The Republic of Iraq Ministry

of Higher Education and Scientific Research

Misan University

College of Dentistry

Fifth Stage

Relationship between salivary alpha amylase activity anthropomorphic indices dietary habits and early childhood dental caries

A Research:

Submitted to College of Dentistry, University of Misan as a requirement for Bachelor's degree in Dentistry

Submitted by:

Zainab hasanin Rheem

Teba kamal abd alzhra

Supervised by:

Dr. Ibtisam Kareem

2024 A.D

1445 A.H

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

وَلَمَّا بَلَغَ أَشُدَّهُ وَاسْتَوَى آتَيْنَاهُ حُكْمًا
وَعِلْمًا وَكَذَلِكَ نَجْزِي الْمُحْسِنِينَ

سورة القصص- الآية 14.

صدق الله العلي العظيم

Dedication

To my mother and father, who never stopped believing in me.”

“For my dad, who taught me the love of stories, and my mom, who lived them.”

“To my parents, who planted the seed of knowledge in my mind and nurtured it.”

“For Mom and Dad, who always reminded me that words have the power to change the world.”

“To my parents, who gave me the gift of dreams and the ability to realize them.”

To all my friends, thank you for being always there for me with all you love and support.

Acknowledgment

I would like to express my deep appreciation and indebtedness particularly to my supervisor

Dr.Ibtisam Kareem

for his support, kind efforts, time, advice and scientific opinions and I'm really proud to be one of his students To all friends and others who in one way or another shared their support , thank you GOD bless all.

Certification

I certify that the preparation of this project entitled:

Relationship between salivary alpha amylase activity anthropomorphic indices dietary habits and early childhood dental caries

Prepared by Fifth-Stage Student:

Zainab hasanin Rheem

Teba kamal abd alzhara

Was made under my supervision at Dentistry Department in partial fulfillment of the Requirements forthe Degree of Bachelor of Science in dentistry.

Supervisor Name:

Date:

Abstract

The most common risk factor is the frequent and prolonged consumption of sugary drinks and snacks, especially when proper oral hygiene practices are not observed.

So because of the bad nutrition of children today and the prevalence of obesity between them the caries absolutely prevalence as black caries .

One answer for the question that why some children develop Early childhood caries while others do not, could be related to the differences between the quantity or quality of salivary components from those children Salivary alpha- amylase is one of the most important components of the oral fluid, which exhibits various biological functions, related to its high affinity for binding to oral streptococci and carbohydrate digestion

On the other hand, the physical features and chemical structure of saliva are reported to have imperative protective mechanisms against dental caries, so that some salivary proteins can act as key biomarkers for dental caries. The most abundant enzyme in human saliva is the salivary alpha-amylase (sAA), which hydrolyzes the starch to glucose and maltose and helps food digestion.

This salivary enzyme of the acquired enamel pellicle regulates bacterial colonization and adds glucose for biofilm formation.

On the other hand, this protein binds to the membrane of cariogenic bacteria and facilitates their elimination from the oral cavity through the salivary clearance, and consequently reduces the risk of dental caries.

List of Contents

List of Figure

List of abbreviations

abbreviations	Meaning
SAA	salivary alpha_amylase
ECC	early childhood caries
BMI	body mass index
SAM	sympathetic-adrenal-medullary
NHAVES	National Health and Nutrition Examination Survey
FITS	Feeding Infants and Toddlers Study
CCHS	Canadian Community Health Survey
HDI	Human Development Index
BS	black stain
FOTI	Fiber-Optic Transillumination

1- Introduction

Predicting caries incidence in young children is becoming increasingly important due to the health care costs and resources constraints. According to the American Academy of Pediatric Dentistry, early childhood caries (ECC) is an important chronic disease, which is progressively caused by the imbalance of various risk and protective factors. This oral health problem is more prevalent among socially deprived communities.
It includes the presence of one or more decayed (non-cavitated or cavitated lesions), missing (due to caries), or filled tooth surfaces of any primary tooth in children ≤71 months. The general etiology of ECC is the same as general dental caries (microbiological, dietetic, and environmental risk factors); however, the predisposing factors are poorly identified.

Severe ECC is said to be the result of a combination of parents' socioeconomic, psychological, and behavioral features. Its high prevalence is chiefly due to incorrect feeding practices, socioeconomic status of the family, parental illiteracy or inadequate education, and inaccessibility of dental care. However, it can be prevented through establishing healthy eating habits, efficient brushing and flossing, receiving regular preventive dental services, and community-based educational programs.

Recently, attentions have been attracted to the possible association between the body composition and dental caries in children. Evidence shows that obesity and dental caries hold the two risk factors of dietary features and socioeconomic status in common. However, contrasting reports exist regarding the association between dental caries and body mass index (BMI) as the measurement of body fatness, particularly in younger children.

On the other hand, the physical features and chemical structure of saliva are reported to have imperative protective mechanisms against dental caries, so that some salivary proteins can act as key biomarkers for dental caries. The most abundant enzyme in human saliva is the salivary alpha-amylase (sAA), which hydrolyzes the starch to glucose and maltose and helps food digestion.

This salivary enzyme of the acquired enamel pellicle regulates bacterial colonization and adds glucose for biofilm formation.

On the other hand, this protein binds to the membrane of cariogenic bacteria and facilitates their elimination from the oral cavity through the salivary clearance, and consequently reduces the risk of dental caries.

Considering the fact that the amylase gene (*AMY1*) shows extensive copy number variations which are directly proportional to the salivary α-amylase content and activity in saliva, evaluation of this enzyme could be a helpful method for determining individuals with high risk of dental caries. Confirming this issue, an association has been reported between some amylase gene (*AMY1*) copy number variations and high occurrence of smooth-surface caries.

Although, high sAA concentration in saliva was found to be positively related with more dental caries incidence in some studies, controversies exist regarding the relationship between the concentration, function, or activity of this protein in saliva in this regard. **(Figure 1-1)**

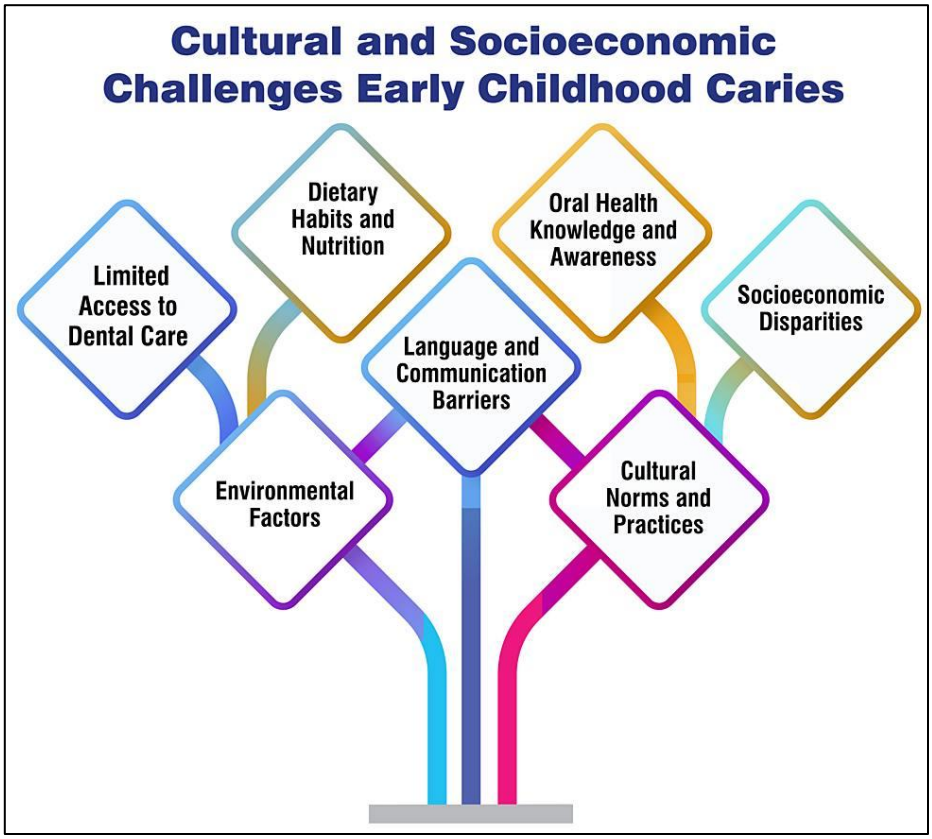

Figure 1-1 | Cultural and socioeconomic challenges early childhood caries

2. Overview of Salivary Alpha Amylase:

Salivary alpha amylase (sAA) is an enzyme produced by the salivary glands, playing a crucial role in the initial breakdown of starches in the mouth. This digestive enzyme is vital in the early stages of food digestion, facilitating the conversion of complex carbohydrates into simpler sugars [1].

Understanding the activity of sAA provides valuable insights into the body's physiological response to stress, food consumption, and overall metabolic health. The regulation and measurement of sAA activity have garnered significant interest in various fields, including psychology, physiology, and nutrition [2].

Salivary alpha amylase, also known as ptyalin, is an enzyme produced by the salivary glands and is fundamental to the initial digestion of starch. Functioning in the oral cavity, this enzyme begins the hydrolysis of dietary starch into maltose and dextrin, aiding in the process of carbohydrate metabolism. Its presence in saliva underscores its significant role in the breakdown of complex carbohydrates, thereby facilitating the absorption and utilization of nutrients within the body [3].

