INVESTIGATION OF SKELETAL RELATIONSHIPS, TOOTH ABNORMALITIES, AND BIOCHEMICAL COMPOSITION OF SALIVA IN CLEFT LIP AND PALATE PATIENTS

MUSTAFA QADEER

UNIVERSITI SAINS MALAYSIA

2023

INVESTIGATION OF SKELETAL RELATIONSHIPS, TOOTH ABNORMALITIES, AND BIOCHEMICAL COMPOSITION OF SALIVA IN CLEFT LIP AND PALATE PATIENTS

by

MUSTAFA QADEER

Thesis submitted in fulfilment of the requirements
for the degree of
Doctor of Philosophy

September 2023

ACKNOWLEDGEMENT

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيم

In the name of Allah, the most beneficent, the most merciful!

First and foremost, I would like to thank Allah for all of the blessings bestowed upon me during my study. I would like to pay sincere gratitude to my supervisor Dr. Saidi Jaafar for his moral support, unparalleled patience, exceptional advice and everything that he has done to help me along the way.

I would also like to thank my co-supervisor Associate Professor Dr. Mohd Fadhli Khamis for his extraordinary guidance, efforts and understanding towards my project. I am especially indebted to my field supervisors Professor Dr. Muhammad Qasim Saeed, for his guidance, insightful comments and encouragement and endless help. I would like to dedicate very special thanks to all my senior colleagues Dr. Haris Khan, Dr. Salman Aziz and Dr. Abdul Samad Khan. They have truly inspired my passion for research, helped me continue believing in myself and strengthened my motivation to continue moving forward along this journey.

Most importantly my family, the love and support of my father, brothers and sister was priceless! Without them it would not have been possible for me to complete this work. I cannot thank them enough for what they have done throughout my entire life! Finally, the understanding of my wife and the love of my daughters Anoushay and Rameen made it all happen! I thank them for standing by my side every hour of the day!

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

LIST OF SYMBOLS

as	Asymmetric vibrations
a.u.	Arbitrary units
δ	Bending vibrations
°C	Degree celcius
µm	Micrometre
µL	Microliter
HU	Hounsfield unit
kV	kilovolt
g	gram
mA	milliampere
mm	millimetre
Str	Stretching
s	Symmetric vibrations
v	Stretching vibrations
cm^{-1}	per centimetre
%	Percentage

LIST OF ABBREVIATIONS

AES	Atomic emission spectroscopy
AMELEX	Amelogenin
ATR	Attenuated Total Reflection
BCLP	Bilateral cleft lip and palate
BP	Bilateral cleft lip and palate Precipitate
BS	Bilateral cleft lip and palate Supernatant
CA	Cluster analysis
CAP	Capillary electrophoresis
CBCT	Cone Beam Computed Tomography
CLP	Cleft lip and palate
CMH	Combined Military Hospital
CP	Control Precipitate
CS	Control Supernatant
DNA	Deoxyribonucleic acid
EDX	Energy-Dispersive X-ray
ELISA	Enzyme linked immunosorbent assay
ENAM	Enamelin
FTIR	Fourier Transform Infrared Spectroscopy
Micro-CT	Micro-computer tomography
MIH	Molar incisor hypomineralisation
NMR	Nuclear magnetic resonance spectroscopy
PCA	Principal component analysis
RID	Radial immunodiffusion

RS	Ramen spectroscopy
SELDITOF	Surface enhanced laser desorption / Ionization Time of Flight Mass Spectrometry
SEM	Scanning Electron Microscopy
T-RFLP	Terminal restriction fragment length polymorphism method
TRFs	Terminal restriction fragments
UCLP	Unilateral cleft lip and palate
UP	Unilateral cleft lip and palate Precipitate
US	Unilateral cleft lip and palate Supernatant
XMT	X-Ray Micro Tomography

PENYIASATAN HUBUNGAN SKELETAL, KEABNORMALAN GIGI DAN KOMPOSISI BIOKIMIA AIR LIUR DALAM KALANGAN PESAKIT SUMBING BIBIR DAN LELANGIT

ABSTRAK

Sumbing bibir dan lelangit (CLP) yang menjejaskan kebanyakan neonat saban tahun, merupakan salah satu kecacatan kelahiran lazim dan didapati berhubung kait dengan pelbagai keabnormalan orofasial lain termasuk hipoplasia enamel gigi dan kecacatan skeletal. Beberapa kajian telah menyiasat keabnormalan gigi yang berkait dengan pesakit CLP. Namun begitu, perincian struktur dalaman gigi (ketebalan enamel, ketumpatan enamel dan ketebalan dentin) serta komposisi biokimia air liur pesakit dengan CLP tidak tersedia. Matlamat menyeluruh kajian ini ialah untuk menilai hubungan skeletal, struktur gigi, saiz gigi dan komposisi biokimia air liur dalam kalangan pesakit CLP. Satu kajian penilaian rekod retrospektif telah dijalankan di Jabatan Ortodontik dan Radiologi Oral, Kolej Perubatan dan Institut Pergigian CMH-Lahore. Sebanyak 4152 pengisihan data tomograpi berkomputer alur kon (CBCT) telah dijalankan dan seramai 73 pesakit yang sumbing dimasukkan dalam kajian untuk mengenal pasti hubungan skeletal dalam jenis sumbing yang berbeza. Sementara itu, seramai 84 pesakit CLP yang tiada sindrom (41 pesakit sumbing bibir dan lelangit unilateral (UCLP) dan 43 pesakit sumbing bibir dan lelangit bilateral (BCLP)) dan 39 orang subjek kawalan telah dipilih untuk dikenal pasti keabnomalan gigi mereka. Ketebalan enamel, ketumpatan enamel, ketebalan dentin dan saiz gigi bagi gigi-gigi kacip maksilari kekal dan gigi-gigi taring telah diukur dari imbasan CBCT. ANOVA dua hala dengan kesan interaksi telah digunakan untuk memeriksa jika jantina mempunyai kesan tambahan kepada sumbing pada pelbagai ukuran gigi. Satu kajian keratan rentas telah dijalankan dimana sampel air liur dikumpul daripada

