)	0.026*
Daily or more than once a day	56 (52%)	28 (50%)	28 (50%)		34 (61%)	22(40%)	
Preterm Birth ***(n,%)***							
Yes	12 (11%)	6 (50%)	6 (50%)	0.39	8 (67%)	4 (33%)	0.74
No	94 (89%)	59 (63%)	35 (37%)		67 (71%)	27 (29%)	
Caregiver or mother or father share utensils with child ***(n,%)***							
Never or rarely	71 (67%)	43 (60%)	28 (40%)	0.82	51 (72%)	20(28%)	0.73
Sometimes, usually or always	35 (33%)	22 (63%)	13 (37%)		24 (68%)	11(31%)	
Dental visit in last 12 months ***(n,%)***							
No	29 (28%)	22 (76%)	7 (24%)	.053*	23 (79%)	6 (20%)	0.22
Yes	76 (72%)	42 (56%)	34 (45%)		51 (67%)	25(32%)	
Maternal tooth decay in last 12 months ***(n,%)***							
No	78 (76%)	48 (61%)	30 (38%)	0.39	55 (70%)	23(30%)	0.54
Yes	25 (24%)	13 (52%)	12 (48%)		16 (64%)	9 (36%)	
Child has plaque on teeth ***(n,%)***							
No	36 (38%)	27 (75%)	9 (25%)	0.025*	31(86 %)	5 (14%)	.012*
Yes	58 (61%)	30 (51%)	28 (48%)		36 (62%)	22 (38%)	

P-values from chi2 statistics for categorical variable and two sample mean test for age variable
Odds ratio from unadjusted binary logistic regression for ECC and S-ECC
** Significant association at α= 0.05 level*

teeth, 25% of the participants' mothers had active tooth decay in last 12 months, 25% visited dentist in last 12 months, 11% were born preterm, and 33% share food utensils with the child sometimes, usually or always. Furthermore, in terms of dietary behaviors, 69% were bottle fed with formula at night for more than six months, 26% ever received a bottle containing beverages other than water or milk at night, and 52% had sugary snack between meals once or more a day.

In regards to the main predictors (shown in Table 1), 24% of the participants included in the analysis were born via C-section, 34% were exposed to ETS (either prenatally, postnatally or both), 68% were ever breastfed, 21% were breastfed overall for more than 6 months, 27.5% were breastfed at night for more than 6 months. Regarding dental caries outcomes, 61% of the study sample had ECC, 30% had S-ECC with mean decayed filled tooth score (DFT) of 1.6 (sd=2.7).

Bivariate Analysis

(Specific Aim I and Aim II)

Bivariate analysis showed significant associations between ECC and the following predictors (shown in Table1): age in years, where mean age for ECC group compared to non-ECC group was 3.1 (sd=1.2) and 4.2 (sd=0.85), respectively (P=0.007); maternal education, where 49% of children from mothers with lesser educational attainment had ECC, compared to 30% of those from mothers with higher educational attainment (P=0.041); overall AAPD CAT, where 63% of children classified as high risk had ECC, compared to 9% of those classified as low risk (P<0.001); sugary snacks per day, where 50% of children who received one or more sugary snacks per day had ECC, compared to 27% of children who had sugary snacks less than once a day (P=0.017);

dental visit in last 12 months, where 45% of children who visited dentist in last 12 months had ECC, compared to 24% of children who did not visit the dentist in last 12 months(P=0.05); and presence of plaque on teeth, where 48% of children with plaque had ECC, compared to 25% of children who did not (P= 0.025). A significant association was found between any ETS exposure and ECC; 54% of children exposed to ETS had ECC compared to 32% whom were not exposed (P=0.026). No significant bivariate associations were found for ECC and mode of delivery, or any of the breastfeeding variables.

Similarly, bivariate analysis for S-ECC showed significant associations for the following predictors (shown in Table 1): age in years (P=0.014), maternal educational attainment (P=0.039), overall AAPD CAT (P<0.001), sugary snacks per day (P=0.026), and presence of plaque on teeth (P=0.012). No significant bivariate associations were found for S-ECC and mode of delivery, ETS, or any of breastfeeding variables.

Moreover, children born via C-section were significantly more likely to have a mother with higher educational attainment (P=0.017) and less likely to have been breastfed (P= 0.032). Those exposed to any ETS were more likely to receive SNAP or food stamps benefits (P=0.041), be of lower income (P=0.008), have a mother with maternal tooth decay in the last 12 months (P=0.029), and be classified as high risk by the CRA (P=0.027). Children who were never breastfed were more likely to be from lower economic status (P<0.001); from a mother with lower educational attainment (P<0.001); and exposed to prenatal maternal smoke (P=0.003).

Bivariate logistic regression for ECC indicated significant unadjusted odds ratios for the following predictors (shown in Table 2): age in years (OR=2.5 [95% CI: 1.6-3.9], P=0.001), higher maternal education (OR=0.442 [95% CI: 0.20-0.97], P=0.043), overall

Table 2
Results from Bivariate Logistic Regression

	ECC	S-ECC
	odds ratio (95% CI) (p-value)	odds ratio (95% CI) (p-value)
Gender		
Female	reference	Reference
Male	.49[.22-1.09] (.083)	.48 [.21-1.13] (.094)
Age in Yrs.	2.5 [1.6-3.9] 0.001*	2.3 [1.4-3.6] 0.001*
Race		
African American	1.4 [0.6- 3.3] (0.419)	1.2 [.4-2.9] (.683)
White, Mexican or other race	reference	Reference
Receives WIC, STAMPS, or income under 20,000$		
Yes	.872 [.34-2.18] (0.770)	.820 [.31-2.15] (.687)
No	reference	reference
Maternal education		
High school graduate, GED or less	reference	reference
Some college, college graduate or professional school	.442 [.20-.97] (.043)*	.413 [.17-.96] (.041)*
Overall AAPD Caries-risk Assessment Tool		
Low	reference	reference
Moderate	2.500 [.497 12.573] (0.26)	3.55 [.30-41.99] (0.314)
High	17.14 [4.65-63.1] (0.0001)*	35.55 [4.54-278.1] (.001)*
Mode of Delivery		
C-section	.582 [.22-1.4] (.257)	.455 [.15-1.33] (.151)
Vaginal birth	reference	reference
Environmental Tobacco Smoke Exposure, Combination of Prenatal and Postnatal Exposure		
No	reference	reference
Yes	2.5 [1.1-5.7] (0.028)*	2.086 [.887-4.903] (.092)
Ever breastfed		
No	reference	reference
Yes	1.10 [.44-2.3] (.978)	.960 [.40-2.29] (0.927)
Duration of Nocturnal Breastfeeding		
Never breastfed	reference	reference
Less than 6 months	1.50 [.60-3.74] (0.381)	1.37 [.52-3.59] (0.522)
More than 6 months	1.13 [.42-3.06] (0.803)	1.004 [.34-2.92] (0.994)