The secretion of salivary alpha amylase serves as an essential component of the digestive system, contributing to the pre-digestion of ingested starches prior to reaching the stomach. Moreover, recent research has highlighted its potential implications in modulating oral microbiota, impacting oral health beyond its traditional role in starch digestion. Additionally, salivary alpha amylase has been linked to the early stages of taste perception, demonstrating its intricate involvement in not just digestion but also sensory experiences.[4]

Furthermore, studies have indicated that salivary alpha amylase levels may be influenced by various physiological and psychological factors, pointing towards its potential involvement in stress responses and as a biomarker for certain health conditions. The multifaceted function of salivary alpha amylase positions it as a key player in the intricate processes of digestion, oral health, and potentially beyond.[5] **(Figure 2-1)**

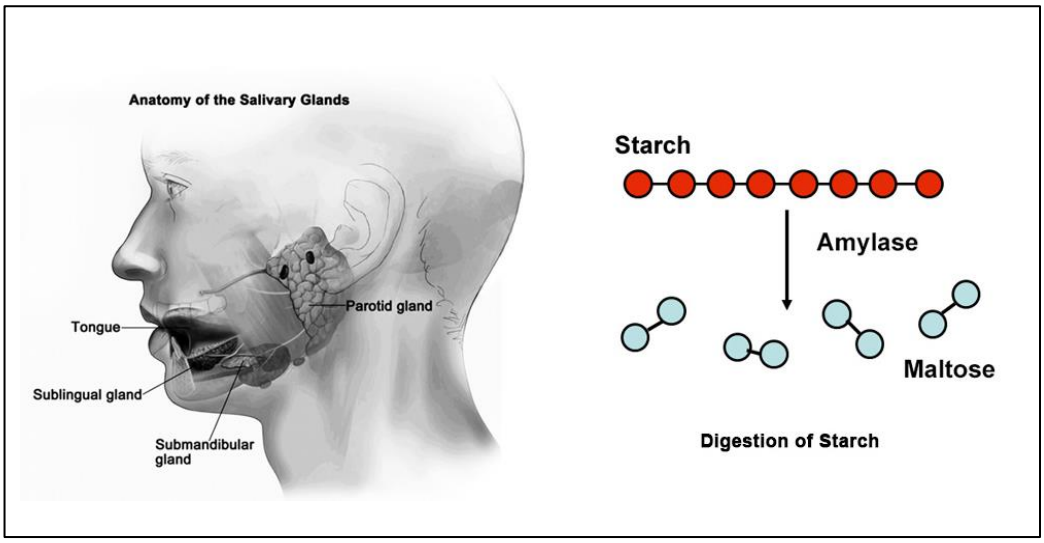

Figure 2-1| Activity of salivary amlyase

2.1 Factors influencing salivary alpha amylase activity:

There are Some factors effect of alpha amylase activity [6]:

Stress: Salivary alpha-amylase (sAA) has been suggested to reflect stress-related body changes. Psychosocial stress increases the release of salivary alpha-amylase, which reflects the activity of the sympathetic-adrenal-medullary (SAM) system. Therefore, it is presumed that sAA measurement is a useful tool for evaluating the SAM system. In addition, previous studies examining the response of sAA levels to SAM system activity showed that increased sAA levels were correlated with increased plasma catecholamine, indicating sympathetic nervous system activation. So far, numerous studies have shown that changes in sAA levels are dependent on

stress stimuli. It is difficult to objectively evaluate the emotional and physical state. sAA measurement can be performed easily and quickly, and therefore, could be used to aid the evaluation of the psychosocial and/or physical stress levels. It is very important to be able to evaluate and understand the level of psychosocial and/or physical stress (distress) experienced by patients. The measurement of sAA is expected to be useful tool as a patient distress [7]

Diet: Food intake regulation in humans involves various central and peripheral mechanisms. In this study salivary a-amylase was examined for functioning as a measure of satiety and food intake. In a 1.25-h session, 32 fasted subjects were given a preload of starch-based custard (849 kJ) followed by ad libitum intake of this custard.Before and after preload intake and after ad libitum consumption, both satiety ratings and a-amylase were analysed. Perceived satiety and a-amylase were increased after preload and ad libitum consumption. Across subjects, the individual amount of ad libitum intake was negatively correlated to a-amylase levels before this intake, whereas it was positively associated with a-amylase activity after ad libitum consumption. In conclusion, salivary a-amylase systematically increases upon food consumption and satiation, and serves therefore as a potential measure of satiety and subsequent food intake [8].

Time of day: Salivary alpha amylase activity can vary throughout the day, with higher levels typically observed in the morning compared to later in the day. Salivary alpha- amylase activity shows a distinct diurnal profile pattern with a pronounced decrease within 60 min after awakening and a steady increase of activity during the course of the day [9].

Health conditions: Various health conditions, including periodontal disease and diabetes, can impact salivary alpha amylase activity levels.

2.2 Measurement methods for salivary alpha amylase activity

2.2.1 Enzyme Activity Assays

Enzyme activity assays are the primary method for measuring salivary alpha amylase activity. These assays typically involve monitoring the breakdown of a starch substrate by the enzyme. One common approach is to use an iodine based colorimetric assay, where the disappearance of a blue color indicates the enzymatic activity. Another method involves the use of chromogenic substrates that generate a colored product when hydrolyzed by the enzyme. These assays provide quantitative measurements of enzyme activity in the saliva [10].

2.3 Normal range of salivary alpha amylase activity

Salivary alpha amylase activity can vary widely among individuals, but a typical normal range is between 20 to 160 units per milliliter of saliva. This range can be influenced by factors such as age, stress levels, and overall health. It's important to note that individual variations and specific measurement techniques may lead to slight differences in the normal range. Overall, a level within this range is considered typical for healthy individuals.[11]

Measuring salivary alpha amylase activity within this range can provide valuable insights into an individual's stress response, overall health, and potential underlying conditions. It serves as an essential biomarker, offering significant implications for research and clinical applications, especially in the context of stress- related studies and health assessments [12].

2.4 Clinical significance of salivary alpha amylase activity

Salivary alpha amylase activity has shown significant promise as a non-invasive biomarker in various clinical scenarios[13]. Its measurement has been linked to several health conditions, including cardiovascular diseases, diabetes, and periodontal diseases. Furthermore, research has indicated its potential in assessing the physiological stress response and its impact on an individual's overall health, [14 -17].

In recent years, a new biomarker for assessing the activity of the sympathetic nervous system (SNS) has been proposed, the activity of salivary α- amylase (sAA) [18,19]. Saliva as a medium is especially attractive, as its sampling is non-invasive, and can be performed by the individual without special training [20]. Alpha-amylase is an abundant protein in human saliva. It is actively secreted by acinar cells of the salivary glands, which are innervated by nerves of both the sympathetic as well as the parasympathetic ganglion, which trigger secretion of salivary fluid and proteins via the principle neurotransmitters acetylcholine and noradrenaline, and hence the use of sAA as an exclusive read-out of sympathetic activity is strongly controversial [21,22] However it could be shown that sAA activity is increased during stress, both physical and psychological [18,19,23], that it correlates with norepinephrine release [24] and that β-adrenoceptor antagonists (BBs) have a reducing effect on sAA activity levels [25,26]. Recent studies have also shown a tendency of higher total output of sAA in hypertensive humans, compared to normotensive ones [27].

Type II diabetes is reported in about 90%–95% of individuals living with diabetes. It is due to a chronic resistance to insulin action in target cells and further relative insulin deficiency leading to hyperglycemia. It is associated with various complications and mainly affects the heart, kidneys, eyes and nerves. It also causes impairment of salivary gland function and thus altering the oral cavity homeostasis. This leads to a decrease in the pH of saliva and xerostomia leading to complications such as caries, gingivitis and periodontitis.

Individuals with any type of diabetes are at an increased risk for developing serious health complications that can affect the heart, blood vessels, eyes, kidneys and nerves. They are also at a higher risk of developing infections, which significantly shortens life expectancy [28]. Furthermore, there exists a considerable heterogeneity within the diabetic population with regard to the development and progression of such complications.[29]

Besides these multi-organ damages, it also impairs salivary functions significantly resulting in qualitative and quantitative changes in salivary composition and flow. This deteriorates the homeostasis of the oral cavity, making it vulnerable to various oral ailments. Studies on various salivary metabolites have been being carried for the diagnosis and prognosis of diabetes.

Studies have demonstrated correlations between salivary alpha amylase levels and the severity of certain diseases, making it a valuable tool for early detection and monitoring of patients. Its role in the clinical setting extends to understanding the body's response to acute and chronic stress, potentially aiding in the management of stress-related disorders [30].

Moreover, the clinical significance of salivary alpha amylase activity extends to its potential as a prognostic indicator in various conditions, thus offering insights into disease progression and treatment outcomes[31,32]. Its non-invasive nature and ease of collection make it an attractive option for clinical assessments and research studies[33,34].

Traditionally, the level of serum amylase has been commonly measured to determine the presence of acute pancreatitis and biliary tract disease in primary clinical settings [35,36]. In contrast, most physicians seldom measure it to determine the degree of advanced chronic pancreatitis, which eventually results in secondary diabetes concomitant with weight loss, lipid diarrhea, and malnutrition[37,38].

The former condition predisposes to higher serum amylase (but not necessarily), whereas the latter condition lowers serum amylase [39].

