PHYSICOCHEMICAL CHARACTERISATION OF REMINERALISED DENTINE TREATED WITH SELF-ASSEMBLING PEPTIDE P11-4 AND CHLORO CALCIUM PHOSPHOSILICATE BIOGLASS IN SALIVA OF OBESE CHILDREN WITH SEVERE DENTAL CARIES IN SAUDI ARABIA

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by

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%R	Percentage remineralisation
α	level of significance
β	Power of study
σ	Standard deviation
δ	Mean difference
δ	Delta axis in NMR

LIST OF ABBREVIATIONS

1D ^{1}H-NMR	One Dimensional-proton NMR
^{1}H-NMR	Proton-NMR
2D GC-MS	2-Dimensional Gas Chromatography Mass Spectrometry
ACC	Arginine & Calcium Carbonate
ACP	Amorphous Calcium Phosphate
AFM	Atomic Force Microscopy
ANOVA	One-way Analysis of Variance
BMI	Body Mass Index
BSD	Backscattered electron detector
Ca	Calcium
CCPS	Chloro Calcium Phosphosilicate
CDC	Centre for Disease Control
CEJ	Cemento-Enamel Junction
CPMG	Carr−Purcell−Meiboom−Gill
CPP-ACP	Casein Phosphopeptide-Amorphous Calcium Phosphate
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
Cu	Copper
D_2O	Deuterium oxide
DMD	Dentine Mineral Density
DMFS	Decayed, Missing, Filled Surfaces
dmfs	Decayed, missing, filled surfaces (deciduous teeth)
dmft	Decayed, missing, filled teeth (deciduous teeth)
DMFT	Decayed, Missing, Filled Teeth
DMP-1	Dentine Matrix Protein-1
DPP	Dentine Phosphoprotein
DSP	Dentine Sialoprotein
EDTA	Ethylene Diamine Tetra Acetic acid
EDX	Energy Dispersive Xray spectroscopy
EELS	Electron energy-loss spectroscopy
EM	Electron Microscopy
ESEM	Environmental SEM
F	Fluoride
FE-SEM	Field Emission SEM
HA	Hydroxyapatite
HEPES	4-(2-HydroxyEthyl)-1-Piperazine Ethane Sulfonic acid
HMDS	Hexa Methyl Disilazane
HPLC-MS	High-Performance Liquid Chromatography-Mass Spectrometry

IASO	International Association for the Study of Obesity
IOTF	International Obesity Taskforce
JEPeM-USM	Jawatankuasa Etika Penyelidikan Manusia of Universiti Sains Malaysia
KSA	Kingdom of Saudi Arabia
LD	Lesion Depth
LMS	Box–Cox transformation, median, coefficient of variation
Mg	Magnesium
MicroCT	Microcomputed Tomography
MMPs	Matrix Metallo Proteinases
MRI	Magnetic Resonance Imaging
Na	Sodium
NCD	Non-communicable diseases
NCD-RisC	Non-communicable diseases-Risk Factor Collaboration
NCHS	National Centre for Health Statistics
NCPs	Non-collagenous proteins
NMR	Nuclear Magnetic Resonance
OCP	Octa calcium Phosphate
OD	Outer Diameter
P	Phosphorus
PA	Phosphoric acid
PBS	Phosphate Buffered Saline
PC	Principal Components
PEG	Polyethylene Glycol
PLS-DA	Partial Least Squares discriminant analysis
PRP	Proline Rich Proteins
Q2	Quality assessment
R2	Goodness of fit
ROI	Region Of Interest
S. mutans	*Streptococcus mutans*
SD	Standard Deviation
SED	Secondary electron detector
SEM	Scanning Electron Microscope
Sr	Strontium
TEM	Transmission Electron Microscopy
TSB	Tryptone Soya Broth
TSP	3- Trimethyl Silyl Propionate
VIP	Variable Importance in Projection
WDS	Wavelength dispersive spectroscopy
WHO	World Health Organisation
WHO-OHA	WHO oral health assessment
WMS	Whole mouth saliva
WSL	White Spot Lesion
Zn	Zinc

LIST OF APPENDICES

PENCIRIAN FIZIKOKIMIA DENTIN YANG DIREMINERALISASI MENGGUNAKAN PEPTIDA P11-4 DAN CHLORO CALCIUM PHOSPHOSILICATE BIOGLASS DALAM AIR LIUR KANAK-KANAK OBES SAUDI DENGAN KARIES GIGI TERUK DI ARAB SAUDI

ABSTRAK

Trend terkini dalam pencegahan karies gigi adalah melalui pergigian regeneratif dengan biomineralisasi serta pertumbuhan kristal hidroksiapatit. Ion kalsium dan fosfat dalam air liur menghalang demineralisasi. Selain itu, air liur adalah cecair biologi yang paling mudah diperoleh untuk dianalisis, memberikan maklumat penting mengenai status kesihatan seseorang. Nuclear Magnetic Resonance (NMR) merupakan teknik yang sangat berkesan dalam menganalisis campuran biofluida dan telah digunakan secara meluas dalam kajian metabolit dengan persediaan sampel yang minimum atau tanpa sebarang penyediaan sampel. Obesiti dalam kalangan kanak-kanak semakin meningkat di seluruh dunia, terutamanya akibat pengambilan makanan terproses dalam jumlah yang berlebihan. Namun, data mengenai profil metabolit air liur dalam kalangan kanak-kanak obes serta kesannya terhadap sifat fizikokimia bahan biomineralisasi regeneratif masih belum mencukupi. Oleh itu, kajian ini bertujuan untuk menganalisis serta membandingkan profil metabolit air liur kanak-kanak obes dengan kanak-kanak sihat, serta menilai kesan air liur mereka terhadap keberkesanan remineralisasi self-assembling peptide P11-4 dan chloro calcium phosphosilicate (CCPS) bioactive glass pada dentin yang mengalami karies secara artifisial. Kajian ini terdiri daripada tiga peringkat utama. Eksperimen 1 melibatkan pengumpulan air liur daripada 20 kanak-kanak obes dan 20 kanak-kanak sihat serta analisis kandungan metabolitnya menggunakan analisis ^{1}H-NMR. Eksperimen 2 pula melibatkan aplikasi

P11-4 peptide dan CCPS pada bahagian dentin yang telah mengalami karies secara artifisial. Eksperimen 3 melibatkan perendaman bahagian dentin yang telah dirawat dalam air liur pesakit yang dikumpulkan dalam Eksperimen 1 selama 28 hari. Keberkesanan remineralisasi dinilai menggunakan Scanning electron microscopy, energy dispersive x-ray spectroscopy, dan microcomputed tomography. Tiga belas (3 asid amino, 4 asid karboksilik, 2 asid ketoa, 1 alkohol dan 3 pelbagai metabolit) daripada 38 metabolit yang dikenal pasti dalam kanak-kanak normal dan obes dengan karies gigi yang teruk, mempunyai skor VIP yang tinggi. Glutamin dan succinylacetone menunjukkan skor VIP tertinggi dengan lapan metabolit meningkat dengan ketara dalam air liur kanak-kanak obes. Kanak-kanak dengan berat badan normal mempunyai paras Ca liur sebanyak 3.24 ± 1.27 mg/dl dan kanak-kanak obes mempunyai 2.37 ± 0.858 mg/dl ($p=0.016$). Salivary P ialah 10.67 ± 2.125 mg/dl dan 8.88 ± 2.729 dalam berat normal dan kanak-kanak obes, masing-masing ($p=0.027$). Selepas 7 hari rawatan, P11-4 dalam air liur kanak-kanak normal lebih berkesan berbanding kumpulan lain, manakala selepas 28 hari, CCPS dalam air liur kanak-kanak normal menunjukkan hasil terbaik. Hasil kajian ini akan membantu dalam menentukan jangka hayat klinikal terapi ini. Dalam jangka masa panjang, kajian ini akan menjadi asas kepada pembangunan protokol yang lebih baik bagi pengurusan karies dentin.

