DENTAL ANOMALIES AND MUSCLE SEGMENT HOMEOBOX 1 (*MSX1*) GENE POLYMORPHISM IN NON-SYNDROMIC CLEFT LIP WITH OR WITHOUT PALATE CHILDREN

AMEL ELDUHMAN B. ELGALI

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DENTAL ANOMALIES AND MUSCLE SEGMENT HOMEOBOX 1 (*MSX1*) GENE POLYMORPHISM IN NON-SYNDROMIC CLEFT LIP WITH OR WITHOUT PALATE CHILDREN

By

AMEL ELDUHMAN B ELGALI

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LIST OF ABBREVIATIONS

BCLP	Bilateral cleft lip and palate
bp	base pairs
Buffer AE	Elution buffer
Buffer BL	Lysis buffer
Buffer BW and TW	Column wash buffer
buffer PB	PCR purification buffer
buffer PE	Column wash buffer
CI	Confidence Interval
CL	Cleft lip
CL±P	Cleft lip with or without cleft palate
CLP	Cleft lip and palate
CPO	Cleft palate only
ddATP	dideoxyadenosine triphosphate
ddCTP	dideoxycytidine triphosphate
ddGTP	dideoxyguanine triphosphate
ddNTP	dideoxyribonucleic triphosphate
ddTTP	dideoxythymine triphosphate
DE	Dens evaginatus
DGGE	Denaturing gradient gel electrophoresis
dH2O	Distilled water
dHPLC	Denaturing high-performance liquid chromatography
DI	Dens invaginatus
DMSO	Dimethyl sulfoxide

DNA	Deoxyribonucleic acid
dNTP	deoxyribonucleotide triphosphate
USM	Universiti Sains Malaysia
IQR	Interquartile range
IRF6	Interferon regulatory factor 6
KRK	Klinik Rawatan Keluarga
LD	Linkage-disequilibrium
LR	Likelihood-ratio
MEE	Medial edge epithelium
MgCl2	Magnesium Chloride
MSX1	Muscle segment homeobox1
NCBI	National center of biotechnology information
NSCL±P	Non-syndromic cleft lip with or without palate
OFC	Orofacial cleft
OPG	Orthopantomogram
PAX9	Paired box 9 gene
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
RARA	Retinoic acid receptor alpha
RFLP	Restriction fragment length polymorphism
RNA	Ribonucleic acid
ROC	Receiver operating characteristic
SD	Standard deviation
SSCP	Single strand conformation polymorphism analysis
SV	Spin vacuum

TGFA	Transforming growth factor alpha
TGFB3	Transforming Growth Factor beta 3
Tm	Melting temperature
TMHA	Temperature-modulated heteroduplex analysis
TNF	Tumor necrosis factor
UCLA	Unilateral cleft lip and alveolus
UCLP	Unilateral cleft lip and palate
ULCLP	Unilateral left cleft lip and palate
URCLP	Unilateral right cleft lip and palate
VWS	Van der Woude syndrome

ANOMALI PERGIGIAN DAN POLIMORFISMA GEN MUSCLE SEGMENT HOMEOBOX 1 (*MSX1*) DALAM KALANGAN PESAKIT DENGAN REKAHAN BIBIR DENGAN ATAU TANPA REKAHAN LELANGIT BUKAN SINDROMIK

ABSTRAK

Rekahan bibir dengan atau tanpa rekahan lelangit bukan sindromik (NSCL±P) ialah satu keadaan anomali karniofasial yang biasa. Lebih kurang 70% daripada pesakit rekahan bibir dan atau tanpa lelangit adalah bukan sindromik. Individu-individu ini dilapurkan mempunyai prevalen anomali gigi yang tinggi dan kajian juga menunjukkan bahawa perkembangan NSCL±P adalah dipengaruhi oleh polimorfisma gen *MSX1*. Tujuan kajian ini dijalankan adalah untuk menentukan kadar prevalen anomali pergigian dan polimorfisma gen *MSX1* 799G>T. Perkaitan antara polimorfisma gen *MSX1* 799G>T dengan pesakit NSCL±P serta yang mempunyai hypodontia berbanding dengan kanak-kanak tanpa rekahan juga dikenalpasti. Kajian hirisan lintang perbandingan telah dijalankan di Hospital Universiti Sains Malaysia bermula September 2014 sehingga September 2015. Keizinan dan persetujuan telah diperoleh. Pemeriksaan gigi secara klinikal ke atas 37 orang pesakit NSCL±P dan 80 orang kanak-kanak tanpa rekahan berumur 7 hingga 13 tahun telah dijalankan diikuti dengan pengambilan orthopantomogram. Kaedah tindakbalas berantai polimerase dan polimorfisma kepanjangan serpihan pembatasan (PCR-RFLP) telah digunakan dalam kajian ini untuk mengenalpasti polimorfisma. Sel bukal dikumpul daripada subjek untuk pengestrakan genomik DNA. Kaedah tindakbalas berantai polymerase (PCR) juga telah digunakan untuk mengamplifikasikan sebahagian gen pada *MSX1* exon 2

menggunakan satu set primer. Produk PCR yang mana tidak boleh dirungkaikan oleh PCR-RFLP telah dihantar untuk analisa jujukan DNA untuk mengenalpasti polimorfisma. Data yang dikumpul kemudian dianalisa menggunakan IBM SPSS versi 22.0. Di dalam kajian ini, purata umur bagi NSCL±P dan kanak-kanak tanpa rekahan adalah di antara 9 hingga 11 tahun. Jumlah perempuan dikenalpasti melebihi jumlah lelaki. UCLP (51.4%) adalah jenis rekahan yang paling tinggi dijumpai dan majoriti daripada pesakit mempunyai CLP pada bahagian kiri (32.4%). Prevalen anomali pergigian dalam kalangan NSCL±P ialah 18.9% (95% CI: 5.7, 32.2) dan kanak-kanak tanpa rekahan ialah 6.3% (95% CI: 0.8, 11.7). Hypodontia dalam kalangan NSCL±P ialah 75% (95% CI: 61.2, 90.2) dan tanpa rekahan ialah 7.5% (95% CI: 1.6, 13.4). Walau bagaimanapun, tiada polimorfisma yang ganjil pada 799G>T, semua sampel (n=117) mengandungi polimorfisma 799G>T yang biasa. Oleh yang demikian, polimorfisma gen *MSX1* 799G>T tidak mempunyai kaitan dengan NSCL±P dan hypodontia. Terdapat perkaitan yang ketara antara NSCL±P dan anomali pergigian dari segi morfologi (p=0.04) dan bilangan (p<0.01). Kadar risiko mempunyai anomali pergigian dari segi morfologi dalam kalangan kanak-kanak NSCL±P ialah 3.5 kali ganda dan dari segi bilangan gigi pula ialah 40 kali ganda berbanding kanak-kanak tanpa rekahan. Kesimpulannya, kadar prevalen anomali gigi dari segi morfologi dan bilangan adalah sangat tinggi dalam kalangan pesakit NSCL±P berbanding kanak-kanak tanpa rekahan. Walaubagaimanapun, perkara tersebut tiada perkaitan yang ketara dengan polimorfisma gen *MSX1* 799G>T. Oleh yang demikian, program penjagaan kesihatan pergigian yang komprehensif adalah sangat disarankan bagi mengatasi impak disebabkan oleh masalah anomali pergigian ke atas kanak-kanak NSCL±P.