In the past decade, low serum amylase has been observed in more common conditions (major contribution) than was previously believed. In this review, a novel interpretation for low serum, salivary, and pancreatic amylase is discussed, particularly in terms of the cardiometabolic conditions of obesity, diabetes, and metabolic syndrome (MetS) [40].

3. Childhood anthropomorphic indices:

The field of anthropometry encompasses a variety of human body measurements, such as weight, height, and size, including skinfold thicknesses, circumferences, lengths, and breadths [41] . Anthropometry is a key component of nutritional status assessment in children and adults. Anthropometric data for children reflect general health status, dietary adequacy, and growth and development over time [42]. In adults, body measurement data are used to evaluate health and dietary status, disease risk, and body composition changes that occur over the adult lifespan [43]. This information obtained from and depend on National Health and Nutrition Examination Survey (NHANES), a complex, stratified, and multistage probability sample of the civilian noninstitutionalized U.S. population [44].

3.1 Anthropometry Measures:

Although portions of the health examination component have varied during the survey's history, much of the anthropometry component methodology has remained consistent over time. The NHANES Anthropometry Procedures Manual describes the protocol, equipment, quality control, and measurement procedures in detail. Weight measured to the nearest 0.1 kilogram. Stature, length, and circumference measurements made to the nearest 0.1 centimeter. Skinfold thickness measures made to the nearest 0.1 millimeter.

Weight measure using a digital floor scale, and an infantometer used to measure recumbent length on infants and young children. Standing height measured with a wall-mounted stadiometer. BMI calculated as weight in kilograms, divided by height in meters squared (kg/m2). Head circumference measurements made using a plastic head circumference measurement tape.

Upper arm length measured with a tape measure from the posterior border of the acromion process to the tip of the olecranon process. The midarm circumference measured with a tape measure. Triceps and subscapular skinfolds measured with a skinfold caliper. Waist circumference measured with a tape measure at the uppermost lateral border of the hip crest (ilium).

The other body measurement data were recorded using computer- assisted data entry [45]. **(Figure 3-1)**

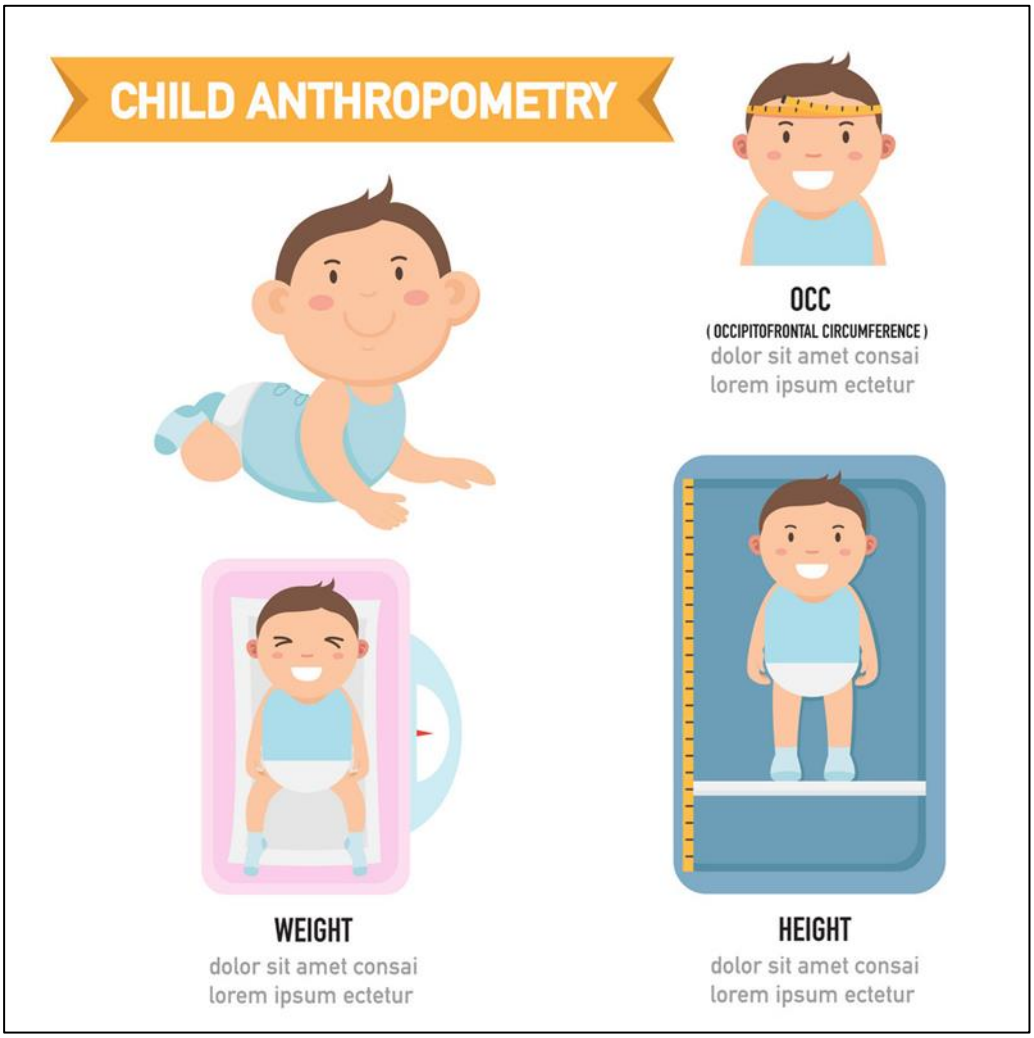

Figure 3-1 | Measurements of child anthropometry

4. Dietary habits in childhood:

Eating behaviours evolve during the first years of life; children learn what, when, and how much to eat through direct experiences with food and by observing the eating behaviours of others. Across human history, undernutrition and food scarcity have been major threats to children's survival, and parental feeding practices have evolved in response to these threats. These feeding practices, which include behaviours such as providing large portions of palatable foods and encouraging children to eat, are still pervasive in most cultures, despite the fact that in many regions the balance has shifted from food scarcity to food excess and over-consumption has become a new threat.

The impact of these ongoing practices on children's dietary intake can be seen in several recent dietary surveys [46].

The Feeding Infants and Toddlers Study (FITS), which provided data on the dietary patterns of 3022 infants and toddlers, revealed that 4 to 24 month old children typically consumed significant amounts of developmentally inappropriate, energy-dense, nutrient poor foods. Of particular concern was the finding that 18% to 33% of infants and toddlers consumed no distinct servings of vegetables on a typical day and when vegetables were consumed the most common choice was french fries. Additionally, reported energy intakes exceeded requirements by 10 to 30%. Unfortunately, there is also evidence that these patterns tend to persist throughout childhood and into adolescence [47], and that diet quality tracks and declines from early childhood through adolescence.

The Canadian Community Health Survey (CCHS) suggests that seven out of ten children aged 4 to 8 years fail to meet the minimum number of servings for vegetables and fruit in Canada's Food Guide to Healthy Eating. These children also fall short of reaching the recommended servings for grains and milk products, thereby suggesting that poor eating habits among children are endemic [48].

The transition into late childhood and adolescence can also be characterized by undesirable changes in eating behaviors such as increased consumption of sugar sweetened beverages (e.g., soda), calorie-dense, nutrient poor snacks and food away from home (e.g., fast food) and a decline in the consumption of milk and other nutrient-dense foods. Meal patterns also tend to change, as teenagers are more likely to skip breakfast and less likely to participate in family dinners. All of these trends

are associated with decreased diet quality and may partially explain the fact that most adolescents are failing to meet the majority of dietary recommendations [49]. **(Figure 4-1)**

Figure 4-1| Healthy and un healthy food

4.1 Influences during Infancy:

The first year of life is a period of rapid physical, social and emotional growth, during which eating patterns also develop. During this first year, infants transition from consuming a single food (i.e., breast milk or formula) to consuming a variety of foods more characteristic of an adult diet. This transition allows infants to learn about food through direct experience, as well as through observation of others' eating behaviours.

Data indicate that breastfeeding and parental modeling in the toddler years play significant roles in establishing longer-term eating behaviours. As reviewed below, children who are not breastfed still derive a significant benefit from the behaviours that their parents impart as they grow and develop.

Breastfeeding is recommended as the optimal feeding method for the first 6 months of life, in part because of the mounting evidence that breastfeeding has a positive impact on the development of a child's later eating behaviours.

Breastfeeding plays a role in the development of a child's response to internal hunger and satiety cues [50], and may foster the development of self- regulatory abilities during feeding. Variations in the composition of breast milk during a single feed, as well as differences in composition across the first months of life, foster this heightened sensitivity to energy intake. Emerging scientific evidence also supports the role of breastfeeding in early metabolic imprinting, which partially accounts for later differences in eating behaviours[51].

Breastfeeding also has a positive impact on later eating behaviour because it may promote acceptance of flavours in the maternal diet that are passed through breast milk. As a result, breastfed infants are exposed to a more varied flavour experience, depending on the variety of the mother's diet and this exposure may affect food acceptance during the transition to solid foods and later in life[52]..

Infants are born with a preference for sweet and salty taste, thus sweet and salty foods have a greater likelihood of being accepted by infants when compared to foods with bitter flavours, such as certain vegetables. Both infants and young children can learn to accept a greater variety of foods and flavours through repeated exposureThus, breastfeeding gives the infant early, repeated exposure to the flavours of the mother's diet, providing a flavour bridge that promotes the infant's acceptance of familiar flavours when they appear in solid foods. As a result, breastfed infants may be more accepting of new foods and likely to consume a more varied diet later in life, depending on the variety of the mother's diet during breastfeeding [53].