PHYSICOCHEMICAL CHARACTERISATION OF REMINERALISED DENTINE TREATED WITH SELF-ASSEMBLING PEPTIDE P11-4 AND CHLORO CALCIUM PHOSPHOSILICATE BIOGLASS IN SALIVA OF OBESE CHILDREN WITH SEVERE DENTAL CARIES IN SAUDI ARABIA

ABSTRACT

The recent trend in caries prevention is regenerative dentistry through biomineralization and growth of hydroxyapatite crystals. Calcium and phosphate ions in the saliva contribute to prevent demineralization and promote remineralization. Saliva is arguably the most accessible biofluid providing significant insights into an individual's health status. Nuclear magnetic resonance (1H-NMR) is highly effective for analysing mixtures and has been successfully utilised in investigations of metabolites within biofluids that necessitate minimal or no sample preparation. Paediatric obesity has been on the rise globally mainly due to excessive intake of processed foods. In obese children, the data on the metabolite profile and how the saliva influences the physical and chemical characteristics of regenerative biomaterials is still lacking. Thus, the aims of this study were to analyse and compare the salivary metabolite profiles of obese children with those of normal weight children, and to assess the effects of their saliva on the remineralising efficacy of self-assembling peptide P11-4 and chloro calcium phosphosilicate (CCPS) bioactive glass on artificially induced dentinal caries. This study comprises three steps. Experiment 1 includes collection of saliva of 20 obese and 20 normal weight children and analysing their metabolite content using 1H-NMR analysis. Experiment 2 includes application of P11-4 peptide and chloro calcium phosphosilicate on artificially induced dentinal caries sections. Experiment 3 involves immersing the treated dentinal sections of the teeth in saliva of patients collected in experiment 1 for 28 days. Their efficacy was assessed using scanning electron microscopy, energy dispersive X-ray spectroscopy and microcomputed tomography. Thirteen (3 amino acids, 4 carboxylic acids, 2 ketoacids, 1 alcohol and 3 miscellaneous metabolites) out of the 38 metabolites identified in normal and obese children with severe dental caries, had high VIP score. Glutamine and succinylacetone showed the highest VIP score with eight metabolites significantly increased in the saliva of obese children. Normal weight children had salivary Ca levels of 3.24±1.27 mg/dl and obese children had 2.37±0.858 mg/dl (p=0.016). Salivary P was 10.67±2.125 mg/dl and 8.88±2.729 in normal weight and obese children, respectively (p=0.027). After 7-days of treatment of dentin discs, P11-4 in normal children saliva was more efficient than other groups whereas after 28-days, CCPS in normal children saliva showed the best results. The outcome of this study would help in determining the clinical longevity of these therapies and in the long run, will pave way in developing a better protocol for the management of dentinal caries.

CHAPTER 1

INTRODUCTION

1.1 Research background

Paediatric obesity is a preliminary risk factor for adult morbidity and mortality. There are two methods of defining obesity in childhood derived from the World Health Organization (WHO) growth reference curve (De Onis et al., 2007) or the one using International Obesity Taskforce (IOTF) definition (Cole et al., 2000). The 2007 Body Mass Index (BMI) standards above +1 SD (For males 25.4 kg/m² & females 25.0 kg/m²) correspond to the 'overweight' status whereas that above +2 SD (29.7 kg/m² for both males and females) correspond to 'obesity' (De Onis et al., 2007). Metabolism of lipids, tyrosine and alanine, along with the urea cycle (Martos-Moreno et al., 2014), and inflammation markers (Butte et al., 2015) are reported to be involved in obesity and its associated conditions. Metabonomics is characterized as "the quantitative assessment of the multiparametric time-dependant metabolic reactions of a complex (multicellular) system to a pathophysiological intervention or genetic alteration" (Nicholson et al., 1999). Nuclear magnetic resonance (NMR) is highly effective for analysing mixtures and has been successfully utilized in investigations of metabolites in biofluids that necessitate minimal or no sample preparation (Fidalgo et al., 2013; K. Wang et al., 2018; Gardner et al., 2020). Salivary metabonomics is increasingly acknowledged as a significant source of biological data for elevated risk of dental caries and a heightened body mass index (BMI). The predominant metabolites identified in dental caries include propionate, butyrate, saccharides, organic acids, and acetone. (Fidalgo et al., 2013; Pereira et al., 2019), whereas obese children showed high amounts of palmitic acid, myristic acid, urea, and N-acetyl galactosamine (Troisi et al., 2019). While the majority of metabolites detectable in saliva via 1H-NMR (proton-NMR) have been found, much effort is still required to elucidate the importance of these various metabolites. Other methods utilised for metabolomic studies include Gas Chromatography-Mass Spectrometry (GC-MS) and High-Performance Liquid Chromatography-Mass Spectrometry (HPLC-MS). Advantages of NMR over these techniques as below:

NMR v/s GC-MS: NMR is non-destructive, analyses complex mixtures without prior separation, needs minimal sample preparation, there is ease of quantification, it is highly reproducible and can be automated. Total acquisition time is approximately 10 min.

NMR v/s HPLC-MS: NMR provides direct quantification, structural information through the chemical shifts and coupling patterns of different nuclei and needs minimal sample preparation.

The tooth's hard tissue, an essential biological mineral component, comprises dentine, enamel, and cementum. Dentine constitutes the primary structure of the tooth, while enamel, the toughest tissue in the body, encases its surface. Dentine principally comprises dentinal tubules, the odontoblastic process, and intercellular substances (Carda et al., 2006), consisting of approximately 70% inorganic materials, predominantly hydroxyapatite (HA), 20% organic materials, and 10% water by weight. Dentinal tubules and odontoblastic processes permeate the entire dentine, while the mineralized collagen fibres inside the intercellular matrix interlace to create a network that constitutes the dentine scaffold. Within the dentine are dentinal tubules with an aperture diameter varying from 10 nm to 3 μm and extending many millimetres in length. The openings of dentinal tubules on the surface are typically obscured by enamel or cementum. Nevertheless, these protective layers may be compromised under specific situations, leading to fluid flow through the exposed tubules due to mechanical, chemical, or thermal stressors, which can induce discomfort and pain. Non-collagenous proteins (NCPs) such as dentine phosphoprotein (DPP), dentine sialoprotein (DSP), and dentine matrix protein-1 (DMP-1) play a crucial role in dentine development (Deshpande et al., 2011).