Table 2 *(continued)*		
Duration of Overall Breastfeeding		
Never breastfed	reference	Reference
Less than 6 months	1.173 [.48-2.82] (0.721)	1.04 [.41-2.6] (0.925)
More than 6 months	.50 [.15-1.59] (0.245)	.581 [.17-1.97] (0.384)
Nocturnal feeding with bottle of formula		
Yes	.92 [.37-2.24] (0.855)	1.29 [.48-3.46] (0.602)
No	reference	Reference
Receives Beverages other than water at night		
Never	reference	reference
Rarely, sometimes, usually or always	.614 [.24-1.5] (0.291)	.522 [.18-1.43] (0.209)
Times sugary snacks are offered between meals		
Never or less than once a day	reference	reference
Daily or more than once a day	2.64 [1.17-5.92] (0.018)*	2.65 [1.10-6.36] (0.029)*
Preterm Birth		
Yes	1.68 [.50-5.63] (0.396)	1.24 [.34-4.46] (0.741)
No	reference	reference
Caregiver or mother or father share utensils with child		
Never or rarely	reference	reference
Sometimes, usually or always	.90 [.39-2.09] (0.820)	1.16 [.48-2.82] (0.729)
Dental visit in last 12 months		
Yes	reference	reference
No	2.54 [.97-6.66] (0.05)*	1.87 [.67-5.20] (0.225)
Maternal tooth decay in last 12 months		
Yes	1.47 [.59-3.66] (0.400)	1.34 [.52-3.48] (0.541)
No	Reference	Reference
Child has plaque on teeth		
Yes	2.80 [1.12-6.98] (0.027)*	3.78 [1.28-11.19] (0.016)*
No	reference	Reference

Odds ratio from unadjusted binary logistic regression for ECC and S-ECC
** Significant association at α= 0.05 level*

AAPD CAT classification of high compared to low (OR=17.14 [95% CI: 4.65-63.1] P<0.001), sugary snacks per day (OR=2.64 [95% CI: 1.17-5.92], P=0.018), dental visits in previous 12 months (OR=2.54 [95% CI: 0.97-6.66], P=0.05), and plaque on the child's teeth (OR=2.80 [1.12-6.98], P=0.027). Any ETS prenatal or postnatal was associated with a significant unadjusted odds ratio for ECC (OR=2.5 [95% CI: 1.1-5.7], P=0.028). No significant unadjusted odds ratio was observed for mode of delivery or any of the breastfeeding variables and ECC.

Likewise, significant unadjusted odds ratios were observed for S-ECC and age in years (OR=2.3 [95% CI: 1.4-3.6], P<0.001), higher maternal educational attainment (OR=0.413 [95% CI:0.17-0.96], P=0.041), overall AAPD CAT classification of high compared to low (OR=35.55 [95% CI: 4.54-278.1], P<0.001), sugary snacks per day (OR=2.65 [95% CI: 1.10-6.36], P=0.029), and plaque on child's teeth (OR= 3.78 [95% CI: 1.28-11.19], P=0.016). No significant unadjusted odds ratio was observed for ETS, mode of delivery or any of the breastfeeding variables and S-ECC (shown in Table 2).

Multi-variable Model for ECC (Specific Aim III)

The final multivariate logistic regression model for ECC –developed through the three block method was significant overall (P<0.001), with a pseudo R-squared of 0.68, model predictive-sensitivity of 77%, specificity of 85.5%, and percent correctly classified of 82.2% (shown in Table 3). Each step was significant and improvement was observed in sensitivity, specificity and pseudo R-squared. Details of each step diagnostics are presented in Table 3. This model indicated that the following variables were significantly independently associated with ECC: age (aOR=5.077 [95% CI: 2.305-11.182], P<0.001), overall AAPD CAT classification of high compared to low (aOR= 18.30 [95% CI: 3.12-107.22], P<0.001), C- section mode of delivery (aOR=0.132 [95% CI: 0.02-0.72], P=0.02), and ETS prenatal or postnatal (aOR=5.39 [95% CI: 1.14-25.33], P=0.033). Duration of overall breastfeeding was not significantly associated with ECC.

Multi-variable Model for S-ECC (Specific Aim III)

The final multivariate logistic regression model for S-ECC developed through the three separate block method was significant overall (P<0.001), with a pseudo R-squared of 0.606, model predictive sensitivity of 67%, specificity of 91.5%, and percent correctly classified of 84.2% (shown in Table 4). The final step for the non-traditional risk was not significant for S-ECC (P=0.063). Details of each step diagnostics are presented in Table 4. The multivariate model for S-ECC indicated that the following variables were significantly independently associated with S-ECC: age (aOR= 3.17 [95% CI: 1.62-6.21], P<0.001), overall AAPD CAT classification of high compared to low (aOR= 24.80 [95% CI: 2.57-239.05], P=0.005), and C-section mode of delivery (aOR=0.141 [95% CI: 0.026-0.748], P=0.021). No significant adjusted odds were found for exposure to ETS or breastfeeding duration and S-ECC.

Table 3
Multi-variable logistic model for ECC

n=101	**odds ratio (95%CI)**	**P-value**	**Block diagnostics**	
Block 1 :Demographics				
Gender			*Overall Block significance*	0.001*
Female	reference		*Pseudo r squared*	0.382
Male	.410 [.10-1.61]	0.203	*Sensitivity*	61.5
Age in Yrs.	5.077 [2.305-11.182]	0.001*	*Specificity*	79
Race			*Percent correctly classified*	72.3
African American	4.201 [.90-19.44]	0.066		
White, Mexican or other race	reference			
Maternal education				
High school graduate, GED or less	reference			
Some college, college graduate or professional school	.605 [.14-2.51]	0.489		
Block 2: CRA (Traditional Risk Factors)				
Overall AAPD Caries-risk Assessment Tool			*Overall Block significance*	0.001*
Low	reference		*Pseudo r squared*	0.584
Moderate	.547 [.05-5.36]	0.605	*Sensitivity*	76.9
High	18.30 [3.12-107.22]	0.001 *	*Specificity*	85.5
			Percent correctly classified	82.2
Bock 3: Non Traditional Risk Factors				
Mode of Delivery			*Overall Block significance*	0.006*
C-section	.132 [.02-.72]	0.02 *	*Pseudo r squared*	0.687
Vaginal birth	reference		*Sensitivity*	76.9
Environmental Tobacco Smoke Exposure, Combination of Prenatal and Postnatal Exposure			*Specificity*	91.9
No	reference		*Percent correctly classified*	86.1
Yes	5.39 [1.14-25.33]	0.033 *	*Model significance*	0.001*
Duration of Overall Breastfeeding				
Never breastfed	reference			
Less than 6 months	4.460 [.65-30.22]	0.126		
More than 6 months	.638 [.10-4.05]	0.634		