5. Overview about Early Childhood Caries:

Early childhood caries, commonly known as baby bottle tooth decay or nursing caries, refers to the presence of one or more decayed, missing, or filled tooth surfaces in any primary tooth in a child under the age of six. This widespread dental condition is a significant public health concern, affecting young children around the world, particularly those from disadvantaged backgrounds [54].

It is important to address this issue early on, as untreated early childhood caries can lead to pain, infections, and difficulty eating, which can negatively impact a child's overall health and well-being. Prevention and early intervention are crucial in combating this condition and promoting the oral health of young children [55].

5.1 Prevalence and Incidence Rates

- **Global Prevalence:** In various regions around the world, early childhood caries affects a significant portion of preschool-age children, with prevalence rates ranging from 20% to 90%. This wide range is influenced by numerous factors such as socioeconomic status, access to dental care, and dietary habits.[56]
- **Incidence Rates:** The incidence of early childhood caries is particularly concerning, with studies showing an increasing trend in certain populations. This rise in incidence is attributed to factors like high sugar consumption, lack of adequate oral hygiene practices, and limited availability of preventive dental services [57].
- **Regional Disparities:** Disparities in prevalence and incidence rates are evident across different geographic regions and communities. Underprivileged areas and marginalized populations often bear a disproportionate burden of early childhood caries, highlighting the importance of targeted interventions and public health initiatives [58].

5.2 Risk factors for early childhood caries

Several risk factors contribute to the development of early childhood caries. The most common risk factor is the frequent and prolonged consumption of sugary drinks and snacks, especially when proper oral hygiene practices are not observed.

Additionally, inadequate exposure to fluoride, whether through water, toothpaste, or other sources, can increase the risk of caries [59].

Furthermore, socioeconomic factors such as low family income, limited access to dental care, and lack of parental education on oral health also play a significant role.

Poor oral hygiene habits, including infrequent brushing and flossing, as well as the presence of cariogenic bacteria in the oral cavity, can exacerbate the risk of early childhood caries [60].

In addition to these primary risk factors, genetic predisposition, developmental disabilities, and certain medical conditions can also heighten the susceptibility to early childhood caries. Understanding and addressing these risk factors is crucial in formulating effective preventive and treatment strategies [61]. **(Figure 5-1)**

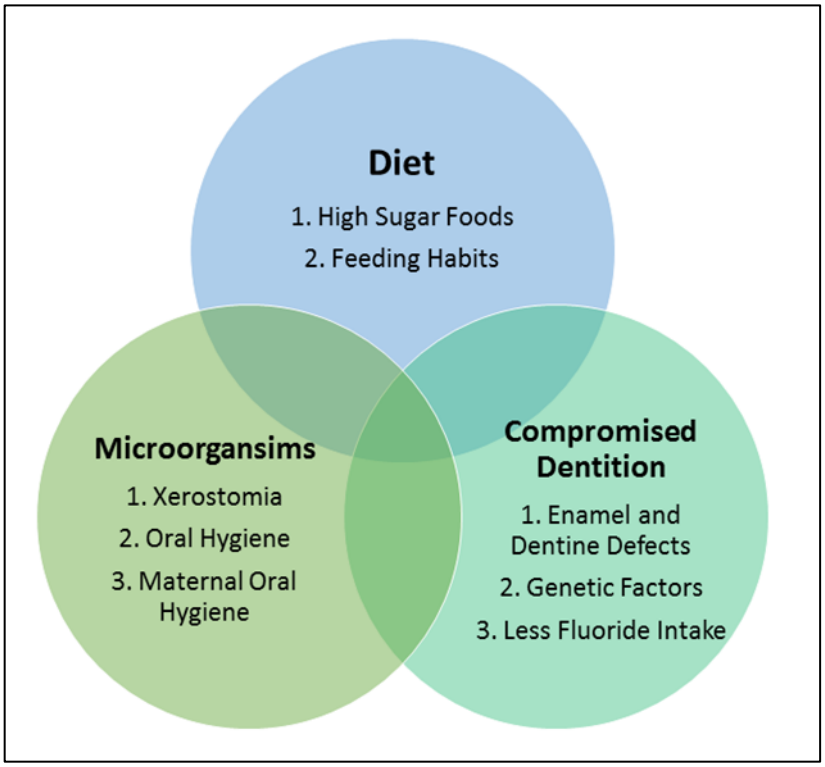

Figure 5-1 | Risk factors for early childhood caries

5.3 Signs and symptoms

- **Toothache**: Dental pain is a relatively common condition in children and impacts the lives of not only children but also their families [62]. Although dentistry has advanced in recent years, reports of toothache are still very frequent, and toothaches lead to suffering, illness, discomfort while eating, discomfort while brushing teeth, sleep disturbance, school absenteeism, and impaired social interactions [63]. Therefore, it is important to assess dental pain due to its impact on children and their family's lives [64]. In addition to its impact on children and their family, dental pain is a common complaint in oral health services [65]. The prevalence of toothache in 5-year-old children ranges from 7- 25% [66]. At the national level, the prevalence of dental pain in preschoolers varies between 7.2% and 22% [67]. Identifying pain in children is challenging because children of preschool age have difficulties in expressing their feelings clearly due to their restricted communication skills. Thus, it is possible for a child to experience toothache at a certain moment and not seek the appropriate treatment, leading to the worsening of this

condition [68]. Some factors have been shown to be associated with dental pain in children aged up to 5 years, such as sociodemographic factors, the Human Development Index (HDI), family income, number of residents per room, parent's educational level, not having a father or mother, age, ethnicity, use of dental services, and clinical factors such as a history of dental caries, oral conditions and the physiological mobility of primary teeth in the exfoliation process [69].

It has been reported that dental caries are a major cause of toothache. A global study found that 573 million children have untreated caries in primary teeth [70].

- **Stained or discolored teeth**: The causes of tooth discoloration are classified according to the location of the stain and are divided into extrinsic, intrinsic, or internalized. Extrinsic discoloration is deposited on the tooth surface or in the acquired pellicle. The compounds that are incorporated into the pellicle produce a stain due to either their basic color or chemical interaction at the tooth surface. Intrinsic stains occur when the tooth structure is penetrated by pigmented materials, usually during tooth development. Internalized discoloration is the incorporation of extrinsic stain within the tooth substance following dental development [71]. A specific type of external discoloration is called black stain (BS). It is characterized as a dark line or an incomplete coalescence of dark dots formed on the cervical third of the tooth and following the contour of the gingival margin, firmly attached to the tooth surface. BS is a common finding in children; however it can be also seen in adults [72]. Studies have shown equal prevalence in both sexes [73]. The characterization of factors contributing to the formation of BS and its nature has become of interest since association between the presence of BS and lower caries experience in children was noted. BS is considered to be a special form of dental plaque with a tendency for calcification [74]. The ultrastructural

examination of this deposit revealed microorganisms embedded in matrix. Almost all of bacteria were Gram-positive rods [75]. The microbiological composition of the BS is thought to be dominated by Actinomycetes [76]. Recent PCR analysis of plaque samples of children with BS showed significantly higher number of Actinomyces naeslundii and lower number of Lactobacillus spp. than in nondiscolored plaque samples [77]. The pigment is suggested to be a black insoluble ferric compound, probably ferric sulfide formed by the interaction between hydrogen sulfide produced by bacteria and iron. The studies on the composition of BS disclosed higher content of calcium and phosphate than in nondiscolored plaque. Spatial chemical analysis using wavelength dispersive spectrometry showed corresponding areas of high concentration of sulfur and copper/iron. This may suggest that sulfur and metal ions form intensely colored compounds [78]. Little data is available on chemical composition of saliva in subjects with BS. However, higher levels of salivary buffering capacity, higher pH, and increased concentrations of calcium and phosphate were reported [79].

The prevalence of BS varies between 2.4% and 18% because of unspecified criteria used for diagnosis and different populations included in the studies. Most of the authors showed that the presence of BS is associated with lower caries experience. The causative factors of BS are not fully understood. Certain types of bacteria seem to be involved in the etiology. It is not clear how the presence of BS on the tooth surface reduces susceptibility to caries. The dominant occurrence on smooth surfaces was not associated with lower caries experience on these surfaces which implies that caries resistance in children with BS is a result of a general lower caries activity rather than a localized effect [80,81].

- **Increased tooth sensitivity:** Affected children may experience heightened sensitivity in their teeth, particularly to hot or cold temperatures.

5.4 Stages of Dental Caries Development

1- Initial Demineralization

The first stage of dental caries development is the demineralization of the tooth enamel. This occurs when acids produced by bacteria in plaque attack the hard outer layer of the tooth, causing it to lose minerals. The initial demineralization is often asymptomatic and may not be visibly apparent during a dental examination. However, it is a crucial early stage that can be halted or reversed with proper oral hygiene and fluoride treatments [82].

2- White Spot Lesions

As the demineralization progresses, it may manifest as white or chalky spots on the surface of the tooth. These white spot lesions indicate the early formation of a cavity and are generally reversible with the implementation of fluoride therapy and improved oral care. It is essential to address white spot lesions promptly to prevent further advancement of the caries process [83].