The most common global health condition affecting more than half of the world's population is dental caries (K. Wang et al., 2018). Dental caries is a biofilm-mediated, diet-modulated, multifactorial, non-communicable, and dynamic disease that results in the net mineral loss of dental hard tissues (Fejerskov, 1997; Pitts et al., 2017). Acidogenic bacteria create dental plaque on the tooth surface and synthesize organic acids. The acids are not readily diluted by saliva, resulting in a substantial fall in the pH value of localized regions on the tooth surface. When this pH level drops to a certain value (below 5.5), the calcium phosphate dissolves, leading to demineralisation (Jones, 2001). Saliva, which is supersaturated with calcium and phosphate ions, subsequently reconstitutes calcium phosphate crystals on the tooth surface by a process termed remineralisation. If the degree of demineralization equals the degree of remineralization, dental caries will not develop in the enamel. Nonetheless, if the extent of demineralisation surpasses that of remineralisation, dental cavities gradually commence to develop. These tissues exhibit limited regenerating potential due to a deficiency of regenerative cells and vascularization. (Pitts et al., 2017). The intricate process of caries advancement, which involves dietary carbohydrates, bacterial metabolism, and demineralization, leads to the exposure and degradation of the collagenous organic matrix by the local bacteria. (Vidal et al., 2014) and bacterial proteases (Featherstone, 2004), permitting the lesion to proliferate. An increase in junk food has not only caused an exponential rise in the incidence of dental caries but has also led to the development of newer strategies in combating this disease.

The primary materials employed for the direct repair of dental hard tissues in clinical practice are glass ionomer cement and composite resin. Although composite resin materials are commonly employed for the direct repair of dental hard tissue defects, concerns like microleakage continue to present challenges for the durable restoration of dental hard tissues in clinical practice. Empirical medical research indicates that the success rate of direct composite resin restorations after a decade is merely 68.7% (Kanzow et al., 2020). The primary causes of failure are secondary caries and repair fractures resulting from microleakage (Sarrett, 2005). Consequently, currently used dental materials fail to satisfy the clinical requirement for durable, stable repair of hard tissue abnormalities, necessitating the innovation of novel materials capable of addressing these disadvantages.

The remineralisation of enamel and dentine can be achieved by introducing regulatory factors into the mineralising solution or by utilising calcium phosphate solid materials for repair. There are many advantages, such as simulating multi-level ordered structures, mimicking the mechanical properties of natural teeth, ensuring firm interface bonding to reduce micro-leakage, and providing good biocompatibility. The most recent trend in caries prevention is remineralisation therapy, which utilises fluoridated and non-fluoridated remineralising agents to regenerate dental tissues (Arifa et al., 2019). Dentine demineralisation exposes the collagen matrix and activates endogenous-bound matrix metal proteinases and cysteine cathepsins, which will lead to the loss of demineralised collagen fibres. Hence, the ideal method to manage dentine caries involves the remineralisation of collagen fibrils to regenerate the dentine microstructure (Xu et al., 2011).

Recently, NCPs and proteoglycans have been extensively studied, and results suggests that they play a crucial role in the process of mineralisation of dentine

(Oosterlaken et al., 2021). There is deposition of HA both inside and between collagen fibres which is regulated by non-collagenous proteins. The amino acids in NCPs are abundant in carboxylic groups, contributing to the negative charge of proteins, hence facilitating their binding with a significant number of Ca+2 ions, thereby preventing the precipitation of the super-saturated calcium phosphate mineralised solution (Deshpande et al., 2011). This indicates that proteins are essential for preserving the solution's stability and creating an appropriate condition for the eventual crystallization of apatite. The particular sequences within NCPs are essential to their functionality. Numerous biomimetic polypeptide compounds have been developed and synthesized, drawing inspiration from the NCP structure (Zhou et al., 2014; Wang et al., 2015; Chien et al., 2017). Biomimetic remineralisation emulates the natural mineralisation process, aiming to replenish demineralised dentine collagen with liquid-like amorphous calcium phosphate (ACP) nano-precursor particles. This bottom-up remineralization procedure doesn't depend on seed crystallites and can be considered as a viable approach for remineralizing demineralized dentine (Cao et al., 2015).

Different authors have studied a variety of analogues to replicate the functionality of NCPs in the mineralisation of dentine. For example, bioactive compounds that release mineral ions to promote apatite production, such as zinc polydopamine or agarose hydrogel system. In addition to the biotic materials, different remineralising mediums are used to provide calcium and phosphate ions. The most commonly used agents are Portland cement, which acts as a calcium and hydroxyl ion-releasing source, artificial saliva, bioactive glass, and calcium chloride solution. These mediums are calcium-containing remineralising agents (Reyes-Carmona et al., 2009; Gandolfi et al., 2019). Some research utilized phosphate-containing remineralizing mediums such as simulated bodily fluid calcium phosphate remineralizing solution, artificial saliva, and phosphate buffered saline (PBS). (Ning et al., 2012; Zhou et al., 2012; Li et al., 2013; Osorio et al., 2014).

In this study, the most easily available biofluid, i.e., human saliva, has been utilised. Saliva is arguably the most accessible biofluid for collection, which is very informative concerning biological status. The composition of saliva offers a potential source of innovative diagnostic indicators for systemic and oral disorders, as most components identified in blood are also present in saliva (Fidalgo et al., 2013; Troisi et al., 2019; Ruth et al., 2021). Saliva serves as body's principal defence mechanism in the oral cavity and is crucial for safeguarding the exposed surfaces of teeth. Saliva can restore the demineralization of exposed tooth surfaces by mechanical washing, antibacterial properties, buffering capacity, calcium phosphate-binding proteins, immunological surveillance, and the release of antimicrobial peptides (Van Nieuw Amerongen et al., 2004). Alongside proteins, the critical microelements present include calcium, sodium, magnesium, zinc, and fluoride; these are essential for the mineralization and development of hard dental tissue. The concentration of calcium and phosphate in saliva becomes supersaturated with calcium and phosphate salts, which exert a protective effect on tooth hard tissues (Hegde et al., 2019).