P-values from multi variable logistic regression

** Significant association at α= 0.05 level*

Table 4
Multi-variable logistic model for S-ECC

n=101	Odds ratio (95% CI)	P-value	Block diagnostics	
Block 1 :Demographics				
Gender			*Overall Block significance*	0.001*
Female	reference		*Pseudo r squared*	0.329
Male	.440 [.11-1.65]	0.224	*Sensitivity*	53.3
Age in Yrs.	3.17 [1.62-6.21]	0.001 *	*Specificity*	83.1
Race			*Percent correctly classified*	74.3
African American	1.63 [.41-6.41]	0.48		
White, Mexican or other race	reference			
Maternal Education				
High school graduate, GED or less	.517 [.12-2.11]	0.358		
Some college, college graduate or professional school	reference			
Block 2: CRA (Traditional Risk Factors)				
Overall AAPD Caries-risk Assessment Tool			*Overall Block significance*	0.001*
Low	reference		*Pseudo r squared*	0.53
Moderate	1.67 [.10-28.11]	0.72	*Sensitivity*	63.3
High	24.80 [0.60-9.25]	0.005 *	*Specificity*	85.9
			Percent correctly classified	79.2
Bock 3: Non Traditional Risk Factors				
Mode of Delivery			*Overall Block significance*	0.063
C-section	.141 [.026-.748]	0.021 *	*Pseudo r squared*	0.606
Vaginal birth	reference		*Sensitivity*	66.7
Environmental Tobacco Smoke Exposure, Combination of Prenatal and Postnatal Exposure			*Specificity*	91.5
No	reference		*Percent correctly classified*	84.2
Yes	2.361 [.60-9.25]	0.218	*Overall Model significance*	0.001*
Duration of Overall Breastfeeding				
Never breastfed	reference			
Less than 6 months	3.088 [.54-17.63]	0.205		
More than 6 months	1.121 [.185-6.77]	0.901		

P-values from multi variable logistic regression
** Significant association at α= 0.05 level*

CHAPTER 5.

DISCUSSION

Main Study Findings

Results from this cross-sectional study showed that in a sample of urban, predominantly low income children two non-traditional factors, ETS and mode of delivery, significantly improved the accuracy of predicting ECC when added to a multi-variable model for caries prediction with the overall AAPD caries risk assessment. In this sample of children with high caries prevalence, the AAPD CRA in itself was highly associated with ECC and S-ECC; those classified as high risk by the CRA were at 18-fold higher chance of having ECC, and 24 fold higher chance of having S-ECC after controlling for demographics. Given the cross-sectional nature of this study, and since risk classification was done at the time of dental exam and outcome measurement, this was a fairly expected result. Similarly in a previous study, the majority of CRA items evaluated were associated with evident decay at time of assessment (Chaffee, Featherstone, Gansky, Cheng, & Zhan, 2016). However, our finding that prediction of dental caries was improved by adding mode of delivery and ETS as risk factors is new. In the final model for ECC prediction, 86% of children were correctly classified for ECC, a 4% percent improvement over the model of demographics and overall CRA only. Further longitudinal studies are needed to assess the models' ability of predicting future dental caries and caries severity.

Exposure to any ETS prenatally, postnatal or both was associated with approximately two-fold increased odds for ECC compared to children who were not exposed, and the association became stronger after accounting for demographics and the CRA classification where the adjusted odds ratio for ECC was 5.39. This association indicates that, although ETS exposure in this sample was associated with more economic disadvantage, there is an independent association between passive smoke exposure and dental caries in young children not explained by differences in economic status. Other biological, behavioral and developmental influences may interplay to explain this association; children exposed to ETS may have more cariogenic microorganisms in their mouth flora, develop weaker less mineralized teeth, possess impaired immunological responses, and possibly practice more deleterious oral habits. Other studies have reported similar findings where exposure to ETS was associated with a significant increase in caries prevalence and severity (Aligne, Moss, Auinger, & Weitzman, 2003; Iida et al., 2007; Majorana et al., 2014). Due to the high correlation between prenatal and postnatal ETS in our study, we could not distinguish the effect time of exposure to ETS may have on the association with dental caries and a larger sample would be needed to assess such differences. No significant associations were found for ETS and Severe-ECC, suggesting that ETS may be associated with onset of disease, but not with its progression.

Mode of delivery was found significantly associated with ECC, where children born via C-section had 80 percent less chance of having ECC compared to those born vaginally. However, this association was significant only in the adjusted model. Similar results were described in three previous studies and have been explained by differences in oral flora composition, socioeconomic status, and possibly more attention given to children born via C-section after a complicated birth (Borowska-Strugińska et al., 2016; Fontana et al., 2011; Pattanaporn et al., 2013). In our sample, children born via C-section were more likely to have a mother with higher educational attainment. Therefore, the observed association may be a result of differences in oral health knowledge and attitudes. This confounding factor has been partially accounted for by adjusting for educational attainment in the multivariate model. Furthermore, it is necessary to view the association between mode of delivery and ECC in light of the study's small sample size where the total number of children born via C-section was n= 27, 25 % of the study sample.

Breastfeeding per se, breastfeeding duration, and nocturnal breastfeeding were not associated with ECC or S-ECC in this study. This is in line with recent research on the subject (Iida et al., 2007; Lavigne, 2013; Mohebbi et al., 2008). Although only breastfeeding duration is reported here for the multivariate model, different models were tested for breastfeeding per se, and nocturnal breastfeeding, separately and combined, and results were unchanged. Furthermore, of the assessed dietary variables, sugary snacks per day was the only factor significantly associated with ECC and S-ECC, where children who received one or more sugary snack between meals per day were at a more than twice the odds for having ECC and more than triple the odds for having S-ECC. Comparable results were described in previous research, and it is likely a direct effect on the etiology of dental caries, where the sugar laden diet creates an ideal environment for cariogenic microorganisms to thrive and produce dental decay (Evans et al., 2013; Prakash et al., 2012a). Furthermore, presence of plaque on a child's teeth increased the odds for ECC by two folds and S-ECC by three folds. This finding is in line with previous research (Mohebbi, Virtanen, Vahid-Golpayegani, & Vehkalahti, 2006).

In terms of demographics, age was an important factor for ECC and S-ECC where each added year increased the child's adjustd odds for ECC by approximately five times, and for S-ECC by more than three times. The longer interplay of various factors with tooth structure over time explains the observed association between age and dental caries prevalence. Tinanoff et al. reported a similar finding in their study where the prevalence of ECC among 5 year olds was 44 %, compared to 13% among 2 year olds (Tinanoff et al., 2002). Although economic disadvantage and race were not significant factors for dental caries prevalence or severity in our sample, maternal education was; children born to a mother who had completed at least some college were at 50% decreased odds of having ECC. This is in line with previous literature and indicates that for a sample of predominantly urban, low income children, maternal education remains a significant protective factor against dental disease, and should be targeted by educational programs to promote better oral health knowledge and behaviors. Finally, in this study dental visit in the last 12 months was significantly associated with disease status, although this association likely reflects previous disease rather than adherence to recommended guidelines on visits.