27 UCLP, 27 BCLP dan 27 individu kawalan. Mendakan dan supernatan sampel air liur telah dianalisis menggunakan spektroskopi inframerah transformasi fourier (FTIR) dan imbasan mikroskopi elektron/sinar-X penyebaran tenaga (SEM/EDX). Keputusan hubungan skeletal menunjukkan bahawa skeletal kelas III didapati mendominasi kedua-dua jantina, diikuti oleh kelas II dan kelas I ($p > 0.05$). Bagi keabnormalan gigi, ketebalan enamel, ketumpatan enamel, lebar mesiodistal, tinggi korona, panjang akar dan panjang gigi telah berkurangan dengan ketara pada pesakit dengan CLP berbanding individu kawalan ($p < 0.05$) sementara dentin tidak menunjukkan perbezaan ketara bagi kebanyakkan gigi yang diukur ($p > 0.05$). Kehilangan gigi kacip lateral (81.4%) didapati lebih banyak bagi BCLP. Bentuk akar atipikal adalah lebih ketara dalam gigi kacip lateral kanan bagi BCLP ($p < 0.05$). Analisis FTIR keatas air liur menunjukkan kehadiran fosfat tidak organic, protin, lipid dan hormone dalam sampel yang diuji. Perbezaan jelas puncak spektrum FTIR diantara kumpulan sumbing dan kawalan diperhatikan, terutamanya untuk fosfat tak organik, amida I dan amida II. Keputusan SEM mendedahkan rangkaian filamen yang saling berkait dengan air liur individu normal. Sementara itu, rangkaian filamen ini tidak terdapat dalam kalangan pesakit CLP. Sebagai tambahan kepada variasi dalam saiz gigi dan ketumpatan enamel yang berkurangan, komposisi biokimia yang tidak normal serta morfologi permukaan air liur boleh menyumbang kepada peningkatan kerentanan karies pada pesakit dengan CLP. Implementasi awal strategi pencegahan penjagaan kesihatan oral untuk menambahbaik status kesihatan mulut adalah penting bagi pesakit CLP.

INVESTIGATION OF SKELETAL RELATIONSHIPS, TOOTH ABNORMALITIES, AND BIOCHEMICAL COMPOSITION OF SALIVA IN CLEFT LIP AND PALATE PATIENTS

ABSTRACT

Cleft lip and palate (CLP) that affects many neonates annually, is one of the most common birth deformities and has been found to be linked with various other orofacial abnormalities including dental enamel hypoplasia and skeletal defects. Several studies have investigated some dental anomalies associated with CLP patients. However, details of the internal tooth structure (enamel thickness, enamel density and dentine thickness) as well as biochemical composition of saliva in patients with CLP are not available. The overall goal of this study is to assess skeletal relationships, tooth structures, tooth size and biochemical composition of the saliva in patients with CLP. A retrospective record review was conducted in the Department of Orthodontics and Oral Radiology, CMH-Lahore Medical College and Institute of Dentistry. 4152 cone beam computed tomography (CBCT) data sorting was performed and 73 patients with cleft were included to identify skeletal relationships in different cleft-types. Meanwhile, 84 'non-syndromic' CLP patients (41 unilateral cleft lip and palate (UCLP) and 43 bilateral cleft lip and palate (BCLP)) and 39 non cleft subjects were selected to identify tooth abnormalities. Enamel thickness, enamel density, dentin thickness, and tooth size of the permanent maxillary incisors and canines were measured from their CBCT scans. Two-way ANOVA with interaction effect was applied to examine if gender has an effect in addition to cleft on various measurements of teeth. A cross-sectional study was conducted where saliva samples were collected from 27 individuals with UCLP, 27 individuals with BCLP, and 27 non-cleft individuals. Precipitate and supernatant of this saliva samples were analised by using

fourier transform infrared (FTIR) spectroscopy and scanning electron microscopy/energy-dispersive X-ray (SEM/EDX). Results of the skeletal relationships showed that the skeletal class III were found to be predominant in both sexes, followed by class II and class I ($p > 0.05$). For tooth abnormalities, the enamel thickness, enamel density, mesiodistal width, crown height, root length and tooth length were significantly smaller in patients with CLP compared to non-cleft individuals ($p < 0.05$) while dentine showed no significant difference in most of teeth measured ($p > 0.05$). Missing lateral incisor (81.4%) was found to be more common in BCLP. Atypical root shape was more prevalent in right lateral incisor in BCLP ($p < 0.05$). FTIR analysis of saliva showed the presence of inorganic phosphates, proteins, lipids, and hormone in the samples tested. Visible difference between FTIR spectra of cleft and non-cleft individuals, specifically in bands of inorganic phosphates, amide I, and amide II were observed. SEM results revealed interconnected filamentous network in the saliva of normal individuals whereas this filamentous network was not present in patients with CLP. In addition to variation in tooth sizes and decreased enamel density, abnormal biochemical composition as well as surface morphology of saliva may contribute to increase caries susceptibility in patients with CLP. Implementation of early oral health care prevention strategy to improve oral health status are essential in patients with CLP.

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Cleft lip and palate (CLP) is the most common birth defect of the face, occurring globally with an incidence of approximately 1 out of every 700 live births (Dixon *et al.*, 2011). CLP is a complex abnormality with both genetic and environmental risk factors contributing to its aetiology (Murray, 2002; Dixon *et al.*, 2011). About 27% of CLP may require orthognathic surgery to correct skeletal problems, while 45% need an orthodontic treatment to improve their dental aesthetics (Ross, 1987). Skeletal anomalies, particularly maxillary hypoplasia, were common in CLP patients with repaired lips (Felemovicius and Taylor, 2009). Other abnormalities such as dental anomalies including enamel hypoplasia and defects of the orbicularis oris muscle were also observed in patients with CLP (Eerens *et al.*, 2001; Jugessur *et al.*, 2009; Tan *et al.*, 2012). In addition, salivary gland aplasia, a very rare developmental defect, as well as changes in saliva composition, have also been reported in patients with CLP (Matsuda *et al.*, 1999; Aizenbud *et al.*, 2008; Reija *et al.*, 2013). Individuals with CLP also seem to be at increased risk for dental caries (Antunes *et al.*, 2014; Wells, 2014; Shashni *et al.*, 2015).