In addition, a lot of research has been done to assess the efficiency of bioactive glass and biomimetic peptides in reducing the susceptibility of dental hard tissue structures, with varied results. This study aims to fill the research gap that exists in assessing the effect of the saliva of obese paediatric children on the surfaces of teeth treated by newly developed remineralising agents like biomimetically acting self-assembling peptide (p11-4 peptide) available as Curodont Repair^{TM} (Kind et al., 2017; Memarpour et al., 2022) or a bioactive glass like chloro calcium phosphosilicate

(CCPS) using Novamin technology available as BioMin C® fluoride free toothpaste (Burwell et al., 2009). It consists of CCPS, which is the active ingredient that facilitates its adhesion to the tooth surface to commence the remineralization process. Bearing that in mind, this research assessed the alterations of the surface anatomy of dentine as well as its chemical properties using P11-4 peptide and CCPS, thereby achieving better protection of dental hard tissue structures against caries in obese paediatric children.

1.2 Problem statement

Obesity during childhood predisposes the individual to obesity and other non-communicable diseases (NCDs) when he/she becomes adult. The NCD Risk Factor Collaboration (NCD-RisC) possesses the most extensive worldwide records on obesity among children aged from 5 up to 19 years (Di Cesare et al., 2019). In 1975, there were 5 million girls and 6 million boys aged 5 to 19 years with obesity across the world whereas the same increased to 50 million girls and 75 million boys by 2016 (Abarca-Gómez et al., 2017). A positive correlation was reported between caries and obesity in many different regions of the world. In Saudi Arabia, obesity (9.3% prevalence in the geographical region of our study) continues to grow at a concerning pace owing to substantial alterations in food habits and lifestyle practices (Alenazi et al., 2023; Adam et al., 2024). Moreover, current reports (2019) indicate that dental caries prevalence in Saudi Arabia is elevated compared to worldwide standards, with rates of 64.9% among 12-year-olds and 71.35% among 15-year-olds. (Al-Rafee et al., 2019). Alshehri and colleagues in their systematic review reported that some studies found a positive association between caries and obesity in some regions of Kingdom of Saudi Arabia whereas others reported conflicting results (Alshehri et al., 2020).

Saliva, a readily obtainable bodily fluid, possesses significant potential in dental diagnostics, and its importance is indisputable. Salivary 1H-NMR has been widely employed in dentistry through the use of saliva in metabonomic research to assist in diagnosing different disorders (Fidalgo et al., 2013, 2015; Singh et al., 2017; Romano et al., 2018; Freitas-Fernandes et al., 2023). Studies have reported metabolite profiles in dental caries. Other studies have reported metabolite profiles in obesity. There is no reported literature on obesity and caries together in an individual. Hence, due to the paucity of information on the spectra of biomarkers for dental caries in obese children, it was intended to explore this research gap.

There is enough evidence to suggest that caries in children spreads at a faster rate and reach dentin earlier. This could be due to thinner enamel in their teeth, more consumption of sugary foods, and less dexterity to effectively brush and floss. Regenerative biomaterials in dentistry such as biomimetically acting P11-4 self-assembling peptide and a bioactive glass (CCPS) have attained prominence recently. Most of the studies have been in-vitro, documenting promising results in remineralisation (Brunton et al., 2013; Kind et al., 2017; Da Cruz et al., 2018; Mohamed et al., 2021; Petrović et al., 2023). There is limited data on the effects of these materials in a natural environment. New technologies in the form of regenerative biomaterials have been studied before. Even though many studies have been done on the remineralisation of dental enamel, yet there is a scarcity of literature on their effects on dentine (Brunton et al., 2013; Farooq et al., 2019; Üstün et al., 2019; Memarpour et al., 2022). As the ultrastructure of enamel and dentine differs considerably, the response of dentine to commercially available remineralising agents also differs. Among these few studies, none of them have explored the response of dentine ex-vivo. Hence, there still exists a research gap in the salivary profile for metabolites in obese children with high caries activity and how their saliva influences the physical and chemical characteristics of dentine discs treated with regenerative biomaterials.

1.3 Justification of study

Paediatric obesity has been on the rise globally, mainly due to the excessive intake of high amounts of processed foods. It has been extensively reported in the literature that children's general health significantly affects their oral health status too (Lifshitz et al., 2016). Dental disease prevalence in obese children has been studied previously. In addition, 1H-NMR was utilized in obese children for comparison of their metabolites profile with those of normal weight paediatric population. But there is a literature deficit on obese and normal weight children with high caries activity; exploring these differences through salivary metabonomics is intriguing. Therefore, it is imperative to study the metabolite profile of the saliva of obese paediatric patients and to assess the effects of regenerative biomaterials on dentinal discs in addition to studying the effect of natural whole saliva on these discs. This enables us to understand the salivary metabonomic profile, specifically in obese children, and the clinical outcome of regenerative therapy (biomimetic remineralisation) on human dentine before and after immersion in the natural whole saliva of these children. The main outcome measures included patients' salivary biomarker profile of dental caries, chemical uptake of calcium and phosphorus by treated dentine after immersion in saliva, and lastly, the superficial and sub-surface physical characterisation of the treated dentine by Scanning Electron Microscope (SEM), Energy Dispersive Xray (EDX) analyses and Micro-Computed Tomography (MicroCT).

1.4 Research objectives

1.4.1 General objective

To create a salivary metabolite profile of obese paediatric patients and assess the role of saliva in the efficacy of regenerative biomaterials in dentinal caries treatment.

1.4.2 Specific objectives

1. To assess, compare and correlate the salivary metabolite profile of obese children those of normal weight children.
2. To compare and correlate the salivary Ca and P levels with Ca and P weight% of treated dentine after saliva immersion.
3. To determine the ex-vivo effects of saliva from obese paediatric patients and normal weight children on the efficacy of self-assembling peptide P11-4 and CCPS on artificially demineralised dentine.

1.5 Research hypotheses

1. The salivary metabolite profile of obese children differs considerably with those of normal weight children.
2. There is a significant difference between the salivary Ca and P levels in obese children and normal weight children that is reflected in the Ca and P weight % in the treated dentine.
3. There is a significant difference between the effects of saliva from obese children and normal weight children in the efficacy of the self-assembling peptide P11-4 and CCPS on dentinal caries ex-vivo.

1.6 Research questions

1. What are the differences in the salivary metabolite profile between obese paediatric patients and normal weight children?
2. Is there any correlation between salivary Ca and P levels with Ca and P weight % in treated dentine?
3. What are the effects of the saliva of obese and normal weight children on the efficacy of the self-assembling peptide P11-4 and CCPS on dentinal caries ex-vivo?

CHAPTER 2

LITERATURE REVIEW

2.1 Background

Childhood obesity is the most common dietary condition affecting children and adolescents globally. Approximately 43 million individuals are classified as obese, with 21-24% of children and adolescents being overweight, and 16-18% of individuals exhibiting abdominal obesity. Obesity during young age causes individuals to develop insulin resistance causing type 2 diabetes, hypertension, hyperlipidaemia, liver and kidney and reproductive dysfunction in adulthood (Xu et al., 2016). Currently, there is a growing interest in discovering and utilizing metabolites as biomarkers for the diagnosis, treatment, and monitoring of diseases. The analysis of saliva with NMR has been extensively employed by numerous authors because to its rapid and non-invasive nature (Silwood et al., 2002; Fidalgo et al., 2013; Singh et al., 2017; García-Villaescusa et al., 2018; Romano et al., 2018). Another oral health implication of paediatric obesity is development of dental caries. High caries index of children predisposes them to early loss of healthy teeth that may adversely affect their occlusion and proper growth of maxillofacial skeleton (Fidalgo et al., 2013). Thus, it is interesting to correlate the findings of salivary NMR in obese children with high caries index which has not been studied previously. Additionally, it is also interesting to analyse the effects of the natural whole saliva of these obese paediatric children on remineralising-agent treated dentine surfaces.