Study Strengths and Limitations

The main strength of this study is in its urban, predominantly low income population, which enabled the assessment of underlying risk factors that contribute to the disparities of dental caries in young children. The high prevalence of dental caries in the study sample enabled statistical analysis with robust estimates despite the small sample size. It is important to note that this high prevalence of ECC (61%) may be a result of the convenience sampling method, where children were recruited from the pediatric dental clinic and may not reflect the true disease prevalence of this population. Furthermore, a wide variety of variables were measured and included in the analysis. Therefore, the reported adjusted associations likely reflect accurate independent associations.

It is central to interpret the results from this study in light of its limitations: 1) The study's cross-sectional design limits the ability to infer causality between dental caries and the assessed exposures. 2) All predictors were self-reported except for the clinical factors from the CRA, therefore there is likely inherent inaccuracy in them, especially for the smoking variables which may underestimate the true exposures. 3) Variables were from recall which could have led to additional inaccuracies when considering that many of the children were 4-5 year olds at time of enrollment, and their caregiver was required to recall events from the first year of life. 4) Limited generalizability of the results reported to other populations because of the specific economic and racial characteristics of this study's population.

CHAPTER 6.

CONCLUSION

This study supports the notion that ECC is a multifactorial disease, and is associated with non-traditional exposures including environmental tobacco smoke, and mode of delivery. ETS exposure could account for some of the disparities observed in ECC. Thus, targeting this factor both prenatally and postnatally by educational and awareness programs is critical, not only for overall health and development of young children, but also as an important factor for oral health in disadvantaged populations. Furthermore, this study suggests that vaginal birth increases the risk for dental caries. However, further research is needed to explain the mechanics of this association and to distinguish if this is a true cause-effect relationship, or a correlate for other underlying factors. Finally, this study underscores the importance of developing evidence-based caries risk assessment systems, and incorporating them into routine dental practice.

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APPENDIX A

PROTOCOL SUBMITTED TO IRB REVIEW

1) Abstract of the study

Early Childhood Caries (ECC) is one of the most common chronic illnesses among young children, effecting around 23% of children 2-5 years old, causing them pain, discomfort, and poor quality of life. It is a multi-factorial disease that develops through a combination of behavioral, environmental, and hereditary exposures. This study aims to investigate the associations between non-traditional factors acquired during the first years of life and the presence of both ECC and Severe-ECC among children 0-5 years old. Additionally the study aims to assess the variation of these exposures by main socio demographic factors. The non- traditional factors measured include mode of delivery, breastfeeding, and Environmental Tobacco Smoke (ETS). A cross-sectional design will be employed for this study, and a sample of 150 caregiver/ child dyads will be recruited from the ongoing flow of patients at (TUKSoD). After obtaining consent subjects will be provided with a self-administered questionnaire on demographics, risk factors, and potential confounders. The patients will then receive a standard intra-oral examination by a student doctor, and classical risk assessment for dental caries will be conducted using the CAMBRA (Caries Management by Risk Assessment) Checklist which collects information on disease indicators, risk factors and preventive factors and yields a risk classification for each patient ("Extreme, High, Moderate, or Low"). Information for the main caries outcomes will be extracted from the participant's dental records. Additionally, a sub sample of (20 -30) patients will be selected randomly for plaque microbiologic testing to assess the count of *S.Mutans*. The relations of these counts to the risk factors will be studied for this sample. Descriptive summaries, and multivariable logistic regression models will be used to determine the adjusted and unadjusted associations between each of the predictors and the caries outcomes.

2) Protocol Title

Early Life Exposures and Childhood Dental Caries among Children 0-5 Years Old

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4) Objectives

Primary Objectives:

The objectives of this study are: 1) To determine exposures during the first years of life that are associated with the presence of Early Childhood Caries (ECC). Specifically, this study aims to test whether mode of delivery, breastfeeding and second/third hand smoking are associated to the presence of ECC and Severe- ECC, and 2) To assess the variation of these early exposures by main socio-demographic factors (caregivers age, gender, SES, race, ethnicity and education).

Secondary Objective

The secondary objective is to determine the exposures during the first year of life that are associated with counts of *S.Mutans* in a plaque sample obtained from the child.

Rationale and Significance

Dental caries is the most common childhood illness affecting around 23% of children 2 -5 years old in the US.(Dye et al., 2015) Early Childhood Caries (ECC) is defined as the presence of one or more decayed, missing or filled tooth surfaces in any primary tooth in a child between birth and 5 years of age.(American Dental Associaton, 2013). In children younger than 3 years of age, any sign of smooth-surface caries is indicative of Severe Early Childhood Caries (S-ECC). From ages 3 through 5, 1 or more cavitated, missing (due to caries), or filled smooth surfaces in primary maxillary anterior teeth or a decayed, missing, or filled score of ≥4 (age 3), ≥5 (age 4), or ≥6 (age 5) surfaces constitutes S-ECC(American Dental Associaton, 2013). Moreover, this disease is associated with poor oral health related quality of life,(M. Y. Li et al., 2015) increased pain, difficulty in biting and chewing for the child,(Filstrup et al., 2003) and is a predictor for the development of dental caries later in the permanent dentition.(Y. Li & Wang, 2002) .

ECC is a multi-factorial disease that develops through the combined effects of bacteria, tooth morphology, fermentable carbohydrates and time.(Ripa, 1988) Since dental caries is a highly preventable disease, identifying those at risk at an early stage is critical, and Caries Risk Assessment (CRA) is increasingly becoming standard of care for dental caries management.(Ramos-Gomez, Crall, Gansky, Slayton, & Featherstone, 2007) (Ref). The Caries Management by Risk Assessment (CAMBRA) is an evidence based method that aims to determine each patient's current and future dental caries by identifying unique disease indicators, risk factors, and protective factors, and assigning a level of risk for each patient; low, high or moderate.(Hurlbutt, 2011) The risk factors included in the CAMBRA tool for children 0-5 years of age are those proven causative in literature; SES, caregivers recent caries status, dietary behaviors, and salivary factors, and each factors is considered to add to the caries risk independently. The sensitivity and specificity of the CAMBRA in predicting caries among the moderate group was found to be 97% and 21% respectively.(Gao et al., 2013)This low specificity could be due to that a single factor can add to the risk factor indicating that a multi variable model approach may be more appropriate. A multivariable model of a combination of 10 risk factors, including a non-traditional factor: tobacco smoke exposure, was validated for prediction of future caries and resulted in a sensitivity and specificity of 79 percent and 81 percent, respectively. Several studies have investigated the associations between dental caries and non-traditional factors including mode of delivery, breastfeeding and environmental tobacco smoke. However, the literature concerning these factors has been inconsistent.