Previous study to examine developmental deformities in patients with CLP used panoramic radiograph as the diagnostic tool (Tortora *et al.*, 2008), however this method did not allow an accurate description of the structural changes in the enamel, dentin and pulp of the tooth. Now, cone beam computed tomography (CBCT) three-dimensional (3D) imaging methods can be utilised to provide better description of internal structures of the tooth with certain measurements (Shah *et al.*, 2014) and can also be used to assess skeletal relationships in patients with CLP. Furthermore, the

structural deficiencies of enamel and dentin in CLP can also be evaluated by comparing them with a non-cleft group in order to identify the differences. This current study provides the way in the detection of skeletal and dental abnormalities at an early stage where the enamel thickness and density in patients with CLP will be observed and compared. It was well established that children with low mineral content can suffer from tooth sensitivity and increased risk of tooth decay (Farah *et al.*, 2010a). Therefore, an early diagnosis of enamel density in the teeth of patients with CLP will be helpful in enhancing the treatment of such patients by providing effective dental care.

There are numerous reports available regarding tooth length in patients with CLP. Bohn (1963) stated that the root of the permanent central incisor on the cleft side was shorter than that of the corresponding tooth on the non-cleft side. Many other researchers using panoramic radiography reported that 5.1% to 44.1% of unilateral CLP (UCLP) patients had abnormal upper incisors with root malformations (Hellquist *et al.*, 1979; Dewinter *et al.*, 2003; Tortora *et al.*, 2008). A later study, also using panoramic radiography measured the root lengths and crown-root (R/C) ratios of permanent teeth in patients with CLP (Al-Jamal *et al.*, 2010). As previously highlighted, panoramic radiographs have some limitations because teeth on these radiographs can appear to be distorted or blurred (Friedland, 1998) which results in inaccuracy in the tooth and root length measurements as well as shape and curvature of the root. CBCT on the other hand gives highly detailed images that can be shown at any angle thus making linear measurement more accurate (Sherrard *et al.*, 2010). The use of CBCT may be helpful in timely diagnosis of tooth and skeletal abnormalities in order to provide better oral health care to the cleft patients.

Saliva analysis is an important tool in diagnosis of various pathological conditions of oral cavity by understanding its immunological and biochemical components (Dowling *et al.*, 2008). Most of the predisposing factors of gingivitis as well as dental caries and surgical wound healing in patients with CLP have been studied intensively (Ahluwalia *et al.*, 2004; Cheng *et al.*, 2007). Along with abnormal tooth morphology that was common in patients with CLP, there were some morphological changes found in their cervical spine (Rajion *et al.*, 2006) as well as issues of the morphology and function of the salivary gland (Tamasas and Cox, 2017). The dysplastic salivary glands and increased salivary cell proliferation result in significantly reduced saliva flow rate and buffering capacity and increased mucus acidity. These changes may result in the alteration of calcium, phosphate ions and salivary proteins in saliva thus increase susceptibility to caries. The purpose of the saliva analysis reported in this current study is to compare the salivary biochemical composition of CLP and control group. Alteration in biochemical composition of saliva may increase caries susceptibility in patients with CLP. Hence, knowledge of saliva composition may play a vital role to provide attentive dental health care to improve oral health status of such patients.

1.2 Justification of the study

CLP is an inborn facial deformity characterised by maxillary growth reduction. This decrement is caused as the result of congenital growth defect, surgical repair, or scar tissue development. The skeletal relationships of patients with CLP in Pakistani population have not been reported and this current study can immensely help in establishing skeletal relationship norms for this population. Moreover, cleft patients should be pre-emptively screened in their early life to avoid any skeletal

complications, and to attain better function and aesthetics. Tooth abnormalities are commonly seen in children with orofacial clefts. Children with clefts tend to have different sizes, shapes, and number of teeth as compared to those without clefts. Detail information regarding internal structure of teeth is still lacking in the literature. A study conducted by Chu and co-workers in mice model with cleft discovered that the incisors of these mice were presented with thinner enamel and reduced enamel density (Chu *et al.*, 2016). To the best of our knowledge, there is no study conducted to assess enamel thickness and density in patients with CLP. Therefore, one of the main purposes of this cross-sectional study was to assess structural tooth abnormalities in different type of clefts and compare it with control non-cleft group to identify any differences. The knowledge of internal structure of the teeth in CLP patients is important for restorative procedures to enhance treatment strategy and dental care of cleft patients. Meanwhile, caries can progress through enamel, dentine and may also affect the dental pulp. By knowing the structural differences, the process of caries can be inhibited to prolong tooth vitality with the help of restorative procedures. Apart from enamel thickness, this current study also examined enamel density in patients with CLP. Teeth with low enamel density are at potentially higher risk for caries and tooth decay. Dental practitioner involved in the treatment and rehabilitation process for these patients should take this into consideration and should support caries prevention protocols.

Previously, panoramic radiograph was utilised to examine developmental deformities of the tooth in patients with CLP. However, the panoramic radiographs have some limitations because teeth on these radiographs can appear to be distorted and/or blurred which results in inaccuracy in the tooth measurements (Friedland, 1998). The use of CBCT in this current study gives highly detailed images that can be shown at any angle thus making linear measurement more accurate. Therefore, other

than enamel thickness and density, CBCT was utilised in this current study to also assessed the tooth in regard to mesiodistal width, crown height, root length along with root shape of patients with CLP. Early diagnosis of malformations in root shape and deficient root length in patients with CLP may influence their orthodontic treatment strategy.

Some studies of patients with CLP have noted an increased prevalence of caries but the underlying cause for this increment is unknown. According to one recent study in the cleft mice model (Tamasas and Cox, 2017), the loss of the interferon regulatory factor 6 (Irf6), a gene that have been repeatedly been associated with CLP can have a significant effect on salivary gland morphology and function. This genetics deficiency resulted in increased oral microbial colonisation and susceptibility to caries as well as alveolar bone loss. Biochemical composition assessment of the saliva in this current study may provide some novel insight into the aetiology of caries in CLP.