2.2 Paediatric obesity

Overweight and obesity primarily result from excessive caloric consumption, inadequate physical activity, or a combination of both factors. Additionally, other genetic, behavioural, and environmental factors contribute to its aetiology. This global epidemic is associated with an elevated risk of numerous chronic and severe diseases, including hypertension with coronary artery disease and stroke, diabetes, cancer, and early mortality (WHO, 2000b). Calle et al. (2005) stated that during a 20-year follow-up, when the potential for confounding factors such as smoking and previous diseases is controlled, overweight and obesity emerge as substantial predictors of mortality from all causes. Another study by Swinburn and co-workers stated that childhood obesity is a precursor of metabolic syndrome, compromised physical health, psychological difficulties, respiratory issues, and glucose intolerance, all of which may persist until adulthood (Swinburn et al., 2011). Prevalence of obese children has increased in recent years around the globe. The International Association for the Study of Obesity (IASO) and the International Obesity Task Force (IOTF) estimate that 200 million schoolchildren are classified as overweight or obese (WHO, 2000a). One of the criteria to assess obesity is by using BMI. Alammar et al. (2020) defined BMI, as "body weight in kilograms divided by the square of height in meters (kg/m2)", which is the most commonly used method to detect obesity. WHO suggested lower BMI cut-off values of 23.5-25 kg/m2, however these cannot be applied to young age groups as each child BMI cut-off values differs according to their age and population standards (WHO, 2000b; Ranjani et al., 2016).

2.3 Growth assessment in Saudi children

The delivery of superior health care necessitates an understanding of normalcy to differentiate between health and illness. The evaluation of nutritional status and growth in children and adolescents through growth charts is a crucial aspect of clinical practice. Numerous nations have developed their own extensive growth charts for children and teenagers (Freeman et al., 1995). In the Kingdom of Saudi Arabia (KSA), Al-Sekait et al. evaluated children's growth, and the findings were published. This study, encompassing 48,000 schoolchildren aged 6 to 18 years, selected through a multistage stratified cluster sampling method, concluded that the growth pattern was delayed by less than one standard deviation in Saudi Arabian children compared to National Centre for Health Statistics (NCHS) standards (Al-Sekait et al., 1992). A countrywide research conducted on schoolboys from 1994 to 1995 indicated a significant prevalence of obesity. (Al-Nuaim et al., 1996). Independent research conducted in several cities and areas revealed that the growth patterns of Saudi youngsters varied from those of their American and European peers. These comprised schoolchildren aged 6 to 16 years from the Eastern Province (Magbool et al., 1993), and children and adolescents residing in the Asir region (4,000 males and 3,660 females, aged from birth to 19 years, predominantly military dependents) (Attallah, 1994). A study by El Mouzan et al. (2007) published extensive data on the growth charts of children and adolescents from 2 to 19 years based on various parameters. Amongst these parameters, weight-for-stature is given below as charts in Figure 2.1

(for boys) and Figure 2.2 (for girls). For every chart, an entire range of percentiles (3rd, 5th, 10th, 25th, 50th, 75th, 90th, 95th, and 97th) were also analysed.

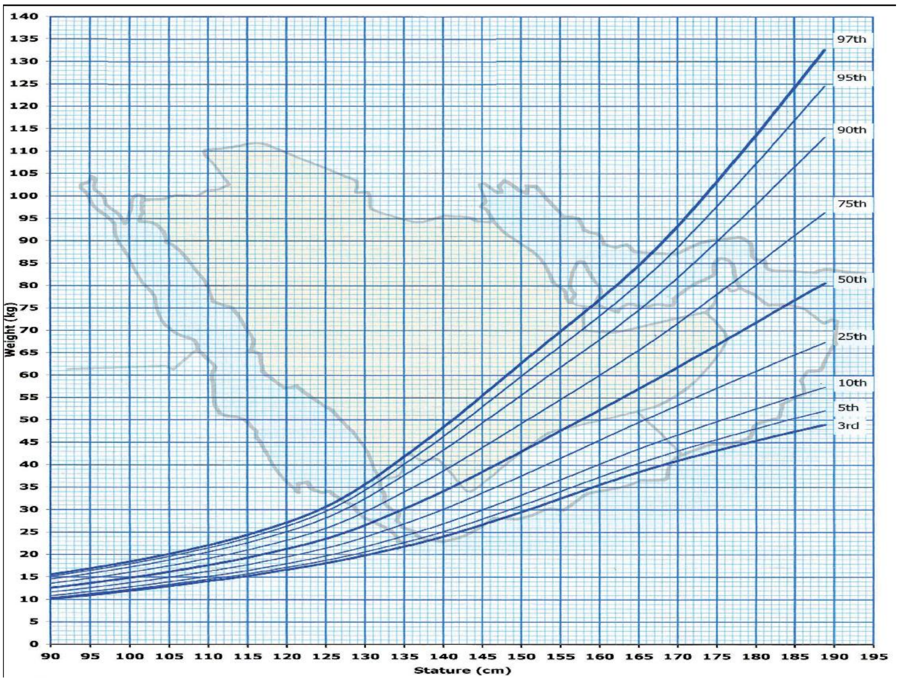


Figure 2.1 Weight-for-stature percentiles: boys, 2 to 19 years (Adapted from El-Mouzan et al., 2007)

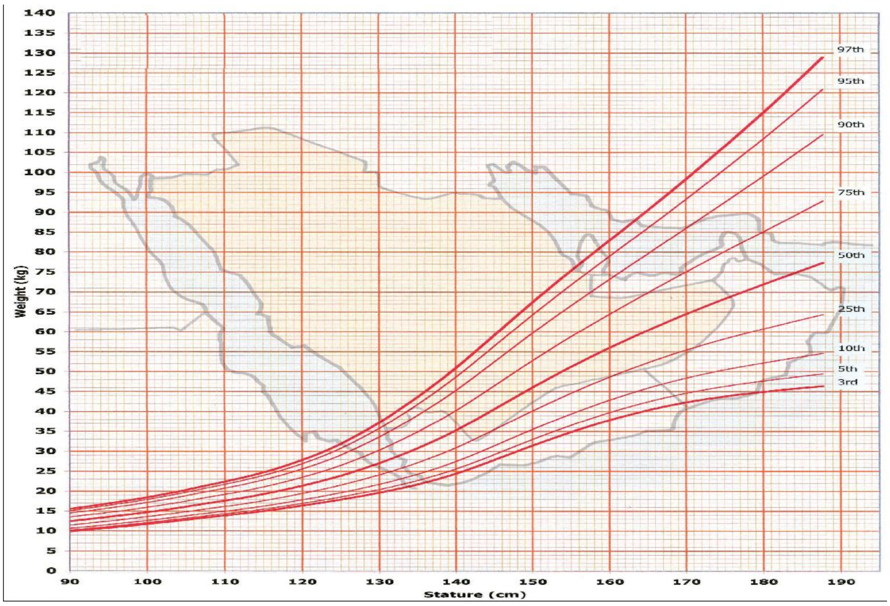


Figure 2.2 Weight-for-stature percentiles: girls, 2 to 19 years. (Adapted from El-Mouzan et al., 2007)