The microbiological component, namely the concentration of *Streptococcus Mutants* (SM) in the child's saliva, plays an important role in the etiology of dental caries, where children with higher counts of SM are more likely to develop ECC, and have more severe forms of disease.(Thibodeau & O'Sullivan, 1996) Perinatal factors including mother's gestational age, child's birth weight, and mode of delivery have been found associated with (SM) levels in the salivary flora. (Y. Li et al., 2005; Zhou et al., 2013) Mode of delivery significantly affects the composition of oral microbiota in six month old infants, where children born vaginally were found to have more diverse flora.(Boustedt et al., 2015; Lif Holgerson et al., 2011) Li et al found children born via C-section were colonized by SM up to a year earlier than those born vaginally, in a prospective cohort design.(Y. Li et al., 2005) Since early colonization with SM is linked to more severe forms of ECC,(Alaluusua & Renkonen, 1983) it is consequently expected that children born via cesarean are more likely to develop ECC. Studies on this relationship have resulted in conflicted findings; whereas some have found no differences in ECC prevalence, (Kuthy et al., 2014; Reed et al., 2014) A prospective study on 3 year old Danish girls reported that among those with ECC mode of delivery affected the mean value of the (dfs) index, an indicator of disease experience, although these differences did not reach statistical significance.(Barfod et al., 2012)

Prolonged and on demand breastfeeding have been linked in several studies to the occurrence of S-ECC, and a higher overall prevalence of dental caries.(Bissar et al., 2014; Chaffee et al., 2014; Prakash et al., 2012b) However, a study using US national representative data found that breastfeeding and its duration were not associated with an increased risk for the disease.(Iida et al., 2007) On the contrary, a recent meta-analysis concluded that breastfed babies were less likely to develop dental caries.(Avila et al., 2015)

Additionally, exposure to prenatal and postnatal maternal smoking has been associated with an increased risk of ECC.(Iida et al., 2007; Majorana et al., 2014; K. Tanaka et al., 2009) Children with high levels of cotinine, an indicator for passive smoke exposure even after birth, were found at an increased risk for having filled and decayed teeth.(Aligne C, Moss ME, Auinger P,Weitzman M., 2003) The association with dental caries was stronger for maternal smoking exposure compared to paternal only exposure.(Hanioka et al., 2008) Moreover, a dose-response relationship was found between cumulative postnatal exposure to environmental tobacco smoke at home and dental caries.(K. Tanaka et al., 2009)

The current study aims to determine the associations between early life exposures and the presence of ECC and S-ECC. This will help in better understanding how prediction of dental caries in children may be affected by the inclusion of these non-traditional disease risk factors among children from a clinic sample in North Philadelphia. Additionally we will investigate in a sub sample the associations between these factors and counts of *S.Mutans* in a plaque sample.

5) Resources and Setting

All patients will be identified, evaluated and treated at the Temple University Kornberg School of Dentistry (TUKSoD). All research staff has CITI training and will be required to read all the documentation related to the study and discuss any questions with the investigators before the initiation of activities.

6) Prior Approvals

N/A

7) Study Design

a) Recruitment Methods

Approximately 150 caregiver/child 0-5 years old dyads will be recruited. There are no restrictions in terms of relative numbers of females and males. All potential subjects will be seeking dental treatment at the TUKSoD.

Potential subjects will be obtained through the ongoing flow of patients to TUKSoD Pediatric Dentistry Clinic by phone or in person. HIPhone numbers will be accessed through axiUm, the electronic patient scheduling program used by various dental clinics at TUKSoD. The research staff will also visit the pediatric clinic during regular operating hours to inform and recruit patients at the lobby. The consent to participate in the study will be provided by the caregiver of the children and caregivers who provide consent will be entered into a contest to receive a $250 dollar gift card if they complete all required assessments. Only one of the participants will receive this payment. Subjects will complete a separate opt in/out form (Appendix G) and none of the information on this form will be used for research purposes, only for the raffle. This is required for compliance with Pennsylvania state laws. Information about the study will also be posted in a flyer (Appendix A) in designated areas at the School of Dentistry and College of Public Health.

b) Inclusion and Exclusion Criteria

There are no restrictions on enrollment in terms of gender, economic status, or ethnic group.

Inclusion criteria

Patients:
Must be 0-5 years of age.
May be new, regular, and emergency patients.

Caregiver:
Must be between 18-65 years old.
Must be able to speak and understand English.

Must be willing to provide consent to participate in the study for himself/herself and his/her child.

Exclusion criteria
Patients:
Older than 5 years of age.
Those whose parents do not provide consent for participation.

Caregiver:
Younger than 18 or older than 65 years of age.
Those who decline to provide consent.

c) Study Timelines

The purpose of this research project is to determine the factors that may be associated to ECC and S-ECC among children under 6 years old, and assess variations by key socio-demographic characteristics.

Table 1: Research Timelines

Approx.Month	***Goals***
June 2016	*IRB submission*
September 2016 (two weeks)	*Pilot testing of the survey*
September 2016- January 2017	*Recruitment and data collection*
February 2017- March 2017	*Data analysis*
April 2017- May 2017	*Finalizing manuscript and poster for publishing*

Pilot Testing
Before the start of the study, pilot testing of the survey questionnaire (Appendix B) will be conducted among 10 caregivers, and data obtained from these caregivers will not be used for the actual study. Pilot testing will require approximately 2 weeks, and will conducted in the same setting as the actual study at the (TUKSoD). The pilot testing will include only administering the survey questionnaire used for the study. This will be done to assess any difficulties among caregivers in comprehending the language used in the survey, or issues in following the instructions of the survey. No information collected during this pilot testing will be used for the study.

Consent and Survey
After obtaining informed consent from the child's caregivers, the questionnaire survey (Appendix B) will be given to the caregiver. The survey is in a written format and will be self- administered by the caregiver, with a member of research staff available to answer any questions.

Intra Oral Exam, Caries Risk Assessment (CRA)
Each child will receive a standard intra-oral examination by a student doctor and classical risk assessment for dental caries will be conducted using the CAMBRA (Caries Management by Risk Assessment) Checklist which collects information on disease indicators, risk factors and preventive factors (Appendix C) and yields a risk classification for each patient ("Extreme, High, Moderate, or Low"). The dental records for participants will be reviewed and data related to the main caries outcomes will be extracted using extraction sheet (Appendix D).

Microbiologic Plaque Testing
For a selected sub-sample of the patients (N:20-30 subjects) collections of plaque samples will be conducted at the pediatric clinic which has 17 operatory chairs. This sub-sample will be selected randomly; a number will be given to each patient by order of enrollment, and if this number corresponds to a number on a list of 30 randomly generated numbers between (1- 150) the participant will be chosen for the microbiologic testing. A "warm room" part of the Endodontology Department will be used for processing the plaque samples to determine the counts of *S.mutans* in plaque.

d) Study Procedures and Data Analysis

Sample and Setting. All research procedures will be performed in the TUKSoD Pediatric Dentistry Clinic. Participation in the research is expected to take several hours in total.