As mentioned above, detail information regarding tooth structure and tooth size abnormalities in patient with orofacial cleft as well as the cause of increase caries incidence in these patients was not available. Therefore, this study performed detail analysis on the tooth structure and tooth size in patients with CLP. As normal morphology and biochemical composition of saliva is important to prevent caries, this current study conducted analysis of the saliva in patients with CLP to establish the possible cause of increased caries incidence in these patients.

1.3 General objective

The general aim of this study was to assess skeletal relationships, and to investigate abnormalities in tooth structure, tooth size as well as biochemical composition of saliva in CLP compared to non-cleft individuals.

1.4 Specific objectives

1. To determine the prevalence of different cleft types in Pakistani population.
2. To assess different types of skeletal relationships in CLP.
3. To determine and compare tooth structure (enamel thickness, enamel densities, dentine thickness) of maxillary anterior teeth between CLP and non-cleft individuals.
4. To determine and compare tooth size (mesiodistal width, crown height, root length, tooth length, root crown ratio) of maxillary anterior teeth between CLP and non-cleft individuals.
5. To assess differences in root shape (typical vs atypical) of maxillary anterior teeth in CLP as compared to non-cleft group.
6. To determine the prevalence of hypodontia in maxillary anterior teeth between CLP and non-cleft individuals.
7. To compare the biochemical composition of saliva in CLP and non-cleft individuals.
8. To compare surface morphology and the chemical composition of the saliva between CLP and non- cleft individuals.

1.5 Research question

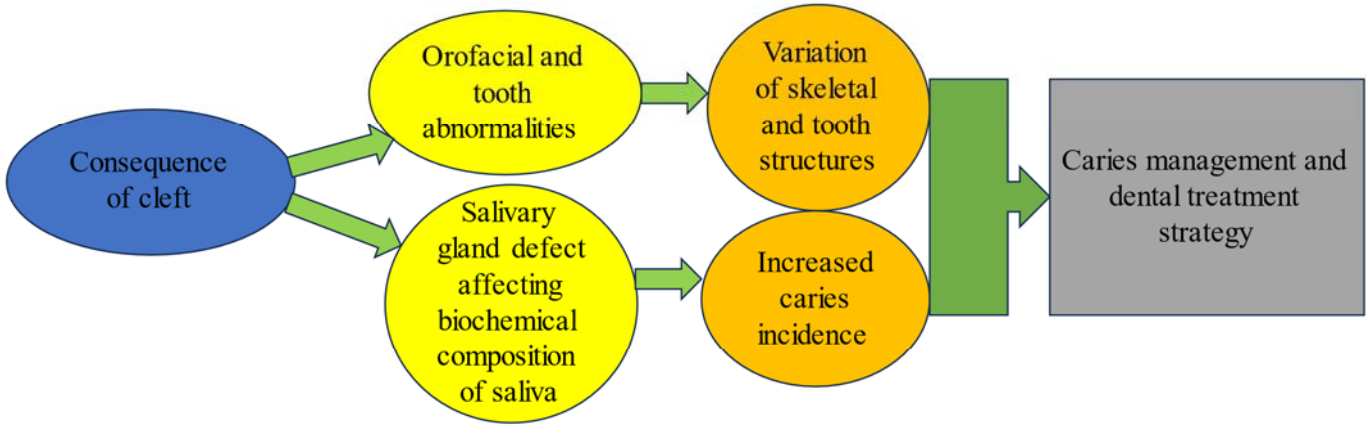
1. What is the prevalence of different types of clefts in Pakistani population?
2. Is there any difference in skeletal relationships in CLP patients?
3. Is there any difference in tooth structure (enamel thickness, enamel densities, dentine thickness) of maxillary anterior teeth between CLP patients and non- cleft individuals?
4. Is there any difference in tooth size of maxillary anterior teeth (mesiodistal width, crown height, root length, tooth length, root crown ratio) between CLP patients and non- cleft individuals?
5. Is there any differences in root shape of maxillary anterior teeth between CLP patients and non- cleft individuals?
6. Is hypodontia is more prevalent in maxillary anterior teeth in CLP patients than non-cleft individuals.
7. Is there any difference in the biochemical composition of saliva between CLP patients and non- cleft individuals?
8. Is there any difference in surface morphology and the chemical composition of the saliva between CLP patients and non- cleft individuals?

1.6 Null hypothesis

1. There is no difference in tooth structure (enamel thickness, enamel density, dentine thickness) of maxillary anterior teeth between CLP patients and non-cleft individuals.

2. There is no difference in tooth size of maxillary anterior teeth (mesiodistal width, crown height, root length, tooth length, root crown ratio) between CLP patients and non-cleft individuals.
3. There is no difference in root shape of maxillary anterior teeth between CLP patients and non-cleft individuals.
4. Hypodontia is not more prevalent in maxillary anterior teeth in CLP patients than in non-cleft individuals.
5. There is no difference in the biochemical composition of saliva between CLP patients and non-cleft individuals.
6. There is no difference in surface morphology and the chemical composition of the saliva between CLP patients and non-cleft individuals.

1.7 Conceptual Framework



CHAPTER 2

LITERATURE REVIEW

2.1 Orofacial clefts

2.1.1 Prevalence and distribution of orofacial clefts

Orofacial clefts (OC) are considered a diversified group of congenital abnormalities of the orofacial structures. Majority of the world's population is affected by the OC which vary significantly among different geographic locations, and ethnic groups. Children born with such anomalies need a greater level of care during their early years. Their speech, hearing, facial appearance, and cognitive impairment can harm their physical health and social life in the long term. Challenges including emotional disturbances, morbidity, social exclusion, and costs of healthcare keep a gateway open for further research in this field (Shaw, 2004; Prahl and Prahl-Andersen, 2007).

The highest number of OC occurrences have been reported in the American Indian population 2.62per 1000 live births. This was followed by Japanese population 1.73:1000, then Chinese population 1.56: 1000 and Caucasian populations1.55:1000 with the lowest rate reported amongst the African population which is 0.58:1000 livebirths. In the Asian population, OC is reported at a ratio of 1.57:1000 live births (Panamonta *et al.*, 2015). Cleft defects show a huge distribution and are affected significantly by variations in ethnicity. Unilateral clefts occur generally four times more frequently than bilateral clefts (Shapira *et al.*, 1999).