BMI is calculated based on Saudi Arabia's sex-specific percentile charts. Previous studies have reported obesity prevalence of 4.1-20.9% (Al-Enazy et al., 2014; Al-Shehri et al., 2016) in school children which is similar to other studies reported from different countries (Marwaha et al., 2006; Dhingra et al., 2011; Karki et al., 2019; Sarokhani et al., 2020). It is measured using the formula BMI = kg/m^2 where kg is a person's weight in kilograms and m^2 is their height in metres squared. Tanner and colleagues described accurately the method of measurement of various components of BMI. The measurement of height is conducted by having the child stand barefoot, ensuring that their heels and back are in touch with a standardized stadiometer board. Their head is positioned so that the child gazes directly ahead, with the Frankfurt horizontal plane (line joining inferior orbital margin with external auditory meatus) is parallel to the floor. Hair clips with head covers were removed. A movable horizontal board is then lowered until its bottom edge contacts the child’s head. The participants are instructed to extend their necks to maximize height. This is recorded as the child’s height up to the last completed 1mm (Tanner et al., 1985). A recent study described paediatric obesity standards in Saudi children using L, M, S and Z scores and gave standard deviation (SD) values for each age group categories in the Saudi children as shown in Table 2.1 (for boys aged 5-18years) and Table 2.2 (for girls aged 5-18years). The LMS parameters are the median (M), the generalized coefficient of variation (S), and the power in the Box–Cox transformation (L). The Box–Cox transformation is used to adjust the distribution of anthropometric data to a normal distribution. This LMS and Z score chart is a reference standard that could be used in population-based studies for accurate estimation of obesity and growth of Saudi children (El-Mouzan et al., 2016). The L, M, and S parameters are calculated and smoothed according to the method of maximum penalized likelihood (Cole et al., 1998).

Table 2.1 L, M, and S parameters and z scores for body mass index for age: Boys 5-18 years (Adapted from El Mouzan *et al.*, 2016)

L, M, and S parameters and z scores for body mass index for age: Boys 5-18 years										
Age (years)	L	M	S	−3	−2	−1	0	1	2	3
5	−1.315	14.380	0.125	10.709	11.588	12.815	14.380	16.472	19.460	23.420
6	−1.275	14.536	0.133	10.635	11.559	12.859	14.536	16.811	20.125	24.640
7	−1.236	14.752	0.142	10.601	11.574	12.954	14.752	17.224	20.895	26.033
8	−1.197	15.114	0.150	10.669	11.701	13.175	15.114	17.817	21.903	27.772
9	−1.157	15.626	0.158	10.834	11.935	13.520	15.626	18.596	23.166	29.891
10	−1.118	16.236	0.167	11.055	12.234	13.944	16.236	19.506	24.621	32.321
11	−1.078	16.899	0.175	11.298	12.561	14.405	16.899	20.495	26.207	34.988
12	−1.039	17.584	0.184	11.542	12.893	14.878	17.584	21.528	27.880	37.834
13	−1.000	18.265	0.192	11.767	13.208	15.339	18.265	22.573	29.598	40.796
14	−0.960	18.949	0.200	11.979	13.513	15.793	18.949	23.636	31.368	43.876
15	−0.921	19.648	0.209	12.184	13.814	16.251	19.648	24.733	33.210	47.092
16	−0.881	20.343	0.217	12.371	14.099	16.697	20.343	25.843	35.092	50.387
17	−0.842	21.015	0.225	12.527	14.354	17.116	21.015	26.937	36.973	53.690
18	−0.803	21.659	0.234	12.651	14.577	17.503	21.659	28.010	38.840	56.964

Table 2.2 L, M, and S parameters and z scores for body mass index for age: Girls 5-18 years (Adapted from El Mouzan *et al.*, 2016)

L, M, and S parameters and z scores for body mass index for age: Boys 5-18 years										
Age (years)	L	M	S	−3	−2	−1	0	1	2	3
5	−1.316	14.282	0.124	10.650	11.521	12.735	14.282	16.348	19.293	23.185
6	−1.295	14.482	0.134	10.600	11.517	12.811	14.482	16.755	20.084	24.656
7	−1.271	14.763	0.144	10.589	11.562	12.947	14.763	17.284	21.088	26.546
8	−1.244	15.173	0.154	10.665	11.701	13.191	15.173	17.978	22.332	28.860
9	−1.211	15.703	0.164	10.820	11.928	13.537	15.703	18.821	23.786	31.528
10	−1.171	16.380	0.172	11.076	12.267	14.010	16.380	19.838	25.451	34.466
11	−1.127	17.213	0.180	11.442	12.729	14.621	17.213	21.027	27.293	37.520
12	−1.085	18.169	0.186	11.907	13.295	15.347	18.169	22.344	29.246	40.587
13	−1.052	19.173	0.191	12.425	13.916	16.125	19.173	23.700	31.211	43.594
14	−1.030	20.100	0.195	12.917	14.499	16.848	20.100	24.942	33.003	46.332
15	−1.015	20.854	0.197	13.316	14.972	17.436	20.854	25.959	34.489	48.656
16	−1.006	21.447	0.200	13.622	15.336	17.892	21.447	26.775	35.721	50.691
17	−0.999	21.860	0.202	13.812	15.571	18.197	21.860	27.368	36.665	52.341
18	−0.988	22.111	0.205	13.894	15.685	18.365	22.111	27.761	37.335	53.569

The accessibility of the aforementioned references facilitates not only a more precise evaluation but also a comparison with other populations utilizing their regional growth chart references. The utilization of the WHO (2008) criteria of growth and nutritional problems in Saudi school-age young population is now feasible using the custom z-score reference. The World Health Organization (WHO) (2008) defines short height as a z score of less than -2 standard deviations (SD), overweight as a body mass index (BMI) greater than +1 SD, obesity as a BMI greater than +2 SD, and thinness as a BMI less than -2 SD.