DENTAL ANOMALIES AND MUSCLE SEGMENT HOMEOBOX 1 (*MSX1*) GENE POLYMORPHISM IN NON-SYNDROMIC CLEFT LIP WITH OR WITHOUT PALATE CHILDREN

ABSTRACT

Non-syndromic cleft lip with or without palate (NSCL±P) is common craniofacial anomalies. About 70% of cleft lip with or without palate is non-syndromic. These individuals are reported to have high prevalence of dental anomalies and studies suggested that the development of NSCL±P and dental anomalies were contributed by *MSX*1 gene polymorphism. The aim of this study was to determine the prevalence of dental anomalies and *MSX*1 gene 799G>T polymorphism. The association between *MSX*1 gene 799G>T polymorphism with NSCL±P as well as hyopodontia compared to non-cleft children were also determined. A comparative cross sectional study was carried out at Hospital Universiti Sains Malaysia from September 2014 to September 2015. The informed consent was obtained from all subjects. Clinical oral examination for 37 NSCL±P and 80 non-cleft children aged 7 to 13 years old were done followed by the orthopantomogram. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was used in this study to identify the polymorphism. The buccal cells were collected from the subjects for genomic DNA extraction. Polymerase chain reaction (PCR) was used to amplify the partial part of *MSX1* exon 2 by using one set of primer. The PCR product which could not be analyzed by PCR-RFLP was sent to DNA sequencing analysis to identify the polymorphism. The data were analyzed using IBM SPSS version 22.0. In the current study, male outnumbered female. UCLP (51.4%) was the common type of the cleft

and the majority of the patients were presented with left side CLP (32.4%). The prevalence of dental anomalies in morphology in NSCL±P was 18.9% (95% CI: 5.7, 32.2) and non-cleft was 6.3% (95% CI: 0.8, 11.7). Hypodontia in NSCL±P was 75% (95% CI: 61.2, 90.2) and non-cleft was 7.5% (95% CI: 1.6, 13.4). However, there was no rare polymorphism of 799G>T gene, all samples (n=117) contained common polymorphism 799G. Therefore, *MSX1* 799G>T polymorphism was not associated with NSCL±P and hypodontia. There was a significant association between NSCL±P and dental anomalies in morphology (p=0.04) and number (p<0.01). The risk of having dental anomalies in morphology in NSCL±P children was 3.5 times and in number was 40 times more than non-cleft children. In conclusion, the prevalence of dental anomalies in morphology and number was very high in NSCL±P compared to non-cleft children. However, it was not significantly associated with *MSX1* 799G>T polymorphism. It is recommended that a comprehensive oral healthcare program is essential to overcome the impacts of these dental anomalies to NSCL±P children.

CHAPTER 1

INTRODUCTION

Craniofacial anomalies or specifically cleft lip with or without palate (CL±P) is the predominant congenital disorder (Murray, 2002). It is characterized by inadequate separation between nasal and oral cavities (Vieira *et al.*, 2008). CL±P can be an isolated birth defect (non-syndromic) or with other congenital malformations (syndromic) (Murray, 2002). Cleft lip and palate (CLP) were the major cleft abnormalities, whereas the least occurrence cleft was cleft palate only (CPO) (Butali *et al.*, 2011). A cleft can take place on either one side (unilateral, 90%) or both sides (bilateral, 10%) of the face, and the majority (66%) of unilateral clefts happen on the left side (Wyszynski *et al.*, 2003). Craniofacial anomalies affect both facial appearance and teeth development, function and aesthetic, in addition, orofacial cleft (OFC) patients demonstrated a lower quality of life scores compared to non-cleft children (Topolski *et al.*, 2005; Wehby *et al.*, 2006). CL±P is demonstrated as a sign in higher than 300 disorders, several of these disorders with a recognized genetic back ground, similar to del 22q syndrome and Van der Woude syndrome (Lace *et al.*, 2006). Many studies had reported that non-syndromic cleft lip with or without cleft palate (NSCL±P) was due to environmental and genetic factors, however, greater part of the cases of the genetic foundation is unknown (Dixon *et al.*, 2011; Murray, 2002; Reiter *et al.*, 2015).

1.1 Epidemiology of cleft lip and palate

The incidence of CLP was estimated to be 1 in 700 live births worldwide, with a wide variability among races and regions (Murray, 2002). Previous study performed in Northern Ireland among Caucasian population showed that the incidence of CLP was reported to occur in 1.28 for every 1000 live births (Zandi and Heidari, 2011), 1.1 for every 1000 live births among Latin American (Blanco-Davila, 2003) and up to 2 for every 1000 live births in Stockholm County in Sweden (Hagberg *et al.*, 1998). Fogh-Anderson (1967) previously reported that in Denmark, there was a significant increment in cleft since 1942 from 1.5:1000 live births to 1.75:1000 live births in 1971 and 1.89:1000 live births in 1981.

There are differences in the prevalence of CLP according to race and sex (McLeod *et al.*, 2004). In general, males are more vulnerable for CL±P compared to females who are more vulnerable to CP (Baek and Kim, 2007). The higher prevalence of CLP had shown among Asian populations approximately 1:500 live births in comparison to 1:2500 live births among African populations, who showed to have the lowest prevalence (Murray, 2002). Japanese is considered to have the highest rate of CLP among Asians population (Murthy and Bhaskar, 2009). Cooper *et al.* (2000) demonstrated that the incidence among Chinese was 1.12 per 1000 live births. Whereas in Thailand, the occurrences of CLP was 1.56 in every 1000 live births (Mutarai *et al.*, 2008). The incidence of CLP in the Philippines was reported to be higher in individuals of the lower socio-economic group in comparison with those in higher economic status (Murray *et al.*, 1997). Interestingly, Filipinos CLP individuals that were born in Hawaii were at 1.46:1000 which were lower than CLP individuals born in Philippine

1.94:1000 (Murray *et al.*, 1997). Therefore studying the relationship between the prevalence OFC and socio-demographic status had given an important information regarding the etiology (Yaqoob *et al.*, 2013) since it was found to be related to other racial factor and gender. In Malaysia, obviously there was an increasing incidence of CLP reported by National Oral Health Surveys occurrences range from one in 941 (NOHS, 1997) to one in 700 births (NOHS, 2007). CLP occurrence was higher in female compared to male among different Malaysian racial groups (Shah *et al.*, 2015). In Kelantan, a study done by Ayu *et al*. (2003) found that the incidence of CLP was 1:500 live births. Another study conducted in Kelantan Combined Cleft and Craniofacial Deformity Clinic (Combined clinic) reported that CLP individuals registered from 1997 to 2000 were about 760 patients (Normastura *et al.*, 2008).