CLP is considered a widespread cleft anomaly, affecting 44% (Jagomagi *et al.*, 2010; Yılmaz *et al.*, 2019) of the total cleft patients. The cause of CLP is the failure of both the medial nasal process and the palatal shelves to fuse with the maxillary process

(Nanci, 2017).The prevalence of CLP varies among different populations. It is more common in males compared to females occurring twice as much in the left side than the right side (Jagomagi *et al.*, 2010). Non-syndromic CLP is more prevalent than the syndromic (Cobourne and DiBiase, 2015).

CP which results due to the failure in the fusion of palatal shelves represents 28.7% of all cleft patients (Yılmaz *et al.*, 2019). It occurs more frequently in females compared to males having a 3:1 ratio (Shapira *et al.*, 1999). Since the palatal shelves in female embryos close slower as compared to their counterparts of the same age, becomes more susceptible and likely to develop CP (Burdi and Silvey, 1969). CL shows approximately 19% of all cleft deformity and occurs more frequently in males than in females. CL results due to lack of union of medial nasal process and the maxillary process. It may affect the lip by forming a minute notch into the vermillion border that may extend up to the nostrils. CL are mostly unilateral and more prevalent on the left side (Jagomagi *et al.*, 2010). Elahi and co-workers in 2014, found that the overall incidence for OC in the Pakistani population is 1.91 per 1000 births. The prevalence of CLP is 0.65 per 1000. The prevalence of CL is 0.80 per 1000 while that of CP is 0.46 per 1000. CL and CLP affect boys more frequently while isolated CP are predominated in girls (Elahi *et al.*, 2004).

2.1.2 Types and classification of orofacial clefts

"Cleft" means separation or split. Cleft formation is due to disturbance during development of an embryo as some of the orofacial structures are unable to unite, and resulted in defects of different types (Nanci, 2017). Great variation occurs in different forms of cleft defects, which can be of primary palate including lip and alveolus. Secondary palate involves the hard and soft palate or both structures simultaneously (Tessier, 1976). Some defects may also extend subcutaneously, hence becoming

difficult in clinical diagnosis (Thornton *et al.*, 1996; Shapira *et al.*, 1999). Cleft may be unilateral (involving one side) or bilateral both sides, and can be complete or incomplete (Thornton *et al.*, 1996). Cleft may also be of syndromic type or may not be related to any syndrome i.e., non-syndromic cleft.

Over the period of years with diversity of OC, a number of different classification systems have been introduced. Inaccurate classifications continue to be a problem. Simple, universal, and practical classification is necessary which must be understandable by the patients, clinicians, and hospital administrators as well. Davis and Ritchie (1922), initially classified clefts based on defect morphology, but Brophy (1921) had an opinion that it should be classified on the basis of understanding of anatomy. Veau (1931) introduced his "landmark Division Palatine", which was more focused on assessment and management of cleft palate. Veau's classification was modified by Fogh-Anderson in 1942, who thought assumed incisive foramen a better landmark rather than alveolar process (Fogh-Andersen, 1942). This classification was supported by Kernahan and Stark in 1958, in which the incisive foramen was declared a useful landmark, and better dividing line according to understanding of facial embryogenesis (Kernahan and Stark, 1958).

In 1960, a committee was made by American Association for Cleft Palate Rehabilitation to develop a new classification system and generated a method that would cover both the embryological and surgical anatomy aspects, thus useful for academicians and clinicians. Kernahan (1971) introduced ''striped-Y'' diagram, that was made principally to ease record keeping. Besides, it also made classification a visual process rather than an abstract and cognitive exercise. The modifications in Kernahan's striped-Y were done by Elsahy in 1973, hence making Kernahan and Stark

classification system predominately applicable in regular clinical practice (Elsahy, 1973).

Furthermore, the classification and standards abbreviations which are recommended by Cleft Palate Craniofacial Journal are valuable in providing new cleft subgroups, that can be useful in future fundamental and clinical research. It was in favour of using simple descriptive terms, cleft lip (CL), cleft lip and palate (CLP), and cleft palate (CP).The addition of different signs i.e. the positive sign (+) to mean ‘‘and,’’ that is, the combination of two features; the positive-or-negative sign (±) mean ‘‘with/without,’’ suggesting the optional inclusion of a second feature; and the virgule (forward slash, or /)mean ‘‘and/or’’(Allori *et al.*, 2017). Suggested groupings are created on the standard abbreviations recommended by Cleft Palate Craniofacial Journal are given below.

- Cleft Lip (CL): Inclusion: Cleft lip only, Exclusion; cleft lip and palate, cleft palate, cleft lip and alveolus

- Cleft palate (CP): Inclusion: Cleft palate only; Exclusion: cleft lip and palate, cleft lip

- Cleft Lip and/or cleft palate (CL/P): Inclusion: Cleft lip + cleft palate cleft lip and palate, Exclusion: no exclusions

- Cleft Lip and palate (CLP): Inclusion: Cleft lip and palate; Exclusion Cleft palate, cleft lip

- Cleft Lip with or without cleft alveolus (CL±A): Inclusion: Cleft lip and alveolus+ cleft lip; Exclusion: Cleft palate, cleft lip and palate, and cleft lip

- Cleft lip with or without cleft palate (CL±P): Inclusion: Cleft lip + cleft lip and palate; Exclusion: Cleft palate

- Cleft palate with or without cleft lip (CP±L): Inclusion: Cleft lip and palate + cleft palate; Exclusion: Cleft lip

The terms that can be added where appropriate are as follows:

i: isolated; U: unilateral; B: bilateral; I: incomplete SM: submucous

2.1.3 Aetiology of orofacial clefts

CLP can result from genetic or environmental factors but usually it has a multifactorial aetiology (Fraser, 1970). The aetiology of syndromic CLP is better understood than non-syndromic CLP (Wong and Hägg, 2004; Krapels *et al.*, 2006). The genes usually involved in syndromic CLP are the one mutated in that specific syndrome. The genes most commonly involved in non-syndromic CLP are mainly transforming growth factor-alpha (*TGFA*) (Ardinger *et al.*, 1989), drosophila msh homeobox homolog-1 (*MSX1*) (Satokata and Maas, 1994), 5,10-methylenetetrahydrofolate reductase (*MTHFR*) (Prescott *et al.*, 2002), and transforming growth factor beta-3 (*TGFB3*) (Vieira *et al.*, 2003). In environmental factors most likely factors involved are smoking (Wyszynski and Wu, 2002), high maternal alcohol use (Munger *et al.*, 1996), folic acid deficiency (Shaw *et al.*, 2002), and seasonal variations (Coupland and Coupland, 1988). When both environmental and genetic factors are present during embryogenic development the chance of CLP increases significantly (van Rooij *et al.*, 2001; Jugessur *et al.*, 2003).