Predictors

Three main predictors will be assessed;

1) Mode of delivery which will be defined as either cesarean birth or vaginal birth,

2) Breastfeeding duration which will be defined as less than 3 months, 3-6 months, 6-12 , more than 12 months,

3) Environmental Smoke Exposure defined as never exposed to smoking (prenatal or postnatal), prenatal smoke exposure only, postnatal smoke exposure only, or both.

Outcome:

Two main outcomes will be evaluated in a dichotomous measurement:

1) ECC Early Childhood Caries: defined as the presence of one or more decayed, missing or filled tooth surfaces in any primary tooth in a child between birth and 5 years of age.

2) (S-ECC) Severe- ECC: defined in children younger than 3 years of age as any sign of smooth-surface caries is indicative of severe early childhood caries (S-ECC), and in ages 3 through 5 as 1 or more cavitated, missing (due to caries), or filled smooth surfaces in primary maxillary anterior teeth or a decayed, missing, or filled score of ≥4 (age 3), ≥5 (age 4), or ≥6 (age 5) surfaces.

For the secondary outcome

The outcome of *S.Mutans* counts will be defined as one of three classes;

Class 0-1 = < 100 000 CFU/ml saliva

Class 2 = 100 000-1.000.000 CFU/ml saliva

Class 3 = > 1 milj CFU/ml saliva

Covariates:

1) Demographics (SES, income, maternal education level, race, ethnicity WIC, stamps)

2) Maternal age at gestation

3) Preterm or full term birth

4) Childs weight at birth

5) Maternal new caries in last 12 months

6) Bottle feeding

7) Sharing of utensils between mother and child
8) Frequency of sugary snacks between meals
9) Drinks other than water at night
10) Medical and dental insurance
11) Overall perceived health
12) Tooth brushing
13) Seen dentist in last year
14) Childs age, and sex
15) Risk classification from CAMBRA checklist

Sample Selection Procedures
All assessments will be conducted after informed consent has been obtained in person. Patients deemed ineligible or who do not wish to participate in the research will be referred for clinical services as appropriate. The decision to participate or not participate in the research will have no effect on the dental treatment that the patient will receive.

Standard Oral Examination and Risk Assessment

Clinical calibration exercises will be conducted to standardize the criteria for detecting dental caries. Likewise, student investigators will be calibrated for collecting objectively information on risk factors and conducting the microbiological testing. Available commercial kits will be used for conducting these tests reducing contact time with patients, improving efficiency of the study flow and reducing disruption to the normal operations at the pediatric clinic. All assistants and others working on the project will be educated about the importance of strictly respecting patients' rights to confidentiality and will have completed training concerning proper practice in accordance with HIPAA regulations.

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Microbiological assessment will be conducted using plaque collected. Counts of *Smutans* will be generated using a commercially available kit Dentocult Strip Mutans Orion Diagnostica. The method is based on the use of a selective culture broth and the adherence and growth of *mutans Streptococci* bacteria on the test strip. Samples are taken to an incubator or kept in a warm room for 48 hours. The schools infection control protocols to handle these specimens will be strictly followed.

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Data and Specimen Banking
Microbiological samples will be discarded after 48 hours once the reading is conducted, following the protocols established by the research lab in the Endodontology Department.

Data Management and Analysis: All demographic information and questionnaires will be administered in person. No data will be collected for this research over the phone. Data will be entered into an excel spreadsheet. Data will be downloaded to an IBM-compatible computer and stored on a password-protected account, and it will be exported to a computer data analysis program (SAS/SPSS). Data will be cleaned and checked for errors prior to analysis; imputation procedures for handling missing data will be conducted depending on the number of the number of cases with missing data. Participant names and corresponding identification numbers will be stored separately in a locked file cabinet or password-protected file.

Data Analysis and Power Calculations.
Initial exploratory analyses will be conducted to determine normality of distributions of study variables. Any necessary transformations will then be conducted. To determine potential covariates, simple differences between groups on continuous variables will be evaluated using analysis of variance, and differences between groups on categorical variables will be examined with Fisher's exact tests and chi- square tests. Statistical significance will be set at a p-value<0.05. Univariate logistic regression will be executed for each of the risk factors with the two outcomes of interest (ECC and S-ECC). Two separate multivariable models for caries prediction will be developed based on the two outcomes of the study (ECC and S-ECC) using the hybrid stepwise method to determine significance in the model with demographics forced in the model.

e) Withdrawal of Subjects

Subjects may be withdrawn from the study because of failure to follow instructions of the research staff or if the principal investigator decides that the research study is no longer in the subject’s best interest.
Details are provided in the consent form.

f) Privacy & Confidentiality

As in any type of treatment or clinical research program, patients' confidentiality must be carefully guarded and respected. The study will use Protected Health Information (PHI) and a HIPAA Authorization form is being submitted with this application (Appendix E).
There will be a separate file with identifiers in receipt of the principal

investigator. All data with identifying information will be stored in locked files or encrypted, password-protected electronic data storage. Data being analyzed will be identified by subject codes (using the subject identification number) and identifying information will be removed. The identity of patients will not be revealed in the presentation or publication of any results from this project. All assistants and others working on the project will be educated about the importance of strictly respecting patients' rights to privacy and confidentiality and will have completed training concerning proper practice in accordance with HIPAA regulations.
Protected Health Information that will be collected includes the patient's name, date they were seen for the study, phone number. The electronic PHI data will be downloaded to a computer using encrypted, password- protected storage of electronic data. All participants will receive a unique random identification number, which will correspond to that person's data. Participant names and corresponding identification numbers will be stored separately in a locked file cabinet or encrypted, password-protected storage of electronic data.

8) Risks to Subjects

This project is a survey based project and no risks are expected. The dental examination and collection of risk factor information including plaque samples are common standard procedures at the dental school.

9) Potential Benefits to Subjects

Patients will be informed about caries risk. There are no other direct benefits to the subject from this study.

10) Costs to Subjects

N/A

11) Informed Consent

See Consent Form in (Appendix F) submitted with this protocol.

An adult who is not the parent of the child is allowed to provide written consent for the study if it has previously been documented in the child's TUKSoD dental chart that they have permission to make decisions about treatment on behalf of the child.
Study will follow "HRP-802 INVESTIGATOR GUIDANCE: Informed Consent" to obtain informed consent.

Study subjects will be entered into a raffle to receive a $250 dollar gift card once they complete their participation in the study. This information has been included into the consent form respectively.

12) Vulnerable Populations

The study involves minors between 0-5 years old. Written consent will be obtained from their accompanying guardian. The permission of one parent is sufficient even if both parents are alive, known, competent, reasonably available, and shares legal responsibility for the care and custody of the child. The consent script will be provided to the parent/guardian. Assent will not be obtained because the capacity of the children is so limited that they cannot reasonably be consulted.