3- Cavitation and Dentin Involvement

If left untreated, the demineralization process advances, and the enamel begins to break down, forming a cavity. At this stage, the decay extends into the dentin, the softer tissue beneath the enamel. The cavity may become noticeable as a dark spot on the surface of the tooth, and patients may experience tooth sensitivity to hot, cold, or sweet stimuli. Cavitation and dentin involvement indicate the progression of dental caries to a more advanced stage, requiring professional intervention such as dental fillings or other restorative treatments [84]. **(Figure 5-2)**

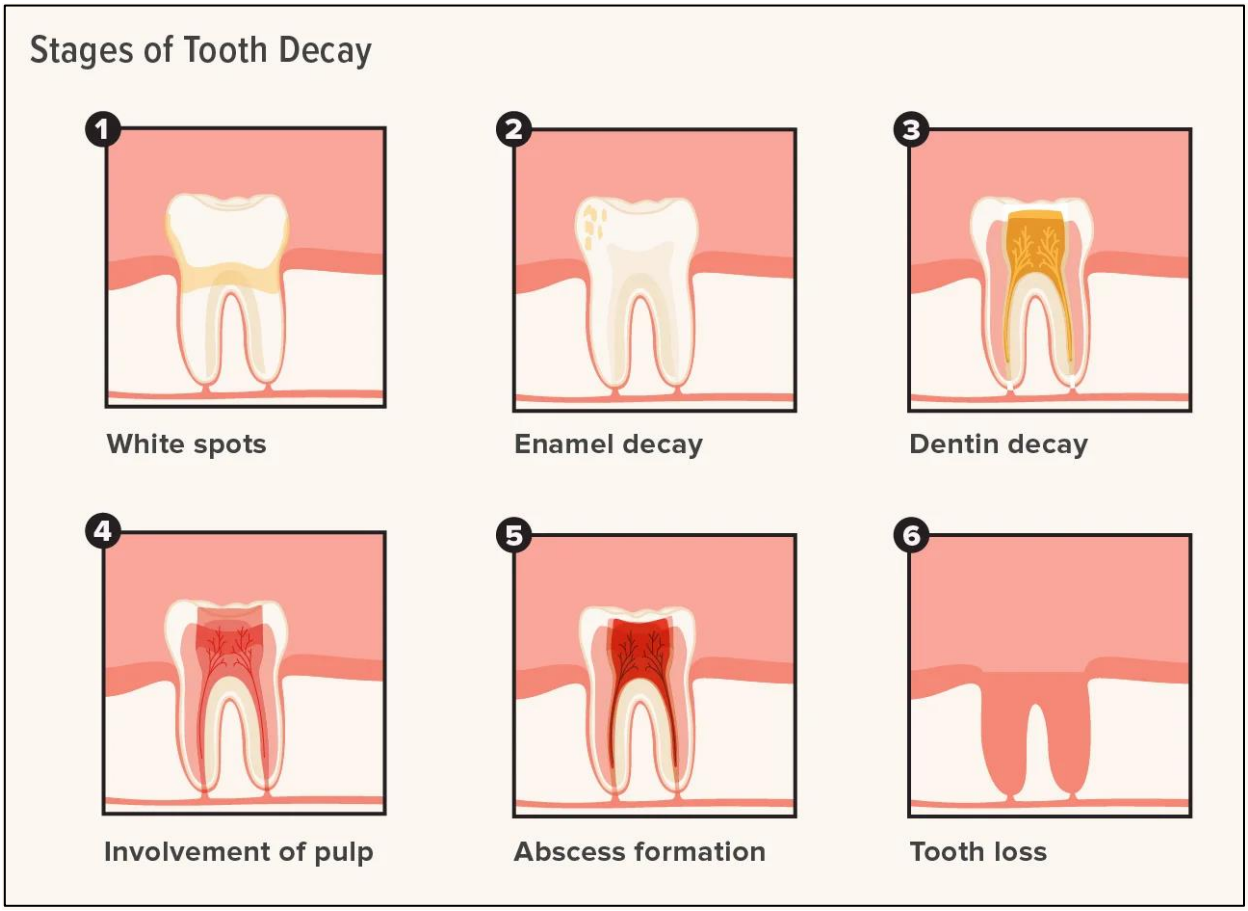

Figure 5-2 | Stages of tooth decay

5.5 Complications of Untreated Early Childhood Caries

- Apical periodontitis
- Periapical abscess
- Periapical granuloma
- Periapical cyst
- Cellulitis
- Abscess
- Periostitis
- Osteomyelitis

Dental caries are not life-threatening, but if the infection spreads through facial planes, patients are at increased risk of sepsis, airway compromise (Ludwig angina), and odontogenic infections, which accounted for 49.1% of cases in a study of deep neck abscesses [85].

Untreated early childhood caries can lead to severe complications, impacting the overall health and well-being of the affected children. The prolonged presence of decay-causing bacteria in the mouth can result in dental abscesses, which are painful and may require invasive procedures for treatment. Furthermore, the decay can spread to the surrounding teeth, leading to additional cavities and dental infections [86].

In some cases, untreated early childhood caries can lead to malnutrition and weight loss, as the discomfort associated with eating can deter children from consuming essential nutrients. Additionally, the pain and discomfort from tooth decay can negatively impact a child's ability to concentrate and perform well in school, affecting their overall academic and social development [87] . **(Figure 5-3)**

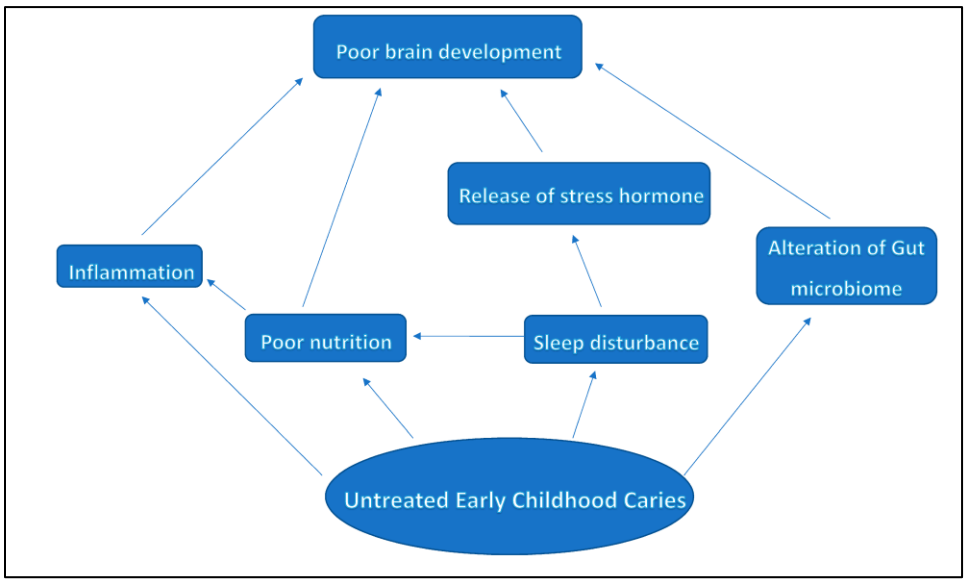

Figure 5-3 |Complications of untreated ECC

5.6 Diagnosis of early childhood caries:

- **Visual Inspection: teeth.**
- **X-rays and Imaging.**
- **Caries Risk Assessment.**
- **Advanced methods:** There were new methods as Laser fluorescence (DIAGNOdent), FOTI (Fiber-Optic Transillumination), Electronic caries monitoring can be used for early caries detection**.**

5.7 Prevention strategies:

❖ Oral Hygiene Practices

Teaching proper oral hygiene practices from an early age is crucial in preventing early childhood caries. This includes regular brushing with fluoridated toothpaste, flossing, and regular dental check-ups.

❖ Dietary Modifications

Limiting sugary snacks and drinks, especially before bedtime, can significantly reduce the risk of developing early childhood caries. Encouraging a diet rich in fruits, vegetables, and dairy products can promote oral health.

❖ Fluoride Supplementation

Fluoride supplementation, as recommended by a pediatric dentist, can help strengthen tooth enamel and prevent tooth decay in young children. This can be in the form of fluoride varnish or supplements.

❖ Sealant Application

Applying dental sealants to the back teeth can provide an added layer of protection against cavities, especially for children at higher risk of developing early childhood caries[88]. **(Figure 5-4)**

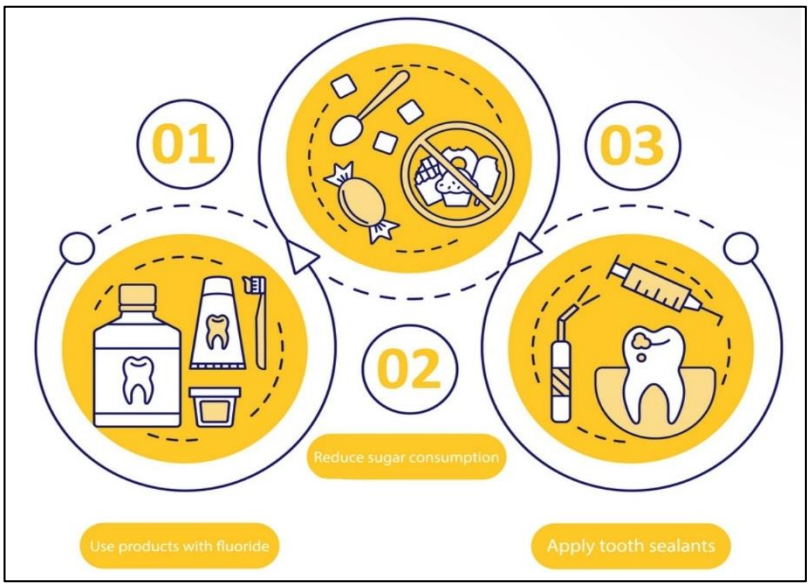

Figure 5-4| Prevention strategies

5.8 Importance of Oral Hygiene in Preventing Early Childhood Caries

Preventing early childhood caries is closely linked to maintaining good oralhygiene habits from a young age. This includes regular brushing with an age- appropriate fluoride toothpaste, flossing, and routine dental check-ups. By instilling these habits early on, parents and caregivers can help reduce the risk of tooth decay in young children. Establishing a consistent oral hygiene routine also promotes overall oral health and sets the foundation for a lifetime of healthy smiles.