2.1.3(a) Genetics factors

TGFA are widely studied family of growth factors. *TGFA* maps on chromosome (2p13) (Ardinger *et al.*, 1989). It has been known to regulate development of palate (Fitzpatrick *et al.*, 1990), and are found to be present at higher level in the medial edge epithelium (MEE) of palatal shelves. *TGFA* mutation along with maternal smoking together might increase the chances of CL/P formation (Shaw *et al.*, 1996). Moreover, Shaw and co-workers in 1998, demonstrated that the children with TGFA genotype, whose mother was not taking multivitamins (folic acid) periconceptionally were at higher risk of giving birth to a CL/P child (Shaw *et al.*, 1998).

TGFB2 and *TGF3* is a representative of the *TGFB* super-gene family. The location of *TGFB2* is at chromosome (1q41) while *TGFB3* is positioned at chromosome (14q24) (Murray, 2002). *TGFB2* plays a vital role in palatogenesis in combination with other *TGFB* family isoforms. It seems to be express in mesenchymal cells near to MEE. *TGFB2* and *TGFB1* control proliferation of mesenchymal cell as well as production of palatal extracellular matrix, whereas *TGFB3* helps in fusion of the palatal seam (Sanford *et al.*, 1997; Alvarez *et al.*, 2002). Tanabe and colleagues in 2000, showed substantial variations in *TGFA2* polymorphism among non-syndromic CL/P in Japanese population (Tanabe *et al.*, 2000). In comparison, research conducted in the Philippines, observed no association among this specific gene and CL/P development (Lidral *et al.*, 1997).

IRF6 mutations is initially thought to be one of the aetiologic factor causing autosomal-dominant Van der Woude syndrome, which may comprise of CL/Por CP only. Moreover, patients can also exhibit tooth anomalies as well as lip fistulas (Kondo *et al.*, 2002). Later, research showed that common alleles in *IRF6* were linked with

non-syndromic form of CL/P (Zucchero *et al.*, 2004). Birnbaum and colleagues in 2009 also observed the function of *IRF6*, and located an area on chromosome (8q24) associated with CL/P (Birnbaum *et al.*, 2009). Some researchers have noticed that mutant *IRF6* mice showed a hyper-proliferative epidermis, unable to differentiate, resulted in multiple epithelial adhesions that may obstruct oral cavity leading to the formation of cleft palate (Ingraham *et al.*, 2006; Richardson *et al.*, 2006). Thus, *IRF6* is an essential factor of keratinocyte proliferation differentiation switch, development of oral periderm, and spatiotemporal regulation which is essential for ensuring appropriate palatal connection (Richardson *et al.*, 2009). Moreover, it has been revealed that *IRF6* is directly targeted by *p63*, which could cause several malformations including clefts. *p63* stimulates *IRF6* transcription via *IRF6* enhancer element, alteration within which enhances the probability of developing CL only (Thomason *et al.*, 2010).

The association among non-syndromic CL/P and marker located on long arm of chromosome (4q25) has found that cleft susceptibility locus can exist within this area (Carinci *et al.*, 2003). Lidral and co-workers in 1998, noticed a link between two (*Msx1/TGFb3*) genes and non-syndromic cleft patients and found its significant role in cleft pathogenesis (Lidral *et al.*, 1998). Moreover, *Msx1* is essential for *Bmp2* and/or *Bmp4* expression, and act as a downstream target of BMP signalling in various embryonic tissues. In mice, it was found CL/P can develop as result of functional loss or absence of BMP receptor type 1 (*Bmpr1a*) in the facial primordia, while lack of Bmp4 may cause cleft lip only (Liu *et al.*, 2005); this demonstrates the different function of BMP signalling in lip development in comparison to secondary palate.

MTHFR is located on chromosome 1q36, an important enzyme that helps in metabolism of folic acid (Mills *et al.*, 1999). The (C677T) mutation of *MTHFR* may resulted in developing neural tube defects as it decreased the folate level in plasma. It has been found in Irish population that the homozygosity for the folate polymorphism associated with thermo-labile form of *MTHFR* is mostly common in CL/P (Mills *et al.*, 1999). It has also been reported that there is increase in incidence of CL/P due to *MTHFR* mutation in mothers of such children (Carinci *et al.*, 2003). Hence, importance of folate intake (periconceptional) was also highlighted in above studies as its inadequate levels can result in CL/P.

The role of B-cell leukaemia/ lymphoma 3 (*BCL3*) in aetiology of CL/P is still not clear. It is associated to different genes which are responsible for the determination of cell lineage as well as cell cycle regulation. The disruption of the epithelial cell around the sides of the forming maxillary process, growth of underlying mesenchyme to create mesenchymal tissue continuity, and seam development are vital in palate formation. *BCL3* mutation may enhance binding to the transcription factor, might be responsible in inhibiting some genes expressions significant in the growth of developing mesenchyme. Thus, the growth retardation in these cells could result in CLP (Gaspar *et al.*, 2002).