2.4 Dental caries

Dental caries constitutes a significant health issue in numerous industrialized nations, affecting a substantial proportion of both children and adults. It is inequitably distributed among populations exhibiting a pronounced socioeconomic gradient. (Schwendicke et al., 2015). Like other disorders classified as NCDs, dental caries arises from a confluence of genetic, physiological, environmental, and behavioural variables (Pitts et al., 2017). The prevention and control of caries as a non-communicable disease necessitate concerted efforts at national, community, and clinical levels. The WHO (2021) Resolution on Oral Health, ratified by the World Health Assembly in May 2021, advocates for nations to transition from a conventional therapeutic model to a preventive promotional strategy that emphasizes risk identification for prompt, comprehensive, and inclusive care. Moreover, the WHO resolution emphasizes eco-friendly and minimally invasive dental practices (Benzian et al., 2021). The dietary advice of paediatricians for reducing obesity in children closely mirror those of dental practitioners for preventing caries (Pitts et al., 2021). Consequently, an early initiation of a healthy lifestyle routine is crucial, and dietary recommendations for caries prevention align with those for obesity prevention. Integrating caries prevention with overall well-being and vaccination programs is thus advantageous when children and parents interact with non-oral health experts.

2.5 Decayed, Missing, Filled Surfaces (DMFS)/Decayed, Missing, Filled Teeth (DMFT) (World Health Organization)

DMFS (permanent teeth) / dmfs (deciduous teeth), a widely recognized and thoroughly documented criterion established in the 1930s, is utilized to quantify the prevalence of caries through the WHO evaluation method. The WHO instrument is the most widely utilized caries assessment tool globally. It ascertains the presence or absence of a cavitated dentine carious lesion. The WHO instrument employs the dmft/DMFT index for the reporting of caries data since its inception in 1938. The WHO instrument documents the existence or nonexistence of a dentine cavity, a restoration, and a tooth lost due to dental caries. This rudimentary delineation was a deliberate choice, as the WHO sought a tool applicable worldwide that yielded comparable results (Frencken et al., 2020). The benefits of utilizing WHO as a mechanism for caries evaluation have been delineated. The manageability and expense of most visual/tactile caries detection equipment are minimal, as they consist of a limited number of hand tools and some consumables. The examination is often concise. The instrument is globally recognized and user-friendly for learning. The reproducibility of this is consistent and dependable. Following adequate examiner training and calibration, both intra- and inter-examiner consistency can attain elevated levels (Frencken et al., 2020). The writers were unable to identify any study that evaluated its face, content, or construct validity. Nonetheless, the codes and descriptions were articulated with clarity, preventing any potential misconceptions. The index has been utilized not only in relation to the WHO instrument but also in most surveys, regardless of the caries evaluation tool employed. Despite decades of criticism, few alternatives to the index have surfaced. Crombag et al. (2018) employed the DMF index to ascertain the prevalence of dental caries. The DMFT index represents the total count of decaying teeth (D), filled teeth (F), and missing teeth (M) attributable to caries. Uppercase letters denote permanent teeth, while lowercase letters signify deciduous (primary) teeth. The WHO categorizes a DMFT of less than 1.2 as very low and greater than 4.4 as high prevalence of caries. A separate study conducted by Guo et al. defined the high caries group as having five or more decaying approximal surfaces (Guo et al., 2022).

Caries Prevalence (Caries Experience): Several studies conducted in Saudi Arabia demonstrated the prevalence of dental caries. A systematic review reported that the mean DMFT index score in primary dentition was 4.14 with average prevalence of 75.43% whereas in permanent dentition it was 1.28 with an average prevalence of 67.7% (Qadir Khan et al., 2024). Calculation of DMFT/dmft*:* At the clinical examination, number of cavities, fillings and missing teeth are to be recorded. Jouhar et al. asserted that the existence of cavities and fillings, referred to as 'caries prevalence,' is a significant factor as it reflects the historical or current equilibrium between resistance factors and caries-inducing factors. A high prevalence of caries indicates that the patient has been vulnerable to the disease in past period of time (Jouhar et al., 2021).

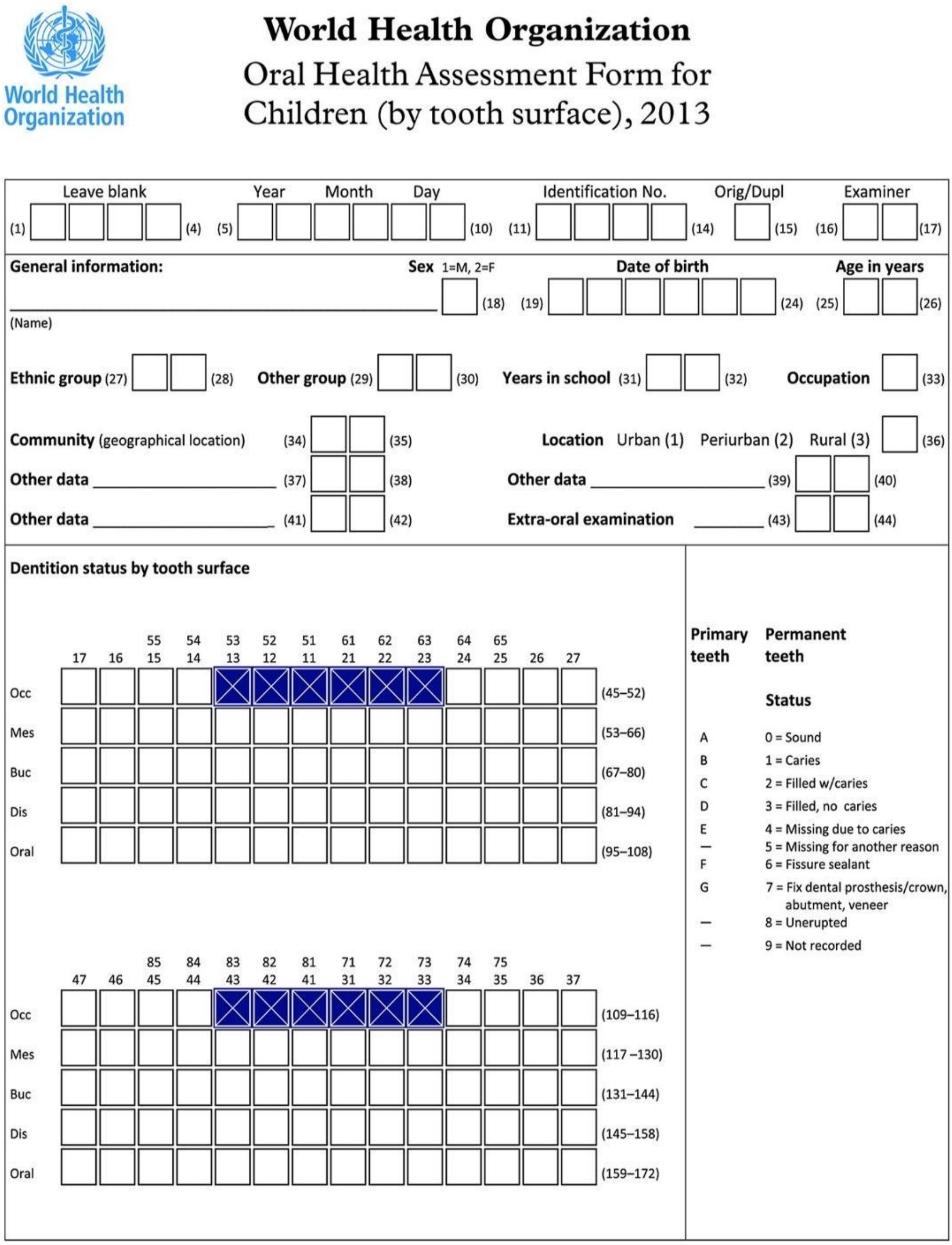
DMFT is occasionally used interchangeably with DMFS, both of which serve as numerical indicators of caries prevalence, derived from the count of Decayed (D), Missing (M), and Filled (F) teeth (T) or surfaces (S). The coding for main teeth employs lowercase letters: Decayed (d), Missing (m), and Filled (f) for teeth (t) or surfaces (s). It is therefore utilized to obtain an estimation demonstrating the extent to which the dentition has been impacted by dental caries. Typically, the calculation is based on 28 teeth, omitting 18, 28, 38, and 48 from the index (WHO, 2013).