It will be emphasized that participation in the study is completely voluntary. The subject's directed guardian will accompany the subject during the evaluation. Minimal risk will be presented to the children.

13) Compensation for Research-Related Injury

Not applicable

14) Economic Burden to Subjects

Not applicable

15) Drugs or Devices

Not applicable.

16) Multi-Site Human Research

Not applicable

17) Sharing of Results with Subjects

Participating subjects will be informed about their dental caries status and risk classification.

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APPENDIX B

SURVEY QUESTIONNAIRE

NON TRADITIONAL EXPOSURES AND CHILDHOOD DENTAL CARIES AMONG CHILDREN 1-5 YEARS OLD

Subject ID Number [__________________________]

Thank you for your participation in this study about children's tooth decay. In this survey you will be asked a few questions about your background and questions related to you and your child's oral health. You will be asked to recall events experienced over the last years of your child's life. Please answer by your best memory.

Please feel free to ask about any question that doesn't seem clear. Whenever "child" is mentioned it refers to your child that is enrolled in the study. Place a check next to the choice that feels most appropriate for each question.

Section A (Demographics)

A1. What is the 5-digit ZIP code at your home address? [__ ___ ___ ___ ___]

A2. What is your gender? [___]

1) __Male
2) __Female

A3. What is your age? *(In years)* [___________]

A4. What is your relation to the child? [___]

1) __Mother
2) __ Father
3) __ Other (_________________________)*please specify*

A5. What is the highest grade of school that you completed? [___]

1)__ less than high school (grades 0-8)

2)__ Some high school (grades 9-11)

3)__ High school graduate (grades 12 or GED)

4)__ Some college (include vocational, trade, or technical school after college, associate degree, No four-year degree)

5)__ College graduate (BS or other four year degree)

6)__ Post graduate or professional school after college

A6. What is your family's income level? [___]

1)__ Less than $19,999

2)__ $20,000 - $49,999

3)__ More than $50,000

A7. What is the child's age? *(in years*)

[____]

A8. Is the child a boy or a girl? [___]

1) Male
2) Female

A9. What race do you consider the child to be? *(Place a check next to the correct answer)*[_____]

1) __ White or Caucasian or European American
2) __ Black or African American
3) __ American Indian or Alaska Native
4) __ Asian
5) __ Native Hawaiian or Other Pacific Islander
6) __ Other (____________________________)

A10. Is the child of Hispanic or Latino origin or descent? [____]

1) ___Yes
2) ___ No

Section B (Main Predictors)

B1. How was the child born? [___]

1) ___Normal (vaginal) birth
2) ___C- section b
3) ___Don't know

B2. Did the child's mother smoke while pregnant with this child? (Including cigarettes, e-cigarettes, cigars, hookah, and any tobacco products)

[___]

1) ___ Yes
2) ___ No
3) ___Don't know

B3. Did the child's mother smoke while pregnant with the child daily, less than daily, or not at all? [____]

1) __Daily
2) __Less than daily
3) __Not at all
4) __Don't know

B4. During the mothers pregnancy with this child, did anyone in the household smoke? (Including cigarettes, e-cigarettes, cigars, hookah, and any tobacco products)

[___]

1) ___ Yes
2) ___ No
3) ___Don't know

B5. During the mother's pregnancy with this child, did she work outside of home?

[___]

1) ___ Yes
2) ___ No/ Didn't work *(Please skip to question B7)*
3) ___Don't know

B6. ***If Yes****:* Did anyone in her workplace smoke indoors? *(Only answer if previous answer is yes)* [___]

1) ___ Yes
2) ___ No
3) ___Don't know

B7. After the child was born, did the child's mother smoke? [___]

1) ___ Yes
2) ___ No
3) ___Don't know

B8. After the child was born, did the child's mother smoke daily, less than daily, or not at all? [___]

1) __Daily
2) __Less than daily
3) __Not at all
4) Don't know

B9. After the child was born, did anyone in the household smoke? [___]

1) ___ Yes
2) ___ No
3) ___Don't know

B10. Was the child breastfed after he/she was born? [___]

1) ___ Yes
2) ___ No
3) ___Don't know

B11. How long was the child breastfed? [____]

1) ___less than 3 months
2) ___3-6 months
3) ___6-12 months
4) ___More than 12 months

B12. Was the child breastfed <u>during the night</u>? [____]

1) ___ Yes
2) ___ No
3) ___Don't know

B13. How long was the child breastfed at <u>night</u>? [___]

1) ___less than 6 months
2) ___6-12 months
3) ___More than 12 months

B14. When was bottle feeding with formula (not mother's milk) first offered to the child? [___]

1)___at birth
2)___between 0- 3 months
3)___between 3-6 months
4)___between 6-12 months
5)___after 12 months

B15. Was your child bottle-fed (with formula) <u>during the night</u>? [___]

1) ___ Yes
2) ___ No
3) ___ don't know

B16. How long was your child bottle-fed (with formula) <u>at night</u>? [___]

1) ___less than 6 months
2) ___ 6-12 months
3) ___More than 12 months

Section C (Covariates)

C1. At birth what was your child's weight approximately? (Measured in pounds) [________________]

C2. Was the child born full term or preterm? [___]

1) ____Preterm (before 36weeks)

2) ____Full term(After 36 weeks)

C3. How old was the child's mother when she had the child? *(in years)*

[__________________]

C4. During the last month, how often has he/she gone to sleep with a bottle containing a beverage other than water (milk, juice, etc)?

[____]

1) ____Never

2) ____Rarely

3) ____Sometimes

4) _____Usually

5) _____Always

C5. During the last month, how many times per day, did the child eat a sugary snack between meals? Including (candy, chocolate, etc.)? [___]

1) ___ never

2) ___ less than once a day

3) ___ 1-3 times a day

4) ___ more than 3 times a day

5) ___ Not sure

C6. During the last month, how often did you (the caregiver), the child's mother, or his/her father share food utensils with the child?

[_____]

1) ____Never

2) ____Rarely

3) ____Sometimes

4) _____Usually

5) _____Always

C7. How many children do you have in the household (the child included)?

[_______________] *[Example: 4]*

C8. Would you say your child's health, in general, is excellent, very good, good, fair, or poor?

[_____]

1)___Excellent

2)___Very good

3)___Good

4)___Fair

5)___Poor

C9. Does your child currently have medical insurance? [____]

1) ___ Yes
2) ____No
3) ____ Don't know

C10. Is your child currently covered with dental insurance? [____]

1) ___Yes
2) ___No
3) ___Don't know

C11. In the last twelve months, has your child been examined or treated by a dentist? [___]

1) ____Yes
2) ____No *(Please skip to question C15)*
3) ____ Don't Know

C12. ***If yes***: Why did your child visit the dental office? [____]

1) Routine checkup
2) Dental trauma
3) Pain
4) Other [_______________] *(specify)*

C13. During the last 12 months has the child's mother (or caregiver), had any tooth decay which caused pain, or received dental treatment for tooth decay? [____]

1) ____Yes
2) ____No
3) ____ Don't Know

Does anyone living in the child's household, receive any of the following?