2.1.3(b) Environment factors

Along with genetic risk factors, various environmental risk factors like teratogenic agents can also be responsible in cleft formation (Merritt, 2005; Kohli and Kohli, 2012).As formation of palate and lip complete in 7th to 9th weeks after conception, these factors might be critical earlier to this time in creating cleft disorder. The role of folic acid supplementation during pregnancy is effective. The insufficient amount of folic acid intake may be associated with defects of neural tube, for example

spina bifida (Jia *et al.*, 2011; Carmichael *et al.*, 2012).The odds ratio for CLP for children of such mothers is 4.36-folds greater as compared to mother using folic acid supplementation (Kelly *et al.*, 2012). The mechanism by which folic acid facilitate the closure of neural tube is still unknown. More than 70% of neural tube defects may be prevented by timely and sufficient consumption of maternal folic acid (Kelly *et al.*, 2012). The aetiopathological relationship between folic acid deficiency and OC may be because folic acid plays important role in the proliferation of neural crest cells and facilitate movement into the facial processes (Loffredo *et al.*, 2001). Females of childbearing age may be recommend to consume additional folic acid (400 μg) everyday preconception and continue to use it 12 weeks of pregnancy (Report of the implementation group on folic acid food fortification to the department of health and children, 2008).

The use of tobacco during early pregnancy may result in several complications such as pre-term, low-birth weight children. Heavy smokers are twice as at risk to give birth to children with OC, particularly CLP. The effect was strongly associated with BCLP when compared to UCLP (Honein *et al.*, 2007). It is not proven yet that exposure of mother to smoke can directly increase the chances of developing cleft or it is associated with certain factors especially genes which enhance the release of some specific enzymes responsible for detoxifying cigarette smoke. There are approximately 4000 compounds found in tobacco smoke that includes aromatic amines, which are known to damage proteins and DNA (Nelson, 2001; Hein, 2002). Lammer and colleagues observed (N-acetyl transferase 1, a vital enzyme related with aromatic amine biotransformation in first trimester) and an increased risk of OC associated with maternal smoking (Lammer *et al.*, 2004). The exposure of mother to cadmium, an important factor in cigarette smoke found to have link with CP in animal

models (Ferm, 1971; Chernoff, 1973). Active smokers with an increased exposure to environmental tobacco smoke (ETS) can also decrease folate levels in serum, and red blood cells than non-smokers (McDonald *et al.*, 2002; Mannino *et al.*, 2003). However, it has been shown, periconceptional folic acid consumption did not significantly alter the association between smoking and OC (Honein *et al.*, 2007). Another factor that enhanced the risk of CL in certain strains of mice can be associated with maternal hypoxia (Millicovsky and Johnston, 1981; Bronsky *et al.*, 1986). The nicotine and hypoxia can cause vasoconstriction of either maternal or fetal blood vessels, resulting in increased chances of developing OC, facilitated by low nutrients supply to the developing embryonic tissues (Ross *et al.*, 1973; van Rooij *et al.*, 2001).

Alcohol drinking during pregnancy, apart from developing fetal alcohol syndrome, craniofacial malformation, central nervous system defects, pre and post-natal growth retardation also increases the chances of forming cleft defects (1.5 to 4.7 times) in the neonatal (DeRoo *et al.*, 2016). The association between cleft defect occurrence and maternal drinking is dose dependent (Munger *et al.*, 1996). According to Shaw and Lammer in 1999, the pregnant women who took five alcoholic drinks at one occasion had almost 3.4 times more chances to deliver an infant with CLP whereas low amount of alcohol consumption did not have significant role in CLP formation (Shaw and Lammer, 1999).

The use of epileptic drugs valproic acid and topiramate by females during pregnancy specially in first trimester, have more chances to give birth to a CLP child as compared to females not taking such medicines (Werler *et al.*, 2011; Margulis *et al.*, 2012). Moreover, increase dosage of retinoids, benzodiazepines, corticosteroids, folate antagonists can also enhance risk of formation of defects in the cleft (Carmichael

and Shaw, 1999; Puhó *et al.*, 2007; Carmichael *et al.*, 2009). The excessive use of vitamin A (over 25,000 IU/day) during pregnancy has been found to have detrimental effects in the neonatal and an increased risk of facial deformities including cleft formation (Kohli and Kohli, 2012; Losee *et al.*, 2015). The healthy diet along with pre-conceptional vitamins must be promoted in pregnant mothers as this can play a significant role in reducing chances of neural tube defects, and cleft deformities in developing embryo (Carmichael *et al.*, 2012). Females having systemic diseases like, diabetes are at a higher risk of developing CL/P in their foetus (Correa *et al.*, 2009). Moreover, maternal exposure to exogenous components such as lead, pesticides, and hazardous waste should be avoided to reduce the risks of developing cleft defects (Moreira *et al*., 2016).

2.2 Skeletal problems in CLP

CLP is an inborn facial abnormality characterized by maxillary growth reduction. This decrease is caused as result of congenital growth defect, pressure (palatal muscle) by palatal surgical repair, or scar tissue development (Sadowsky *et al.*, 1973; Hayashi *et al.*, 1976; Öztürk and Cura, 1996). CLP frequently show abnormality in lip structure, raised muscle tension that might harm the development and function of facial structures (Lin *et al.*, 2016). Liu and colleagues in 2011, observed that UCLP operated paitents developed craniofacial defects, and the growth of craniofacial structures is affected mainly in maxilla (Liu *et al.*, 2011). Regarding the jaw relationship, different skeletal patterns have been observed in cleft patients. Romanini and co-workers in 2014 have noticed Class II skeletal relationship before the pubertal growth spurt in UCLP (Romanini *et al.*, 2014) whereas other studies have shown Class I skeletal relationshipto be dominant in various age groups (Scheuer *et*

al., 2001; Heliövaara and Rautio, 2011). Furhermore, some studies also found Class III skeletal pattern post-pubertal growth (Doğan *et al.*, 2006; Liu *et al.*, 2011).

Patients affected from CLP shows defeciency in overall proportions of the maxilla and mandible specifically maxilla in retruted form is seen. The complete cleft's most frequently occurring feature is the impaired anteroposterior growth of the maxilla (Capelozza Jr *et al.*, 1993). Children with clefts showed increase in the inter ocular width, whereas basal and facial maxillary width is observed to be similar with people not having cleft (Smahel and Brejcha, 1983). Patients affected by complete clefts may also show vertical facial changes, decrease in mid facial height posteriorly, and enhanced lower facial height anteriorly.