Significance of DMFT and dmft Indices:

- Treatment Planning: These indices are valuable for clinicians in devising treatment plans. By understanding the extent of dental caries in an individual's permanent or primary teeth, dentists can determine the most appropriate interventions, which may include restorations, extractions, or preventive measures. While the DMF does not accurately reflect treatment necessity, the amount of decay within the DMF (D/DMF) serves as a proxy for unfulfilled treatment requirements, and the proportion of filled DMF (F/DMF) can be interpreted as an indicator of access to care (Shulman et al., 2007).
- Public Health Initiatives: DMFT and dmft indices serve as critical tools in public health assessments. Governments and organizations use these data to allocate resources effectively, implement community-based preventive programs, and track trends in oral health over time. Brazilian law known as Organic Health Law of 1990 claims that the use of epidemiology is a necessary tool for the establishment of priorities, allocation of resources and programmatic guidance. The main index for epidemiological surveys in oral health has been DMFT (Lessa et al., 1994).
- Prevention and Education: By identifying caries experience using these indices, oral health professionals can educate individuals and communities about oral hygiene practices and lifestyle choices that can help prevent tooth decay (Petersen, 2008; Zandi-Ghashghai et al., 2020).

To conclude, DMFT and dmft indices are indispensable tools for assessing dental caries experience in permanent and primary dentition. They provide essential data for individualized treatment planning, public health initiatives, and preventive measures. The careful monitoring of dental caries experience through these indices contributes to improved oral health outcomes and overall well-being for individuals of all ages. Regular dental check-ups and early intervention guided by these indices can ultimately lead to a healthier and happier population with fewer dental caries-related issues.

The WHO Oral Health Assessment (WHO-OHA) Form for Children contains 128 boxes, numbered from 45 to 172, categorized by tooth surface. Letters A to G indicate the condition of primary teeth, while digits 0 to 9 represent the condition of permanent teeth (WHO, 2013). Nguyen et al determined the dmft index for an individual by tallying the instances of B, C, D, and E codes in the Oral rows (boxes 95-108 and boxes 159-172) as shown in Figure 2.3 (Nguyen et al., 2023).



World Health Organization

World Health Organization
Oral Health Assessment Form for
Children (by tooth surface), 2013

Leave blank (1) (4) Year Month Day (5) (10) Identification No. (11) (14) Orig/Dupl (15) Examiner (16) (17)

General information: Sex 1=M, 2=F (18) Date of birth (19) (24) Age in years (25) (26)

(Name)

Ethnic group (27) (28) Other group (29) (30) Years in school (31) (32) Occupation (33)

Community (geographical location) (34) (35) Location Urban (1) Periurban (2) Rural (3) (36)

Other data (37) (38) Other data (39) (40)

Other data (41) (42) Extra-oral examination (43) (44)

Dentition status by tooth surface

	17	16	55 15	54 14	53 13	52 12	51 11	61 21	62 22	63 23	64 24	65 25	26	27	
Occ					X	X	X	X	X	X					(45–52)
Mes															(53–66)
Buc															(67–80)
Dis															(81–94)
Oral															(95–108)

	47	46	85 45	84 44	83 43	82 42	81 41	71 31	72 32	73 33	74 34	75 35	36	37	
Occ					X	X	X	X	X	X					(109–116)
Mes															(117 –130)
Buc															(131–144)
Dis															(145–158)
Oral															(159–172)

Primary teeth	Permanent teeth Status
A	0 = Sound
B	1 = Caries
C	2 = Filled w/caries
D	3 = Filled, no caries
E	4 = Missing due to caries
–	5 = Missing for another reason
F	6 = Fissure sealant
G	7 = Fix dental prosthesis/crown, abutment, veneer
–	8 = Unerupted
–	9 = Not recorded

Figure 2.3 WHO caries assessment chart for DMFS/dmfs (Adapted from WHO, (2013); Nguyen et al., (2023)

Nguyen et al. (2023) also documented further details on calculating DMFS/dmfs and DMFS/dmfs as specified in Table 2.3.

Table 2.3 Codes and box numbers in calculating dmft/DMFT and dmfs/DMFS in WHO Oral Health Assessment Form for Children (by tooth surface)

Index	Codes	Box numbers
dmft	B, C, D, and E	#95-#108 and #159-#172
DMFT	1, 2, 3, and 4	#95-#108 and #159-#172
dmfs	B, C, D, and E	#45-#94 and #109-#158
DMFS	1, 2, 3, and 4	#45-#94 and #109-#158

DMFT and dmft: decayed, missing, and filled teeth for permanent and primary teeth; DMFS and dmfs: decayed, missing, and filled tooth surfaces for permanent and primary tooth surfaces.

2.6 BMI and dental caries

Diet, poor health behaviours, and other genetic and environmental risk factors are believed to be general contributors to both obesity and dental caries (Palmer, 2005). Empirical findings indicated a positive correlation between obesity and dental caries. (Gerdin et al., 2008). However, this observation has been challenged by previous studies (Lempert et al., 2014; Li et al., 2015). The relationship between BMI and dental caries is an important area of study, as both obesity and dental caries are prevalent health concerns in children worldwide. A Study by Li et al. (2017) in adolescents of Hong Kong concluded that that tooth brushing frequency at 15-18 years of age was correlated with their BMI, waist circumference and waist-to-hip & waist-to-height ratio. In Saudi Arabia, these issues are also of particular concern due to changing dietary habits and lifestyles.

BMI is a commonly utilized instrument for evaluating nutritional health of children. It is calculated by dividing an individual's weight in kilograms by the square of their height in metres. A study by Khanna et al. (2022) assessed BMI in children and further stated that it is age- and sex-specific, and is categorised into underweight, normal weight, overweight, and obesity. Another study by Satman et al. stated that while BMI in general is primarily used to assess weight status, it is also indicative of an individual's overall health, as it can be influenced by factors such as diet, physical