1.2 Etiology of cleft lip and palate

Even though the genetic and environmental factors have an essential role for NSCL±P, the causes of CLP are not completely understood (Yaqoob *et al.*, 2013). There may be several factors or multifactorial etiology involved.

1.2.1 Genetic factors

CLP is a complex defect and its etiology is considered to include both minor and major genetic effect as well as the environmental factors (Murray, 2002). Historically, Fogh-Andersen was recognized as the first researcher approaching the evidence that orofacial clefting has a strong genetic component. 12% to 20% of non-syndromic orofacial clefts are caused by genetic factors with the rest are caused by the

environmental factors or gene and environment interaction (Fogh-Andersen, 1967). Dixon *et al.* (2011) stated that CLP is found to be caused by a single mutant gene, others by chromosomal abnormalities, and the majority is due to the interface between genetic factors and environmental factors. However, the genetic variations in any particular gene contributed to oral clefts is complex due to possible participation of several genes and different modes of inheritance (Carinci *et al.*, 2007). Most of the previous research suggested that gene-gene and gene-environment reactions almost obviously play a part in CLP development (Murray, 2002; Prescott *et al.*, 2000; Spritz, 2001). With the development of new techniques in molecular biology and methods for studying genetics, advancement has been made to distinguish and identify some of the genes associated with CLP and how they affect the embryonic development of the facial component (Cobourne, 2004).

1.2.2 Environmental factors

Even though genes have a fundamental role in facial embryogenesis, the environment has an important role in modifying genetic effects (Murray and Schutte, 2004). Three main classes of environmental causes have been analyzed. Maternal smoking is one of the teratogens that is found to have a significant impact on cleft development (Little *et al.*, 2004). Romitti *et al.* (2007) reported that the consumption of corticosteroid during the periconceptional period until three months after conception increases the risk of NSCL±P. Another pharmaceutical such as the anticonvulsant drugs and benzodiazepines may increase susceptibility to CLP by maternal ingestion (Murray and Schutte, 2004). It has been demonstrated that a maternal folic acid supplement can significantly decrease the risk for CLP as it is necessary for the synthesis of ribonucleic

acid (RNA) and deoxyribonucleic acid (DNA). Thus, it is essential for embryonic cell proliferation differentiation, and growth, as well as for host defense (Wong and Hägg, 2004). In contrast, other authors reported that folic acid consumption during pregnancy has no effect on CLP occurrences (Hayes *et al.*, 1996). Moreover, vitamins and cholesterol metabolism are also important in influencing human embryonic development (Kumar *et al.*, 2012a; Wong and Hägg, 2004).

1. 3 Dental anomalies in cleft lip and palate

Various researches have stated the occurrence of dental anomalies in contribution with a variety of cleft types, cleft lip (CL), CPO or both (Akcam *et al.*, 2010). CLP individuals are reported to have higher predominance of dental anomalies compared to general populations (Ribeiro *et al.*, 2002). However, different studies stated that dental anomalies related to either microform of OFC or generalized developmental deformities (Harris, 2002). These dental anomalies include the variations in number (hypodontia, supernumerary), morphology (peg-shaped tooth, fusion, gemination, evaginatus, invaginatus), size, and position of developing teeth (Akcam *et al.*, 2010). It may also have a damaging effect on dentition resulting in problems related to aesthetic, phonation and respiration. In addition, it has deterioration effect on chewing and deglutition and other complications related to hearing, speech, appearance, social communication (Glenny *et al.*, 2004; Mossey *et al.*, 2009). Dental anomalies may happen in both permanent and deciduous teeth (Mukhopadhyay and Mitra, 2014). However, previous study demonstrated permanent teeth at the maxillary dental arch have a higher prevalence of dental anomalies compared to mandibular arch (Akcam *et al.*, 2010). The interference of odontogenesis due to causative factors; most commonly

environmental and genetic factors during pre and post-natal lead to dental anomalies (Cakan *et al.*, 2013). Genetic factors have been demonstrated for the dental anomalies seen with craniofacial developmental patients and for isolated tooth malformations (Klein *et al.*, 2013). Dental anomalies take place during various stages of teeth development i.e. numerical anomalies occur during initial formation of the tooth germ. While position anomalies such as ectopic and impaction occurs during tooth eruption. In addition, morphology anomalies take place during morphodifferentiation (Kathariya *et al.*, 2013).

1.4 Genetic study of cleft lip and palate

Genetic epidemiological studies demonstrated that few associating loci incorporating the major gene that was involved in the etiology of CLP (Dixon *et al.*, 2011). Different candidate genes for OFC have been screened for linkage-disequilibrium (LD) with CLP or CPO, similarly, included the etiology of congenital dental anomalies (van den Boogaard *et al.*, 2000). Along these lines, Vieira *et al.* (2008) reported that dental anomalies data and the genetic investigation of CLP gave new chance to map the tendency loci for CLP development. This approach might help in recognizing the evidence of genetic variants that contribute to cleft formation, which would be a conclusive procedure that may permit the improved assessment of recurrence risks for individual families (Vieira *et al.*, 2008). NSCL±P known as a complicated trait resulted by multiple interacting loci, and environmental changes, approximately three to 14 interacting loci present a good quality model for genetic effects in CLP (Vieira *et al.*, 2005). *MSX1* is widely expressed at the sites where epithelial mesenchymal interactions take place during developing vertebrate embryos. The process of palatal

fusion is also controlled by interactive signaling from the mesenchyme to the epithelium, which is mediated by growth factors and extracellular matrix proteins (Reddy *et al.*, 2014). Lidral and Murray (2004) used complete sequencing, extensive scale family and linkage studies proved the importance of *MSX1* in the development of the maxillofacial region, thus *MSX1* polymorphism might be the causative factor for the development of NSCL±P. Interestingly, identification of specific influences has developed with the availability of epidemiological and molecular studies (Jezewski *et al.*, 2003).