Furthermore, emphasizing the importance of a balanced diet and limiting sugary and acidic foods and beverages contributes to the prevention of early childhood caries. Encouraging children to drink water and consume nutrient-rich foods can minimize the detrimental effects of plaque and acid on their teeth, ultimately preventing tooth decay.

Educating parents, caregivers, and young children about the significance of oral hygiene practices not only prevents early childhood caries but also cultivates a positive attitude towards dental care. Creating a nurturing and supportive environment for oral health awareness sets the stage for long-term dental wellness and well-being in early childhood. It is essential to highlight the role of regular dental visits and professional cleanings in the prevention of early childhood caries. Dental professionals play a vital role in educating families about effective oral hygiene techniques and providing preventive treatments like fluoride varnishes and sealants, which further safeguard children's teeth from decay[89].

6. Relationship between salivary alpha amylase activity anthropomorphic indices, dietary habits and early childhood dental caries:

Early childhood caries is a multifactorial disease associated with multiple variables. One answer for the question that why some children develop Early childhood caries while others do not, could be related to the differences between the quantity or quality of salivary components from those children [90]. Salivary alpha- amylase is one of the most important components of the oral fluid, which exhibits various biological functions, related to its high affinity for binding to oral streptococci and carbohydrate digestion [91]

The present study tried to find if Early childhood caries is related with Salivary alpha- amylase enzyme activity, BMI, nutritional and oral hygiene related variables. The Salivary alpha-amylase activity was found to be significantly higher in caries-free children, indicating an inverse relationship between the Early childhood caries and Salivary alpha-amylase; which was consistent with Borghi et al. findings . Similarly, Scannapieco et al. [92] proposed that Salivary alpha-amylase could attach to cariogenic bacteria and facilitate their elimination from the oral cavity, and consequently reduce the incidence of Early childhood caries.

However, another study reported the mean amylase activity, total protein concentrations, and total IgM to be similar in caries-free and Early childhood caries groups. This could be due to their limited sample size (20 in each group) which precluded any significant difference [93].

Sitaru et al. [94] detected that caries-active children had higher levels of salivary enzyme activity compared with caries-free groups, and pronounced Salivary alpha-amylase as a predictive biomarker in preventive dentistry. Their study was conducted on age group of 10 to 14 years old with different criteria for determining caries in comparison with our study which was related to Early childhood caries.

Generally, diversity of the findings of the clinical studies in relation to salivary constituents are usually common due to the difficulties in standardizing sampling methods and laboratorial tests [95].

Controversies also exist about the concentration of this protein in saliva. Compared with caries-free controls, higher concentrations of Salivary alpha-amylase were detected not only in children with Early childhood caries, but also in caries-susceptible young adults, particularly overweight adolescent girls. Seemingly,

excessive amounts of Salivary alpha-amylase contribute to hydrolysis of starch and acid release by cariogenic bacteria, and thereby raise the risks of dental caries. In contrast, Mojarad et al. [96] concluded that Early childhood caries might also be developed in case of decreased Salivary alpha-amylase concentration. Such a controversy can be justified by the multicomponent nature of human saliva (water, several electrolytes, mucus, glycoproteins, enzymes, and antibacterial compounds), which incorporates a confounding effect that does not allow assessing the effect of a single component in such a media [97].

The function and activity of a specific component in saliva is not necessarily related to its concentration in this media. Furthermore, the concentrations of the salivary constituents are regulated by salivary flow, which is hard to be measured in young children. Thus, based on these controversial results, it would be relevant to investigate how activity of Salivary alpha-amylase behaves in saliva of children with Early childhood caries and if Salivary alpha-amylase can be considered as a predictor for Early Childhood Caries in preschoolers [98].

Carbohydrates and sugar are extensively proven as the chief dietary elements that account for the incidence of dental caries. Similarly, the current findings confirmed that dental caries are significantly associated with brushing and all dietary habits, particularly the amount and frequency of sugar consumption. The frequency of sugar intake (restricted to main meals or between meals) is reported to play an important role in both dental caries and childhood malnutrition. Moreover, improper feeding practices, lack of parental education, and poor oral hygiene are known to raise the risk of Early Childhood Caries. Oral health is imperative for children to maintain the oral functions such as eating and speech, as well as developing a positive self-image [99].

Pediatric growth disorders have always been a multidisciplinary clinical concern for the specialists, dentists included. Recently, more investigations have been focused on the metabolic effects of obesity on oral health like higher risks of caries and periodontal diseases . Although the relationship between Early Childhood Caries and BMI has been formerly evaluated, the mean BMI, BMI percentiles, or mean weight have been assessed in populations of different ages and mixed sexes [100]. However, in the present study, adjusting the z-scores for both age and sex by Health Watch Pro software yielded more logical report of means.

In line with some previous studies, the current findings revealed the dental caries to be more prevalent among underweight children; however, this was not statistically

significant. noted that the socioeconomic level affected the association between BMI and dental caries. Accordingly, overweight children of high socioeconomic families had less dental caries than the normal-weight children.

Conflicting results have been obtained regarding the relationship between dental caries and BMI in children . While some studies rejected any association between dental caries and obesity [, a systematic review provided evidence that dental caries is relatable to both low and high BMI [60]. Some studies noted the dental caries to be more frequent in obese children (BMI >30) than those with normal body weight (BMI <25).

It is recommended to emphasize the importance of oral hygiene provide nutritional counseling and take appropriate preventive measures for the children with nutritional imbalance leading to abnormal BMI. Both malnutrition and dental caries can have lifelong negative repercussions for children. An interdisciplinary approach between the pedodontists and primary health care providers or pediatricians can offer a good opportunity to prevent chronic oral diseases and treat these childhood diseases [100].

Conclusion

There are many factors related to the development or causing the dental caries in the children and the most common is the diet and sugar consumption and we can observe that by the anthropomorphic indices in particular the weight because it's some time related to the bad diet habits.

Another indicator for caries is the salivary alpha amylase, it's act as key biomarkers in preventive dentistry.

Salivary alpha- amylase could attach to cariogenic bacteria and facilitate their elimination from the oral cavity, and consequently reduce the incidence of Early childhood caries.

Recommendation

1.It is recommende provide nutritional

counseling and take appropriate preventive measures for the children with nutritional imbalance leading to abnormal BMI.

2. Commitment to good oral hygiene by brushing your teeth after every meal and using dental floss regularly

3. Visit the dentist periodically and regularly

4. Applying dental sealants to the back teeth .

5.use fluoride supplements.

References

1.Peyrot des Gachons, C., & Breslin, P. A. (2016). Salivary amylase: digestion and metabolic syndrome. Current diabetes reports, 16, 1-7.

2.Cozma, S., Dima-Cozma, L. C., Ghiciuc, C. M., Pasquali, V., Saponaro, A., & Patacchioli, F. R. (2017). Salivary cortisol and α-amylase: subclinical indicators of stress as cardiometabolic risk. Brazilian journal of medical and biological research,

3.Ahsan, H. (2015). Ptyalin or Human Salivary Amylase: What's in a Name? Journalof Medical Erudite, 3(2), 87-94.

4.Mosca, A. C., & Chen, J. (2017). Food-saliva interactions: Mechanisms and implications. Trends in Food Science & Technology, 66, 125-134.

5.Liao, C., Chen, X., & Fu, Y. (2023). Salivary analysis: An emerging paradigm for non-invasive healthcare diagnosis and monitoring. Interdisciplinary Medicine, e20230009.

6.Nater, U. M., Rohleder, N., Schlotz, W., Ehlert, U., & Kirschbaum, C. (2007). Determinants of the diurnal course of salivary alpha amylase. Psychoneuroendocrinology, 32(4), 392-401.

7.Grassi G, Esler M. How to assess sympathetic activity inhumans. J Hypertens. 1999;17(6):719–34.

8.Schwartz, M. W., Woods, S. C., Porte, D., Seeley, R. J., & Baskin, D. G. (2000). Central nervous system control of food intake. Nature, 404, 661–671.

9.Nater UM, Rohleder N, Schlotz W, Ehlert U, Kirschbaum C. Determinants of the diurnal course of salivary alpha-amylase. Psychoneuroendocrinology. 2007 May;32(4):392-401. doi: 10.1016/j.psyneuen.2007.02.007. Epub 2007 Apr 5. PMID: 17418498.42

10.McCleary BV. Measurement of Polysaccharide Degrading Enzymes Using Chromogenic and Colorimetric. Chemistry in Australia. 1991 Sep.