C14. a) Food Stamps or SNAP (Supplemental Nutrition Assistance Program) Benefits [____]

1) ____Yes
2) ____No
3) ____ Don't Know

C15. b) WIC Program benefits (Women, Infant and Children Food Supplement)? [_____]

1) ____Yes
2) ____No
3) ____ Don't Know

Thank you for your time, we appreciate your participation.

APPENDIX C

INFORMED CONSENT FORM

Title of research*: Early Life Exposures and Childhood Dental Caries among Children 0-5 years old*

Investigator and Department:

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Logan Mariano, Predoctoral Student Kornberg School of Dentistry

Jacob P Sebastian, Predoctoral Student Kornberg School of Dentistry

Chintan Patel, Senior Student, Kornberg School of Dentistry

Why am I being invited to take part in this research?

We invite you to take part in a research study because you have a child younger than 5 years old, coming for dental treatment at the TUKSoD pediatric dental clinic, and we would like to understand the factors that may be related to your child's tooth decay.

If the research involves children, "you" or "your" refers to your child.

What should I know about this research?

Someone will explain this research to you.
Whether or not you take part is up to you.
You can choose not to take part.
You can agree to take part and later change your mind.
Your decision will not be held against you.
You can ask all the questions you want before you decide.

Who can I talk to about this research?

If you have questions, concerns, or complaints, or think the research has hurt you, contact the research team at *Marisol Tellez Merchan, Ph.D., at 215-707-1773*, or write to her at the following address:

Maurice H Kornberg School of Dentistry

Temple University

Department of Public Health Sciences

3223 North Broad Street, Room 307

Philadelphia, PA 19140.

This research has been reviewed and approved by an Institutional Review Board. You may talk to them at (215) 707-3390 or e-mail them at: irb@temple.edu for any of the following:

Your questions, concerns, or complaints are not being answered by the research team.
You cannot reach the research team.
You want to talk to someone besides the research team.
You have questions about your rights as a research subject.
You want to get information or provide input about this research.

Why is this research being done?

Tooth decay is one of the most common chronic illnesses affecting young children. It can lead to the child's pain and difficulty biting and chewing and is associated with a higher risk of tooth decay as the child grows into an adult. Many factors are associated with this disease including sugary beverages, brushing the child's teeth, and the caregiver's oral health. Understanding which risk factors contribute to tooth decay is very important to identify those at risk, and deliver the timely and appropriate treatment.

The purpose of this study is to assess how nontraditional factors acquired during the first years of life may be related to early carious lesions, and severe dental caries in young children under 5 years old. Primarily we want to investigate if second and third hand smoking, breastfeeding, and mode of delivery may be associated with the child's tooth decay.

Additionally because dental caries is mainly a bacterial infection we want to assess how these factors affect the microbiological composition of the child's saliva. To do this we will select a number of the research participants to undergo plaque microbiological testing. You will be informed by research personnel if your child has been chosen for this procedure.

How long will I be in this research?

We expect that you will be in this research for a few hours.

How many people will be studied?

We expect about ___150__ people will take part in the research.

What happens if I agree to be in this research?

Survey and Dental Exam

First you will be given a written survey with questions about events and exposures that may be related to your child's tooth decay disease. All answers are confidential and you have the option of not answering any question you don't feel comfortable with. The survey is self-administered but you can ask research staff about anything that seems confusing. Your child will then undergo a standard intra-oral exam, and a traditional caries risk assessment called the CAMBRA (Caries Management by Risk Assessment) Checklist. The CAMBRA is a short set of questions asked by the dental examiner about tooth brushing, behaviors and other factors related to tooth decay. Afterwards the child will undergo regular dental treatment as needed by his/ her diagnosis.

Plaque Microbiological Testing

If your child has been chosen for the plaque testing you will be informed by research staff. This test will take place at the dental chair and no pain or discomfort is associated with it. During this procedure a plaque sample will be taken from your child's mouth and used for testing.

What happens if I agree to be in this research, but I change my mind later?

If you agree to take part in the research now and later decide to stop, it will not be held against you. It will in no way affect your relationship with the dentists, dental students or staff at TUKSoD.

If you stop being in the research, already collected data may not be removed from the study database. You will be asked whether the investigator can collect data from your routine medical care. If you agree, this data will be handled the same as research data.

Is there any way being in this research could be bad for me?

The interviews and questionnaires that you will receive during the course of the study involve no specific risks or discomfort beyond those of a standard clinical interview, such as feeling upset at a review of your mental status, boredom, or fatigue. The interviews and questionnaires may be time-consuming, and they ask about personal matters. It is possible that you will feel upset, tired or anxious. If this happens, you can choose not to answer specific questions or ask to have the assessment stopped.

The study team will communicate any new information of which they become aware that may affect your willingness to participate in this study.

Will being in this research help me in any way?

We cannot promise any benefits to you or others from taking part in this research. However, possible benefits include knowing your child's risk of dental caries. The indirect benefit will be the help you provide in advancing knowledge about factors associated with tooth decay in young children.

What happens to the information collected for this research?

To the extent allowed by law, we limit the viewing of your personal information to people who have to review it. We cannot promise complete secrecy. The IRB, Temple University, Temple University Health System, Inc. and its affiliates, and other representatives of these organizations may inspect and copy your information. Microbiological samples will be discarded after 48 hours once the reading is conducted, following the protocols established by the research lab in the Endodontology Department.

The sponsor and its agents (if applicable), monitors, auditors, the IRB, and the Food and Drug Administration will be granted direct access to the portion of your medical records which are related to this research study for verification of the research procedures and date. By giving your consent, you are authorizing this access. You will also need to sign a separate "Authorization to use and disclose your protected health information" to be a part of this research. We may publish the results of this research. However, we will keep your name and other identifying information confidential.

Federal law provides additional protections of your personal information. These are described in an attached document titled "Authorization to use and disclose your protected health information".

Can I be removed from the research without my permission?

The person in charge of the research study can remove you from the research study without your approval. Possible reasons for removal include failure to follow instructions of the research staff or if the principal investigator decides that the research study is no longer in your best interest.

We will tell you about any new information that may affect your health, welfare, or choice to stay in the research.

What will I be paid for taking part in this research?

If you agree to take part in this research, you will be entered into a raffle to win 350$ for your time and effort. Federal tax law requires to you to report this payment as income to the Internal Revenue Service.

You may be asked to tell us your social security number. If payments are more than $599.00, we will report them to the Internal Revenue Service and send you a Form 1099-MISC. If you do not give us your social security number, you may take part in this research if you agree to not be paid.