1.5 Justification of the study

In Malaysia, until today, there is limited study done on the prevalence of dental anomalies, and no known study on the association between dental anomalies and *MSX1* 799G>T polymorphism in NSCL±P and non-cleft children. Therefore, this study is conducted to determine the prevalence of these anomalies among NSCL±P patients and non-cleft children attended the Dental Clinic of Hospital Universiti Sains Malaysia (HUSM). By understanding these anomalies, it would help in the planning of better management for NSCL±P and non-cleft children. This is because, CLP patients with various dental anomalies will lead to various dental issues such as occlusal vertical dimension (Paradowska-Stolarz and Kawala, 2014), esthetics and malalignment of teeth (Mossey *et al.*, 2009). Crowding causes limited access for the tooth brush and the normal cleansing of the teeth by the saliva and tongue especially at the interproximal region, that affects their oral hygiene (Cheng *et al.*, 2007). Thus, early prevention of complications due to dental anomalies can be part of the prevention strategies and genetic counseling during management of CLP children in the

multidisciplinary clinic. Since the occurrence of CLP and dental anomalies are during the first trimester of gestation, both defects can be characterized as a result of genetic abnormalities. Various studies showed that tooth agenesis observed in NSCL±P were associated with the mutation of *MSX1* gene (Carinci *et al.*, 2007; Satokata and Maas, 1994). However in Malaysia, there is no known study done to show this association. Knowing this association will provide a new understanding on the gene that controls the tooth formation. The finding will also help in estimating the risk of getting dental anomalies. Therefore, it is hoped that the protocol for genetic screening for our own Malaysian population could be developed.

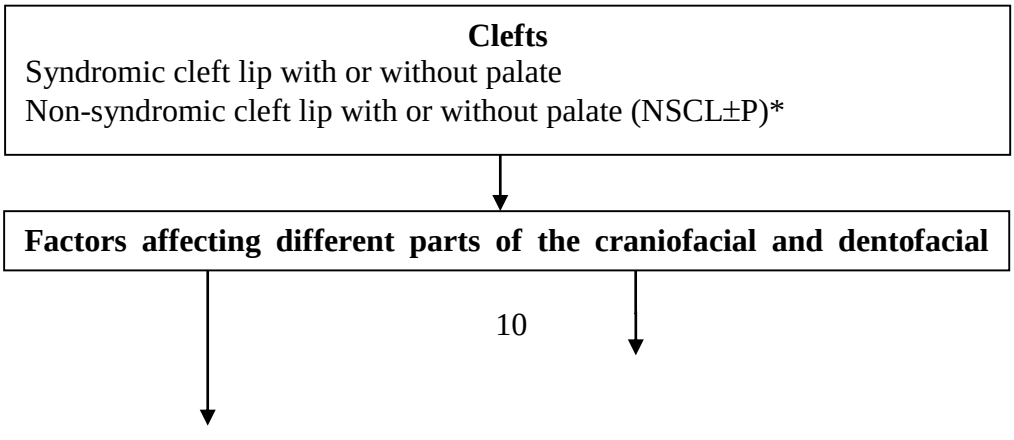
1.6 Conceptual framework

OFC and dental anomalies are due to both environmental and genetic factors (Brook, 2009; Schutte and Murray, 1999). Several candidate of genes contributed to the development of NSCL±P in different populations, and *MSX1* gene was one of the genes strongly related to NSCL±P (Singh and Ramu, 2012). Different variations and mutations of *MSX1* gene have been revealed (rs3775261, rs1042484, rs12532, rs6446693, rs4464513 and rs1907998). 799G>T is one of the variations for *MSX1* gene (Tongkobpetch *et al.*, 2006). Multiple studies have shown that a number of genes may be involved, as well as environmental factors, including parental age, medication use during pregnancy, smoking, and vitamin deficiency such as folic acid deficiency (Jaruratanasirikul *et al.*, 2008; Spritz, 2001). For instance, Little *et al.* (2004) stated that there was a strong evidence that smoking during pregnancy was related to CLP. Furthermore, the maternal smoking (tobacco use) and maternal nutrition during pregnancy would affect intrauterine growth and birth weight (González *et al.*, 2008).

In contrast, it has been reported that the maternal smoking during the first trimester of pregnancy did not contribute to a high risk for oral cleft development (Lieff *et al.*, 1999). Interaction of environmental factor and candidate genes affect the tooth development individually, a group of teeth or the whole dentition. Since the development of the tooth germ undergoes several critical stages, if it fails or it is late, the tooth germ will suffer apoptosis or prevent the development (Brook, 2009). Furthermore, congenital dental anomalies are significantly predominate in individuals with CLP compared to the general population (Shapira *et al.*, 1999).

Dental anomalies in the area of the cleft, include anomalies of number (hypodontia or supernumerary), morphology (peg-shape, tooth fusion, tooth gemination, dens evaginatus and dens invaginatus), time of formation and/or eruption, size, direction of tooth growth, formation and mineralization of enamel (Baek and Kim, 2007), as well as possible ectopic eruption of the canines on the affected side (Aizenbud *et al.*, 2005; Al Jamal *et al.*, 2010). The hypodontia is the most common in individuals with CLP (Ribeiro *et al.*, 2002). This is followed by supernumerary teeth, which represented as the second highest occurrence dental anomaly (Menezes *et al.*, 2010). Peg-shaped tooth is described as the most common morphological anomalies (Al-Kharboush *et al.*, 2015). Both permanent and deciduous dentitions are affected, but these anomalies occur at a greatly higher rate in the permanent dentition (Shapira *et al.*, 1999). The higher incidences of supernumerary teeth and the limited dental arch space attributed to the underdeveloped maxilla which may lead to malalignment of teeth in the CLP patients. Crowding leads to plaque accumulation, and causes gingival problems due to the difficulty to perform good oral hygiene (Al Nuaimi *et al.*, 2014). These patients might need a comprehensive prosthetic and restorative management to recover proper

function, comfort as well as esthetics (Dhanrajani and Jiffry, 1998). Psychological effects of OFC can be interrelated. Nervousness, depression, and palpitations were accounted for about twice in subjects with CLP in comparison with non-cleft children, where these psychological problems were connected with worries about appearance, dentition, and speech (Sousa *et al.*, 2009). Although, the availability of effective and successful treatment of the corrective orofacial cleft abnormality functionally and esthetically but it is still challenged, due to long and expensive of different treatment, especially dental, surgical and speech therapies (Rajendran, 2009).