11.Rohleder, N., & Nater, U. M. (2009). Determinants of salivary α-amylase in humans and methodological considerations. Psychoneuroendocrinology, 34(4), 469-485.

12.Nagy, T., Van Lién, R., Willemsen, G., Proctor, G., Efting, M., Fülöp, M., ... & Bosch, J. A. (2015). A fluid response: Alpha-amylase reactions to acute laboratory stress are related to sample timing and saliva flow rate. Biological psychology, 109,111-119.

13.Ali, N., & Nater, U. M. (2020). Salivary alpha-amylase as a biomarker of stress in behavioral medicine. International journal of behavioral medicine, 27, 337-342.

14.Cohn JN, Levine TB, Olivari MT, Garberg V, Lura D, et al. (1984) Plasma norepinephrine as a guide to prognosis in patients with chronic congestive heart failure. N Engl J Med 311: 819-823.

15.Schrier RW, Abraham WT (1999) Hormones and hemodynamics in heart failure. N Engl J Med 341: 577-585.

16.lervasi G, Pingitore A (2009) Thyroid and heart failure from pathophysiology to clinics. Springer-Verlag Italia.

17.Clerico A, Recchia FA, Passino C, Emdin M (2006) Cardiac endocrine function is an essential component of the homeostatic regulation network: physiological and clinical implications. Am J Physiol Heart Circ Physiol 290: H17-29.

18.Nater UM, Rohleder N (2009) Salivary alpha-amylase as a non-invasive biomarker for the sympathetic nervous system: Current state of research Psychoneuroendocrinol 34: 486-496.

19.Granger DA, Kivlighan KT, El-Sheikh M, Gordis EB, Stroud LR (2007) Salivary43alpha-amylase in biobehavioral research: recent developments and applications. Ann NY Acad Sci 1098: 122-144.

20.Rohleder N, Nater UM (2009) Determinants of salivary a-amylase in humans and methodological considerations. Psychoneuroendocrinology 34: 469-485.

21.Proctor GB, Carpenter GH (2007) Regulation of salivary gland function by autonomic nerves. Auton Neurosci 133: 3-18.

22.Bosch JA, Veerman EC, de Gus EJ, Proctor GB (2011) a-Amylase as a reliable and convenient measure of sympathetic activity: don't start salivating just yet! Psychoneuroendocrinology 36: 449-453.

23.Ljungberg G, Ericson T, Ekblom B, Birkhed D (1997) Saliva and marathon running. Scand J Med Sci Sports 7: 214-219.

24.Chatterton RT, Vogelsong KM, Lu YC, Ellman AB, Hudgens GA (1996) Salivary alpha-amylase as a measure of endogenous adrenergic activity. Clin Physiol 16: 433-448.

25.Van Stegerena A, Rohleder N, Everaerd W, Wolf OT (2006) Salivary alpha amylase as marker for adrenergic activity during stress: Effect of betablockade. Psychoneuroendocrinology 31: 137-141.

26.Nederfors T, Dahlof C (1992) Effects of the B-adrenoceptor antagonists atenolol and propranolol on human whole saliva and composition. Arch Oral Biol 37:579- 584.

27.Strahler J, Kirschbaum C, Rohleder N (2011) Association of blood pressure and antihypertensive drugs with diurnal alpha-amylase activity. Int J Psychophysiol 81: 31-37.

28.Mrag M, Kassab A, Omezzine A, Belkacem CR, Ben FI, Douki N, et al. Saliva diagnostic utility in patients with type 2 diabetes: Future standard method. J Med44 Biochem 2020;39:140-8.

29.Panchbhai AS, Degwekar SS, Bhowte RR. Estimation of salivary glucose, salivary amylase, salivary total protein and salivary flow rate in diabetics in India. J Oral Sci 2010;52:359-68.

30.Kurdi, M. S., Ramaswamy, A. H., Kumar, L. A., Choukimath, S. M., & Jangi, A.A.(2021). Use of a non-invasive biomarker salivary alpha-amylase to assess the roleof probiotics in sleep regulation and stress attenuation in surgical patients: A randomised double-blind clinical trial. Indian Journal of Anaesthesia, 65(5), 390.

31.Kameya A, Hayakawa T, Noda A, Kondo T. Differential determination of serum isoamylase using an amylase inhibitor and its clinical application. Am J Gastroenterol. 1985;80:54–59.

32.Pieper-Bigelow C, Strocchi A, Levitt MD. Where does serum amylase come from and where does it go. Gastroenterol Clin North Am. 1990;19:793–810.

33.Worning H. Chronic pancreatitis: pathogenesis, natural history and conservative treatment. Clin Gastroenterol. 1984;13:871–894.

34.Waljee AK, Dimagno MJ, Wu BU, Schoenfeld PS, Conwell DL. Systematic review: pancreatic enzyme treatment of malabsorption associated with chronic pancreatitis. Aliment Pharmacol Ther. 2009;29:235–246.

35.Flood JG, Schuerch C, Dorazio RC, Bowers GN. Marked hyperamylasemia associated with carcinoma of the lung. Clin Chem. 1978;24:1207–1212.

36.Sosnoff DR, Friend RB, Berkovic M, Rasansky RJ, Hoffman SM. Salivary amylase-producingmultiple myeloma: case report and review of the current literature. J Clin Oncol. 2013;31:e309–e311

37.Domschke W, Tympner F, Domschke S, Demling L. Exocrine pancreatic function in juvenilediabetics. Am J Dig Dis. 1975;20:309–312.

38.Swislocki A, Noth R, Hallstone A, Kyger E, Triadafilopoulos G. Secretin- stimulated amylase release into blood is impaired in type 1 diabetes mellitus. Horm Metab Res. 2005;37:326–330.

39.Ogawa Z, Hasegawa A. Amylase. Rinsho Byori. 2001;Suppl 116:36–44.

40.Skrha J, Stěpán J. Clinical significance of amylase isoenzyme determination. Acta Univ Carol Med Monogr. 1987;120:1–81.

41.Liao, C., Chen, X., & Fu, Y. (2023). Salivary analysis: An emerging paradigm for non-invasive healthcare diagnosis and monitoring. Interdisciplinary Medicine, e20230009.

42.Vineetha, R., Pai, K. M., Vengal, M., Gopalakrishna, K., & Narayanakurup, D. (2014). Usefulness of salivary alpha amylase as a biomarker of chronic stress and stress related oral mucosal changes–a pilot study. Journal of clinical and experimentaldentistry, 6(2), e132.

43.Nater, U. M., La Marca, R., Florin, L., Moses, A., Langhans, W., Koller, M. M., &Ehlert, U. (2006). Stress-induced changes in human salivary alpha-amylase activity— associations with adrenergic activity. Psychoneuroendocrinology, 31(1), 49-58.

44.Casadei K, Kiel J. Anthropometric Measurement. 2022 Sep 26. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan–. PMID: 30726000.

45.Centers for Disease Control and Prevention. National Health and Nutrition Examination Survey (NHANES) anthropometry procedures manual. 2009. Available from: http://www.cdc.gov/nchs/data/nhanes/ nhanes_09_10/BodyMeasures_09.pdf.

46.Nielsen SJ, Popkin BM. Patterns and trends in food portion sizes, 1977-1998. Jama. 2001;289(4):450–453.

47.Fox MK, Pac S, Devaney B, Jankowski L. Feeding infants and toddlers study: What foods are infants and toddlers eating? J Am Diet Assoc. 2004 Jan;104(1 Suppl 1):s22-30. doi: 10.1016/j.jada.2003.10.026. PMID: 14702014.

48.Garnguet D. Statistics. Health Statistics Division; Canada: 2004. Nutrition Findings from the Canadian Community Health Survey Overview of Canadians' Eating Habits.

49.Fiorito LM, Mitchell DC, Smiciklas-Wright H, Birch LL. Dairy and dairy-related nutrient intake during middle childhood. J Am Diet Assoc. 2006;106(4):534–542.

50.Wright P. Hunger, satiety and feeding behavior in early infancy. In: Boakes R, Popplewell D, Burton M, editors. Eating habits: Food, physiology and learned behavior. John Wiley and Sons; New York: 1987. pp. 75–95.

51.Savino F, Costamagna M, Prino A, Oggero R, Silvestro L. Leptin levels in breast- fed and formula-fed infants. Acta Paediatr. 2002;91(9):897–902.

52.Birch LL, McPhee L, Shoba BC, Pirok E, Steinberg L. What kind of exposure reduces children's food neophobia? Looking vs. tasting Appetite. 1987;9(3):171– 178.

53.Galloway AT, Lee Y, Birch LL. Predictors and consequences of food neophobia and pickiness in young girls. J Am Diet Assoc. 2003;103(6):692–698.

54.Çolak, H., Dülgergil, Ç. T., Dalli, M., & Hamidi, M. M. (2013). Early childhood caries update: A review of causes, diagnoses, and treatments. Journal of natural science, biology, and medicine, 4(1), 29.

55.Fung, H. T. M., Wong, M. C., Lo, E. C., & Chu, C. H. (2013). Early childhood caries: a literature review. Oral Hygiene & Health.

56.Singh, S., Vijayakumar, N., Priyadarshini, H. R., & Shobha, M. (2012). Prevalenceof early childhood caries among 3-5 year old pre-schoolers in schools of Marathahalli,Bangalore. Dental research journal, 9(6), 710.