2.2.1 Reasons affecting facial development in CLP

Maxillary morphology and the severity of cleft have an impact on facial growth. Larger cleft and small size maxilla can badly affect growth of the maxilla (Suzuki *et al.*, 1993). UCLP patients usually have an asymterical anterior maxilla with elevated premaxillary area. The nasal septum deformation was also present with protusion and upward elevation of the anterior nasal spine. A Computed Tomography (CT) study conducted by Kane and co-workers in 2007 on UCLP patients revealed severe bony dysmorphology, alongwith displacement of premaxilla towards the non-cleft side .This study also supported that the entire facial development may be affected in UCLP. They observed quantitative morphometric analysis of UCLP patients by applying matrix analysis of CT landmarks (Kane *et al.*, 2007). In majority of the cases, abnormalities were found to be apparent from the initial phase after lack of union among facial processes (Latham, 1973). These abnormalities may have occurred due to deficient mesenchymal tissue, defeciency of nasal capsules, impingment of septum on the airways (Precious *et al.*, 2001). In CLP patients, nasal airway is under influnce

of phyryngeal, and adenoid flaps. Large flaps can affect size of nasal airway, and compromise facial development (Ren *et al.*, 1993). Children with UCLP due to constricted maxillary arch, commonly tend to have lowered tongue position and experience mouth breathing (Precious *et al.*, 2001).

The size, function, and form of nose in CLP patients is of great importance as this may hinder with normal functions of respiration, particle infiltration, humidification, phonation and olfaction (Howard and Rohrich, 2002). The reduced size of nasal airway can alter speech (Dalston *et al.*, 1992; Warren *et al.*, 1992), smell perception in CLP patients (Richman *et al.*, 1988). The adult UCLP patients may suffer from nasal defects due to initial cleft defect, or early treatment. The operated UCLP may present with narrowing of vestibule on the cleft side vestibule, maxillary restriction, and breakdown of alar attachment on affected side, deviated bony nasal septum and dorsum side of the nose (Verwoerd *et al.*, 1995). These nasal abnormalities constrict nasal cavity and raise nasal resistance to breathing. The reduction in size of nasal airway has influence on breathing mode, and increased rate of mouth breathing (Warren *et al.*, 1990; Drake *et al.*, 1993), which may have an impact on growth and development, and growth of orofacial tissues (Linder-Aronson, 1979; Löfstrand-Tideström *et al.*, 1999).

While the first evaluation of cleft patients by craniofacial teams should take place right after birth, morphological rehabilitation begins with a lip surgery after 3 months of birth and a palate surgery at the age of 1 year (Semb and Shaw, 1998). Primary surgical repair is essential for regular anatomical and functional features. Primary lip and palate repair may also cater to nasal deformities in these patients (Daw and Patel, 2004). Primary plastic surgery, however, may inhibit the anteroposterior

maxillary growth as the tension applied by cheiloplasty on the reconstructed lip along with the scar limit the maxillary growth (Liao and Mars, 2006). This compromised growth of the maxilla can lead to skeletal Class III patterns (Semb, 1991; Semb and Shaw, 1991).

Animal studies provide evidence that the greater pressure and restriction from the restored lip hinder the maxillary growth (Bardach and Mooney, 1984; Bardach and Kelly, 1988). Same type of studies have been done on the humans to link the outcome of lip and palate surgeries on UCLP patients. These studies were carried out in countries like Sri Lanka (Mars and Houston, 1990) and China (Yoshida *et al.*, 1992) and reported a profoundly reduced maxillary length. These studies highlighted that since the surgical impact is greater on the maxillary base and the dental arches, the maxillary anterio-posterior vertical dimensions are decreased rather than the transverse dimensions (Bergland and Sidhu, 1974; Dahl *et al.*, 1981; Enemark *et al.*, 1990).On the other hand, these studies also discovered that various surgical techniques applied during the repair could impact the extent of malocclusion and propotion of scar formation. Some authors have also focused on the association between the contraction of collagen fibers in the granulation tissue and hampered maxillary growth (Mars and Houston, 1990; Kuijpers-Jagtman and Long Jr, 2000).

The effect of surgery on maxillary growth is still a core problem to be resolved (Long Jr *et al.*, 2011). There are disagreements regarding the effect of surgery on maxillary growth and whether lip and palate surgery has a more severe impact on children with CL/P (Mars and Houston, 1990; Normando *et al.*, 1992). Moreover, other aspects related to surgery, such as the type of surgical technique, the timing of the surgery, and the surgeon's skill, may also have an impact on the maxilla. It has

been reported that superior skills of the surgeon decrease the risks of formation of scar in patients with UCLP, and it is more probable that high volume operators have better surgical skills than low volume operators (Prahl-Andersen and Ju, 2006).

2.3 Tooth Abnormalities in CLP

Tooth abnormalities may occur due to genetic and environmental factors. However, the factors that trigger abnormalities most often are specific gene defects, prenatal, and post-natal aetiological factors, which are linked to aberrations in the dimension, shape, structure, and position of teeth (Garn *et al.*, 1963; Sofaer, 1979). It has been seen that the abnormalities are found to be more pervasive in CLP patients as compared to the normal population. These abnormalities are mostly localized in the cleft defect area (Haring, 1976; Lourenço Ribeiro *et al.*, 2003) . The anomalies in CLP may be because of cleft itself or the early rectification of the defects through surgery. Additionally, the severity of abnormalities is significantly related to cleft severity (Schroeder and Green, 1975). The extent of tooth abnormalities differ in various cleft types, gender, and ethnicity (Paradowska-Stolarz *et al.*, 2014; Al-Kharboush *et al.*, 2015).

While the embryo is in its developmental stage, the occurrence of the cleft palate and the development of tooth germs are closely linked to each other at chronological as well as anatomical level (Tonge, 1967). The association between dental anomalies and CLP may be attributed to their adjacent anatomy, and the timing of tooth development as well as the timing of cleft formation. Some genes may perform a significant role in the occurrence of congenital tooth anomalies and OC. For example, two signalling molecules that influence the shape and position of teeth are PAX9 and Msx1 (Nakatomi *et al.*, 2010; Wu *et al.*, 2011). Thus, the aetiology of OC