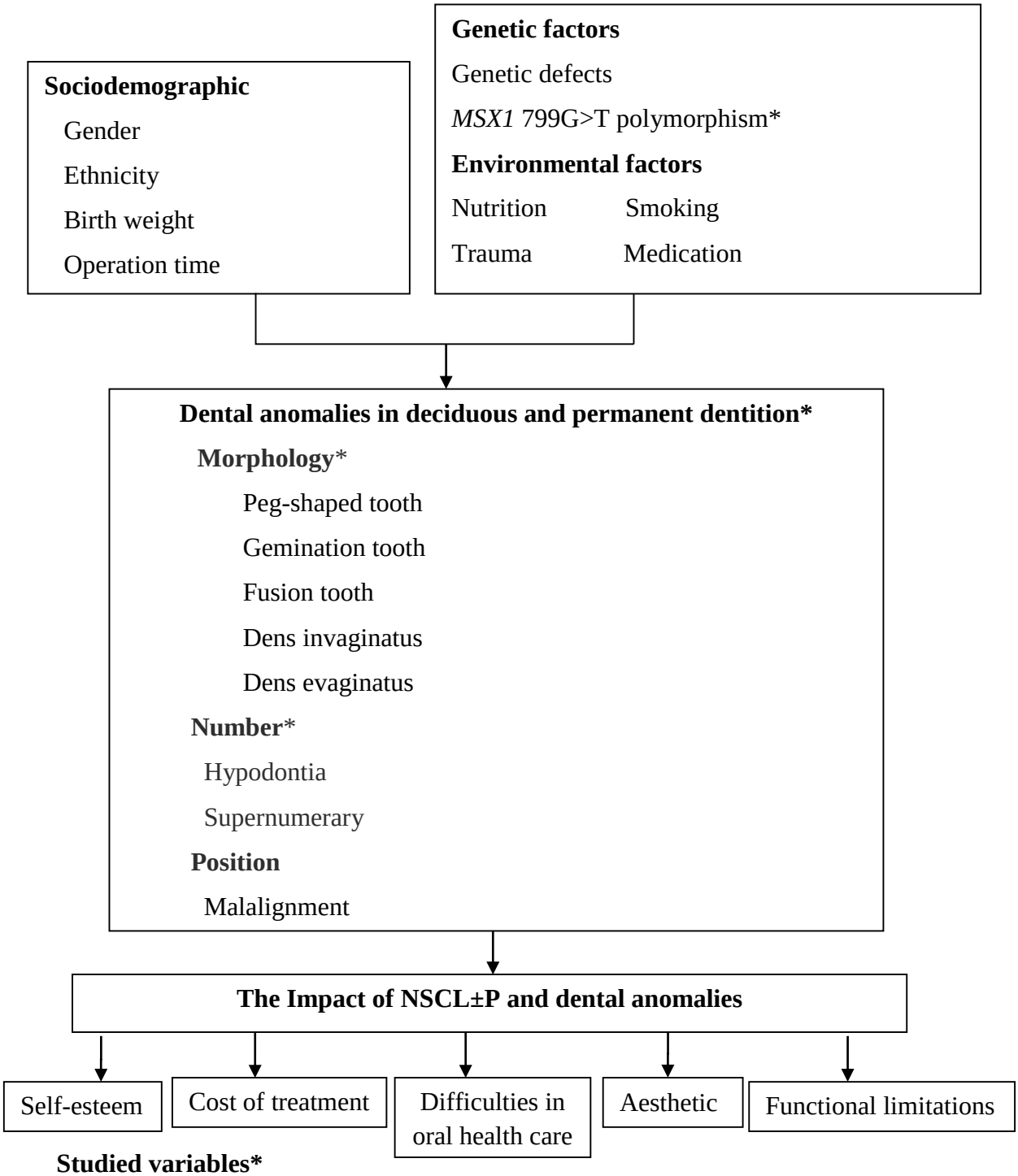


Figure 1.1: Conceptual framework of the study

1.7 Objectives

1.7.1 General objective

To study the dental anomalies and *MSX1* gene polymorphism in NSCL±P children compared to unrelated non-cleft children.

1.7.2 Specific objectives

1. To determine the prevalence of dental anomalies in NSCL±P children and unrelated non-cleft children.
2. To determine the prevalence of *MSX1* 799G>T polymorphism in NSCL±P children and unrelated non-cleft children.
3. To determine the association between dental anomalies and NSCL±P children.
4. To determine the association between *MSX1* 799G>T polymorphism and hypodontia in NSCL±P children.

1.7.3 Research questions

1. What is the prevalence of dental anomalies among NSCL±P children and unrelated non-cleft children?

2. What is the prevalence of *MSX1* 799G>T polymorphism among NSCL±P children and unrelated non-cleft children?

1.7.4 Research hypothesis

1. There is a significant association between dental anomalies and NSCL±P children.

2. There is a significant association between *MSX1* 799G>T polymorphism and hypodontia in NSCL±P children.

CHAPTER 2

LITERATURE REVIEW

2.1 Overview of the cleft lip and palate

Orderly, to realize common types of cleft and their etiology, it is necessary to understand the embryological development and the sequence of lip and palate formation.

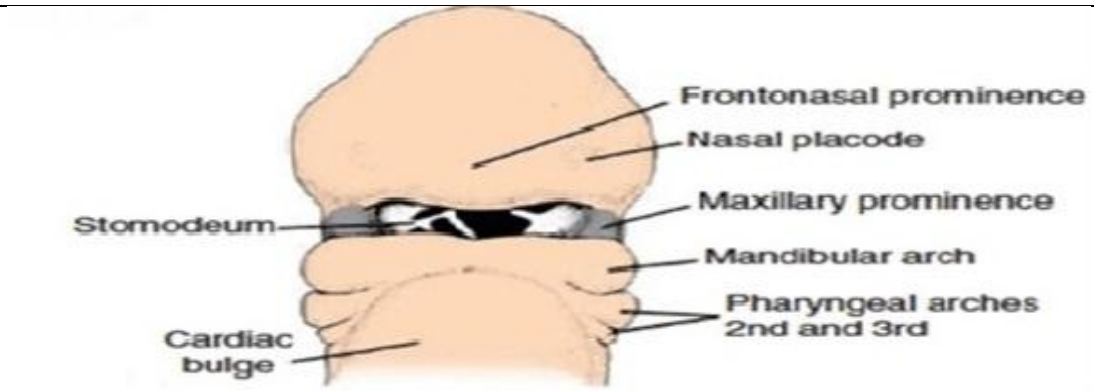
2.1.1 Embryological progress of the lip and palate

The formation of the face starts between fourth to eight weeks of embryogenesis. By the end of the fourth week, the facial primordial prominences are formed, which consists of mainly neural crest that derived from mesenchyme. These facial primordia comprise of five swellings appear around the stomodeum and have a significant role in face development. The prominences are one frontonasal prominence, two maxillary prominences and two mandibular prominences (Sadler, 2011) (Figure 2.1.A). Nasal (olfactory) placode is the thick portion on each side of frontonasal prominence. If the movement of the neural crest and the creation of the prominences are failed or interrupted, it can influence the development of facial structures and bring out clefts or other craniofacial abnormalities. In the fifth week, the nasal placode forms the nasal pit by invagination and form two nasal prominences (medial and lateral nasal prominences) (Figure 2.1.B). Around six to seven weeks of gestation, the maxillary prominence and the medial nasal prominence fuse to form the upper lip (Figure 2.1.C). Hence, the maxillary processes form the lateral parts of the upper lip and the medial