57.Anil, S., & Anand, P. S. (2017). Early childhood caries: prevalence, risk factors, and prevention. Frontiers in pediatrics, 5, 157.

58.Kanagaratnam, S., & Schluter, P. J. (2019). A review of early childhood caries, risk factors and preventive strategies. New Zealand Dental Journal, 115(4), 132- 50.

59.Hui Bin, S. U., Zhang, W., & Zhou, X. B. (2017). Risk factors associated with early childhood caries. Chin J Dent Res, 20(2), 97-104.

60.Kagihara, L. E., Niederhauser, V. P., & Stark, M. (2009). Assessment, management, and prevention of early childhood caries. Journal of the American Academy of Nurse Practitioners, 21(1), 1-10.

61.Losso, E. M., Tavares, M. C. R., da Silva, J. Y., & Urban, C. D. A. (2009). Severeearly childhood caries: an integral approach. Jornal de pediatria, 85, 295- 300.

62.Moure-Leite FR, Ramos-Jorge J, Ramos-Jorge ML, Paiva SM, Vale MP, Pordeus IA. Impact of dental pain on daily living of five-year-old Brazilian preschool children: prevalence and associated factors. Eur Arch Paediatr Dent. 2011;12:293–7.

63.Benoliel R, Svensson P, Evers S, Wang SJ, Barke A, Korwisi B. The IASP classification of chronic pain for ICD-11: chronic secondary headache or orofacial pain. Pain. 2019;160:60–8.

64.Freire MDCM, Corrêa-Faria P, Costa LR. Effect of dental pain and caries on the quality of life of Brazilian preschool children. Rev Saude Publica. 2018;52:01– 10.

65.Corrêa-Faria P, Daher A, Freire MDCM, de Abreu MHNG, Bönecker M, Costa LR. Impact of untreated dental caries severity on the quality of life of preschool children and their families: a cross-sectional study. Qual Life Res. 2018;27:3191– 8.

66.Qiu RM, Tao Y, Zhou Y, Zhi QH, Lin HC. The relationship between children's oral health-related behaviors and their caregiver's social support. BMC Oral Health. 2016;16:86.

67.Boeira GF, Correa MB, Peres KG, Peres MA, Santos IS, Matijasevich A. Caries is the main cause for dental pain in childhood: findings from a birth cohort. Caries Res. 2012;46:488–95.

68.Clementino MA, Gomes MC, Pinto-Sarmento TC, Martins CC, Granville-Garcia AF, Paiva SM. Perceived impact of dental pain on the quality of life of preschool children and their families. PLoS One. 2015;10:e0130602.

69.Boeira GF, Correa MB, Peres KG, Peres MA, Santos IS, Matijasevich A. Caries is the main cause for dental pain in childhood: findings from a birth cohort. Caries Res. 2012;46:488–95.

70.Kassebaum NJ, Smith AGC, Bernabé E, Fleming TD, Reynolds E, Vos T. Global, regional, and national prevalence, incidence, and disability-adjusted life years for oral conditions for 195 countries, 1990-2015: A systematic analysis for the global burden of diseases, injuries, and risk factors. J Dent Res. 2017;96:380– 7.

71.Watts A., Addy M. Tooth discolouration and staining: a review of the literature. British Dental Journal. 2001;190(6):309–316.

72.Reid J. S., Beeley J. A., MacDonald D. G. Investigations into black extrinsic tooth stain. Journal of Dental Research. 1977;56(8):895–899. doi: 10.1177/00220345770560081001.

73.Chen X., Zhan J. Y., Lu H. X., et al. Factors associated with black tooth stain in Chinese preschool children. Clinical Oral Investigations. 2014;18(9):2059–2066. doi: 10.1007/s00784-013-1184-z.

74.Reid J. S., Beeley J. A. Biochemical studies on the composition of gingival debris from children with black extrinsic tooth stain. Caries Research. 1976;10(5):363–369. doi: 10.1159/000260217.

75.Theilade J., Slots J., Fejerskov O. The ultrastructure of black stain on human primary teeth. Scandinavian Journal of Dental Research. 1973;81(7):528–532.

76.Slots J. The microflora of black stain on human primary teeth. Scandinavian Journal of Dental Research. 1974;82(7):484–490.

77.Heinrich-Weltzien R., Bartsch B., Eick S. Dental caries and microbiota in children with black stain and non-discoloured dental plaque. Caries Research. 2014;48(2):118–125.

78.Tantbirojn D., Douglas W. H., Ko C.-C., McSwiggen P. L. Spatial chemical analysis of dental stain using wavelength dispersive spectrometry. European Journal of Oral Sciences. 1998;106(5):971–976. doi: 10.1046/j.0909-8836..t01-8-.x.

79.Surdacka A. Amount and pH of the saliva in children and adolescents with black tartar. Czasopismo Stomatologiczne. 1989;42(6):381–386.

80.Heinrich-Weltzien R., Monse B., van Palenstein Helderman W. Black stain and dental caries in Filipino schoolchildren. Community Dentistry and Oral Epidemiology. 2009;37(2):182–187. doi: 10.1111/j.1600-0528.2008.00458.x.

81.Bhat S. Black tooth stain and dental caries among Udaipur school children. International Journal of Public Health Dentistry. 2010;1:13–15.

82.Zero, D. T. (1999). Dental caries process. Dental Clinics of North America, 43(4),635-664.

83.Khatri, M., Kishore, S., Nagarathinam, S., Siva, S., & Barai, V. (2021). White Spot Lesions and Remineralization. In Oral Health Care-An Important Issue of theModern Society. IntechOpen.

84.Fejerskov, O., Nyvad, B., & Kidd, E. A. M. (2008). Pathology of dentalcaries.Dental caries: the disease and its clinical management, 3, 7-9.

85.Daramola OO, Flanagan CE, Maisel RH, Odland RM. Diagnosis and treatment of deep neck space abscesses. Otolaryngol Head Neck Surg. 2009 Jul;141(1):123- 30.

86.CHI, D. L. (2019). Dental Health and Oral Disorders. Burns' Pediatric Primary Care E-Book, 319.

87.Naidoo, S., & Myburgh, N. (2007). Nutrition, oral health and the youngchild. Maternal & child nutrition, 3(4), 312-321.

88.de Farias D. b., Barreto Bezerra A. C. Salivary antibodies, amylase and protein from children with early childhood caries. Clinical Oral Investigations . 2003;7(3):154–157. doi: 10.1007/s00784-003-0222-7.

89.Sitaru A., Tohati A., Pop A. M., Bica C. Correlation between the salivary level of alpha-amylase and the risk for dental caries in young permanent teeth. Revista de Chimie . 2017;68(12):2984–2986. doi: 10.37358/RC.17.12.6022.

90.Scannapieco F. A., Torres G., Levine M. J. Salivary α-amylase: role in dental plaque and caries formation. Critical Reviews in Oral Biology and Medicine . 1993;4(3):301–307. doi: 10.1177/10454411930040030701.

91.Singh S., Sharma A., Sood P. B., Sood A., Zaidi I., Sinha A. Saliva as a prediction tool for dental caries: an in vivo study. Journal of Oral Biology and Craniofacial Research . 2015;5(2):59–64. doi: 10.1016/j.jobcr.2015.05.001.

92.Monea M. O., Vlad R. A., Stoica A. L. Analysis of salivary level of alpha- amylase as a risk factor for dental caries. Acta Medica Transilvanica . 2018;23(1)

93.Balekjian A. Y., Meyer T. S., Montague M. E., Longton R. W. Electrophoretic patterns of parotid fluid proteins from caries-resistant and caries-susceptible individuals. Journal of Dental Research . 1975;54(4):850–856. doi: 10.1177/00220345750540042501.

94.Anil S., Anand P. S. Early childhood caries: prevalence, risk factors, and prevention. Frontiers in Pediatrics . 2017;5(5):p. 157.doi: 10.3389/fped.2017.00157.

95.Salamonowicz M., Zalewska A., Maciejczyk M. Oral consequences of obesity and metabolic syndrome in children and adolescents. Dental and Medical Problems . 2019;56(1):97–104. doi: 10.17219/dmp/102620.

96.Davidson K., Schroth R. J., Levi J. A., Yaffe A. B., Mittermuller B.-A., Sellers E.A. C. Higher body mass index associated with severe early childhood caries. BMC Pediatrics . 2016;16(1):p. 137. doi: 10.1186/s12887-016-0679-6.

97.Hilt A., Daszkowska M. Dental status of normal and overweight persons from secondary schools of Lodz. Dental and Medical Problems . 2012;49(3):351–357.

98.Hooley M., Skouteris H., Boganin C., Satur J., Kilpatrick N. Body mass index and dental caries in children and adolescents: a systematic review of literature published 2004 to 2011. Systematic Reviews . 2012;1(1):57–26. doi: 10.1186/2046-4053-1-57.

99.Hong L., Ahmed A., McCunniff M., Overman P., Mathew M. Obesity and dental caries in children aged 2–6 years in the United States: national health and nutrition examination survey 1999–2002. Journal of Public Health Dentistry . 2008;68(4):227–233. doi: 10.1111/j.1752-7325.2008.00083.

100.Bud A., Bud E., Esian D., et al. Interrelation between salivary pH, buffer capacity and dental caries in underweight, normal weight and overweight children. Revista de Chimie . 2017;68(6):1255–1258. doi: 10.37358/rc.17.6.5652.