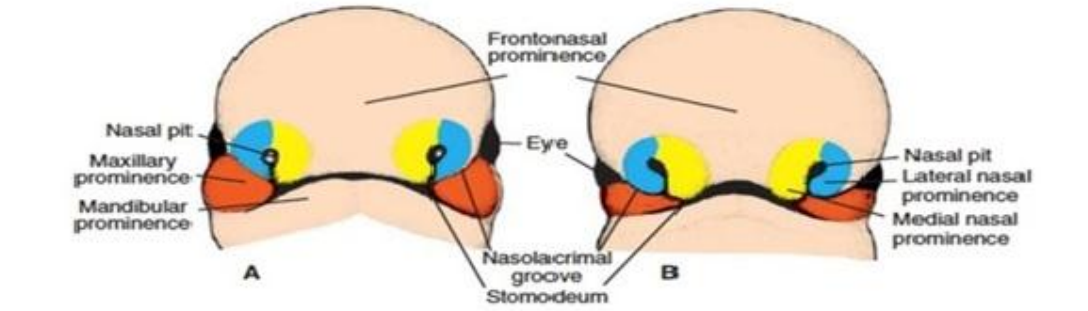
nasal process form the medial part (philtrum). The mandibular prominences fuse across the midline and below the stomodeum to form the jaw and lower lip (Sadler, 2011).

Intermaxillary segment is created by the fusion of two lateral nasal prominences, which consists of philtrum, four upper incisor teeth and primary palate. Secondary palate formed at the beginning of the eighth week of development. Prior to palate formation, the tongue is high and in the area of the nasal cavity and the palatal shelves are vertical and lie on each side of the tongue. Gradually the tongue starts to go down by seventh or eighth week, when this occurs, the palatal shelves move slowly starting a vertical to a horizontal situation and combine. The process of combination proceeds between the palatal shelves moving in a posterior direction from the incisive foramen along the median suture line lead to complete the formation of the hard palate (Figure 2.1.D). The incisive foramen is the landmark between primary and secondary palate. The vomer, forms the portion of the nasal septum, moves downward and fuses with the superior surface of the hard palate, thus completes the separation of the nasal cavities. Around week twelve of gestation and after the formation of the hard palate, the velum and uvula are shaped (Jiang *et al.*, 2006).

A

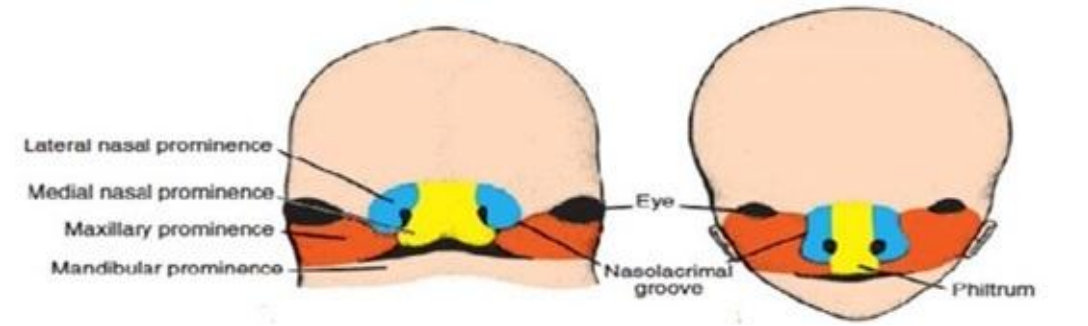


B



Frontal aspect of the face. **A.** 5-week embryo. **B.** 6-week embryo. The nasal prominences are gradually separated from the maxillary prominence by deep furrows.

C



D

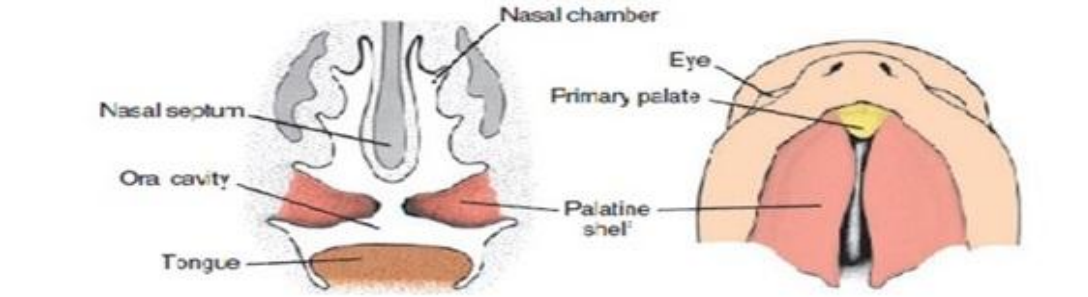


Figure 2.1 The diagram express the embryological development of lip and palate (Sadler, 2011). A. By the end of forth week. B. Fifth and sixth week. C. Seventh to eighth week. D. Palate development.

2.1.2 Pathological embryology of cleft lip and palate

A cleft is a gap or an abnormal opening in an anatomical structure that is ordinarily closed. Generally, patients with clefts have both insufficiency and displacement of normal tissue (Bishara, 2002). Cleft that occurs in the secondary palate only is definitely diverse in mechanism of cleft of primary palate which includes the lip and/or palate (Murray, 2002). A cleft lip occurs when an epithelial bridge fails, as a result of lack of mesodermal production and proliferation from nasal and maxillary processes (Bishara, 2002). Furthermore, it prevents tongue migration to take place earlier, subsequently inhibit horizontal alignment and combination of the palatal shelves. Secondary palate cleft is resulted from the default of union of the lateral palatine processes (palatal shelves), and constantly occurs posterior to the incisive foramen, whereas the primary palate cleft takes place frontal to the incisive foramen (Bishara, 2002).

2.2 Classification of cleft lip and palate

Historically, Kernahan and Stark (1958) have introduced the anatomical classification based on embryological development with the incisive foramen as the dividing point. The CLP are divided into: clefts of primary palate (unilateral, median or bilateral), clefts of secondary palate (incomplete or submucosal, complete) and clefts of secondary and primary palate (median or bilateral, unilateral). A primary palate

structure which is located anterior to incisive foramen combines approximately seven weeks of gestation and involves the lip and alveolus. A primary palate cleft may be bilateral or unilateral. In the case of bilateral cleft of the lip lead to complete division of the tissue, it would usually form philtrum. This abnormal separation for philtral tissue resulted from bilateral cleft is known as prolabium. The separation of triangular shaped premaxilla bone occurs due to bilateral cleft that crosses through both incisive structure lines in alveolus to incisive foramen. Cleft of the secondary palate occur in structures posterior to incisive foramen, the structures consolidate roughly nine weeks of gestation and include velum and hard palate exclusive of the premaxilla (Kummer, 2008).

Bailey and Johnson (2006) from University of Iowa have grouped cleft classification into IV groups based on anatomical structures (Figure 2.2):

Group I: Cleft lip (unilateral or bilateral). It can be incomplete or submucosal, or complete cleft lip.

Group II: Cleft palate that extends from posterior to the incisive foramen, includes secondary palate only.

Group III: Cleft lip and cleft palate include both secondary and primary palate.

Group IV: Alveolar cleft that extends anteriorly of the incisive foramen includes primary palate only.

Thornton *et al.* (1996) stated that surgeons like to describe the cleft according to the location of the cleft lip or cleft palate and may describe it as complete or incomplete. Lidral and Murray (2004) classified OFC into NSCL±P or isolated and syndromic CL±P according to the existence of other developmental retardation or congenital abnormalities. It has been reported that 20–30% of CLP and 40–60% of CPO are

contributed as one of over 400 syndromes (Gorlin *et al.*, 2001) which was in consensus with Murray (2002) who suggested that 70% of CL±P patients are non-syndromic.

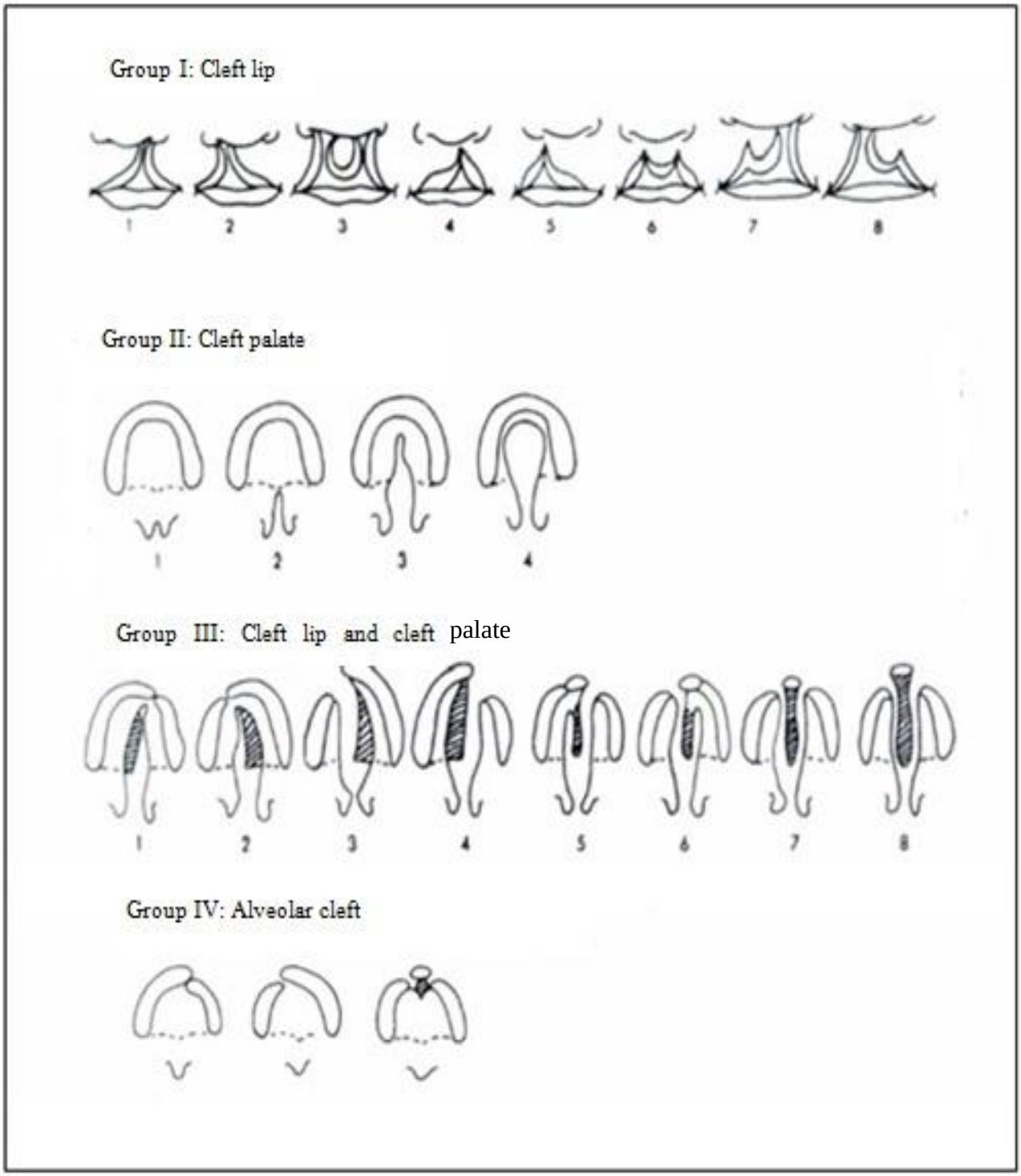


Figure 2.2: Classification of oral clefts (Bailey and Johnson, 2006)**.**

2.3 Dental anomalies associated with CLP

It has been reported that individuals with NSCL±P exhibit a higher frequency of dental anomalies than non-cleft individuals (Lai *et al.,* 2009). These dental anomalies can be in number or morphology (Wong *et al.*, 2012).

2.3.1 Anomalies related to the number of teeth in CLP patient

2.3.1. (a) Hypodontia

The term hypodontia is used when one to six teeth are missing, excluding third molars (Sivakumar Nuvvula *et al.*, 2010). The etiology of hypodontia reported to be as a result of disturbances during the early stages of development and is suggested as a mild dysplastic expression of the ectoderm (Aktan *et al.,* 2010). Genetics plays a crucial role in congenital missing teeth (Fekonja, 2005). The environmental factors which include infection, trauma and drugs were also reported to be associated with missing teeth (De Coster *et al.*, 2009; Goya *et al.*, 2008). A study conducted in Belgium and Netherlands by Dewinter *et al.* (2003) published that at the cleft side, the missing of the lateral incisor was presented higher than 50% of the subjects, compared to missing of second premolars (27.2%) and/or lateral incisors outside the cleft region. It was also reported that the left side was affected twice than the right side and the number of affected teeth was associated with the cleft severity. Rahman *et al.* (2004) in a study conducted on NSCL±P children in Kelantan showed that missing teeth were more common in the bilateral cleft lip and palate (BCLP) compared to unilateral cleft lip and palate (UCLP) and non-cleft group. In addition, the susceptibility of getting

hypodontia for CLP children was 15.3 times higher compared to non-cleft subjects. This result was similar to the finding by Shapira *et al.* (2000) who found that prevalence of hypodontia was 77%, which was significantly higher than non-cleft subjects. The most commonly missing teeth in the cleft area were maxillary permanent lateral incisors, while maxillary second premolars were the most commonly missing teeth in the non-cleft side. The prevalence of hypodontia was 67.7% among Israeli CLP patients, mostly missing of maxillary lateral incisor tooth (Aizenbud *et al.*, 2005), which was also reported in Turkey at 70.8% - 97% (Akcam *et al.*, 2010). Among Taiwanese CLP patients, the prevalence of missing maxillary lateral incisor was 65.8% in the BCLP group, whereas it was 56.7% in UCLP group, and the percentage of missing maxillary second premolars was 19.2% in the UCLP group, and only 7.6% in the BCLP group (Wu *et al.*, 2011). Lekkas *et al.* (2000) suggested that the surgery technique for the closure of the hard palate in early childhood is the most notable etiological factor for the hypodontia in the early operated cleft patient.

2.3.1(b) Supernumerary teeth

Supernumerary teeth refer to those teeth that appear more than a regular number of teeth (Akcam *et al.*, 2010). According to their position in the dental arch, they are divided into mesiodens which positioned between the maxillary central incisors, paramolar that is positioned in the interproximal space buccal to maxillary second and third molars, and distomolar which is known as fourth permanent molar due to its position placed distal to the third molar. Furthermore, it is also classified based on their morphology into conical, tuberculate and supplemental (Meighani and Pakdaman, 2010). The etiology of supernumerary teeth are not well understood and most of the

theories attribute the etiology to the genetic and environmental factors which lead to hyperactivity of dental lamina near the regular tooth bud (Cantín and Fonseca, 2013). Supernumerary teeth were found to be inversely correlated to cleft severity and it presented 16.1% in CLP compared to only 2.8% in non-cleft patients, the frequency of hypodontia was twice the frequency of supernumerary teeth in the general population and CLP patients (Lopes *et al.*, 1991). Another study evaluated dental anomalies in CLP children who attended the London Hospital and St. Andrews Hospital, which revealed that 15.8% of the patients had supernumerary teeth and most of the supernumerary teeth were found in the CL group (Tahir, 1998). The result of other study conducted among Brazilian children with CLP, showed that supernumerary teeth were found in 11.7% of the cases (Tereza *et al.*, 2010).

Among the Caucasian NSCL±P patients, the supernumerary lateral incisors were found in 7.3% of the UCLP patients compared to central incisor at 1.2%. The occurrence was reported as 6.7% for supernumerary lateral incisors and 1.7% for supernumerary central incisors. They found no supernumerary teeth outside the cleft area (Tortora *et al.*, 2008). It was in agreement with the study done in Japan, among UCLP patients, which showed that lateral permanent incisor was the most common tooth to be a supernumerary (Suzuki *et al.*, 1992). However, Galie *et al.* (2009) reported that no supernumerary teeth detected among unilateral cleft lip and alveolus (UCLA) patients. Study done by Akcam *et al.* (2010) in patients with UCLP, observed a highest percentage of supernumerary teeth presented in cleft region, as well as, they stated that the disintegration of the dental lamina during cleft development lead to formation of supernumerary teeth. Taiwanese CLP patients also showed the same trend where the prevalence among UCL group was approximately 15%, followed by the

UCLA group 9.7% and the UCLP group 4.8% (Wu *et al.*, 2011). They concluded that the prevalence of supernumerary teeth in this study was the highest in the UCL group and decreased as the risk of the cleft increased. Supernumerary teeth usually asymptomatic and may be detected by a chance on the radiograph (Mukhopadhyay, 2011; Simoes *et al.*, 2011). Therefore, clinical examination followed by radiographic examination was indicated for the purpose of diagnosis developmental dental anomalies (Correia *et al.*, 2013).

2.3.2 Anomalies related to the morphology of teeth in CLP patient

2.3.2 (a) Peg-shaped tooth

Microdontia or specifically peg-shaped is a tooth that does not fill its space in the dental arch, or a tooth that appears small because of the absence of expected shape (Sousa *et al.*, 2009). Most common forms of localized microdontia is that which affects the maxillary lateral incisors (Chanchala and Nandlal, 2012). According to Shafer, Hine, and Levy (Bargale and Kiran, 2011) microdontia is classified into three types:

Type I: Microdontia included a single tooth only.

Type II: Proportional generalized microdontia.

Type III: Generalized microdontia.

A study done among Taiwanese CLP patients found that the prevalence of peg-shaped lateral incisor was the highest in the UCLA group (61.3%), followed by BCLP (58%), the UCLP (48.2%), UCL (45%) and the lowest percentage was in CPO group (10%) (Wu *et al.*, 2011). Among CLP patients in Kelantan, the abnormalities in the

morphology of teeth were the highest in CLP children (24.5%), in comparison to the non-cleft children (10.1%) (Rahman *et al*., 2004). In addition, according to the different CLP types, an abnormality in dental morphology was 40% in the BCLP compared to only 20.5% in UCLP children. Interestingly, the risk of having morphological teeth anomalies in CLP children was almost four times higher than non-cleft children (Rahman *et al.*, 2004). Lai *et al.* (2009) and Akcam *et al.* (2010) reported that microdontia was higher in BCLP compared to UCLP in Chinese and Turkish children respectively.

2.3.2(b) Fusion and gemination tooth

Fusion is a developmental abnormality produced as a result of two dental germs which were individually developed and then combined during the initiation stage of the tooth development. Tooth gemination is an attempt by the tooth bud to divide that leads to an incomplete splitting into two teeth resulting in a bifid crown with a single root. These anomalies of conjoined teeth is known as double teeth (Saraf, 2006).

In addition, they were commonly found in unilateral mandibular lateral incisor region, and the fusion was more common than germination (Wong *et al.*, 2012). The etiology of fusion is uncertain. The affecting factors such as the physical forces or pressure from dental arch growth lead to close contact between two teeth during tooth development as well as genetic and environmental factors have been demonstrated as probable causes (Veeraiyan and Fenton, 2009; Yücel and Güler, 2006). Among the CLP patients, King *et al.* (2010) reported that double teeth occurred in 0.4% of CLP individuals and 0.1% to 0.8% in normal Chinese individuals which are in agreement