3D-CT CRANIOFACIAL MORPHOMETRY AMONG MALAY DOWN SYNDROME AND NORMAL SUBJECTS IN HOSPITAL UNIVERSITI SAINS MALAYSIA

HAIDER ALI HASAN Al-SHAMMARI

UNIVERSITI SAINS MALAYSIA

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by

HAIDER ALI HASAN Al-SHAMMARI

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LIST OF SYMBOLS, ABBREVIATIONS AND ACRONYMNS

CBCT	Cone beam computed tomography
CT	Computed tomography
DICOM	Digital Imaging and Communications in Medicine
DS	Down Syndrome
3D	Three-dimensional
2D	Two-dimensional
HU	Hounsfield Unit
HUSM	Hospital Universiti Sains Malaysia
ICC	Intra-class correlation coefficient
IGF	Insulin-like growth factor
Mb	Mega base
mm	Millimeter
n	Sample size
SD	Standard deviation
USM	Universiti Sains Malaysia

3D-CT MORFOMETRI KRANIOFASIAL ANTARA SINDROM DOWN DAN SUBJEK NORMAL MELAYU DI HOSPITAL UNIVERSITI SAINS MALAYSIA

ABSTRAK

Kajian ini adalah kajian pertama tomografi berkomputer 3-dimensi (3D-CT), sepanjang pengetahuan penyelidik, terhadap morfologi kraniofasial subjek Sindrom Down (DS) Malay. Maklumat ini diharapkan dapat meningkatkan pemahaman ciri-ciri fenotip kuantitatif DS dalam menambahbaik strategi yang diperlukan untuk pengurusan pesakit-pesakit ini. Untuk mengkaji saiz struktur kraniofasial yang melibatkan pengukuran linear dan angular dalam kalangan subjek DS Malay dan untuk membandingkannya dengan subjek normal. Sebagai tambahan, dimorfisme seks dan perbezaan umur subjek normal dan DS juga telah disiasat.Kajian ini adalah kajian keratan lintang 3D-CT terhadap 240 orang rakyat Malay (Normal=180, DS=60) yang berumur antara 0 hingga 35 tahun. Format awal 2D telah dibentuk semula kepada 3D menggunakan perisian 'Mimics V17.0'. Dimensi linear dan angular struktur kraniofasial setiap subjek telah diukur menggunakan definisi petanda yang konsisten. Perbandingan telah dibuat antara subjek DS dan normal, antara lelaki dan perempuan dan dalam kalangan kumpulan umur yang telah dipilih. Umur yang telah dipilih ialah kanak-kanak (0 hingga 6 tahun), pra-remaja (7 hingga 12 tahun), remaja (13 hingga 20 tahun) dan dewasa (21 hingga 35 tahun). Kebolehpercayaan pemeriksa yang tinggi dalam menjalankan pengukuran telah dikesan dengan nilai ICC dalam lingkungan 0.8 hingga 1. Secara amnya, setiap bahagian kraniofasial menunjukkan pola pertumbuhan unik yang dicerap dari bayi

hingga dewasa. Kebanyakan pengukuran kraniofasial dalam subjek DS menunjukkan nilai yang lebih kecil dari nilai normal sejak dari bayi hingga dewasa. Dimorfisme seks telah dicerap untuk variabel linear dan angular pada kedua-dua subjek DS dan normal.Jumlah variabel yang menunjukkan dimorfisme seks meningkat dari bayi hingga dewasa. Perbezaan saiz antara lelaki dan perempuan adalah tidak ketara semasa bayi dan hanya beberapa variabel menunjukkan perbezaan yang signifikan. Semasa peringkat pra-remaja dan remaja, hanya beberapa variabel yang menunjukkan perbezaan saiz yang signifikan antara jantina. Dimorfisme seks pada bahagian kraniofasial adalah paling ketara semasa dewasa dengan lebih banyak variabel menunjukkan perbezaan signifikan antara jantina.Majoriti variabel kraniofasial meningkat pada saiz secara signifikan dari sejak dilahirkan hingga berumur 35 tahun dalam kedua-dua subjek DS dan normal. Kajian ini menyediakan deskriptif yang komprehensif dimensi kraniofasial subjek DS Malay. Kajian ini telah menjana data rujukan kraniofasial untuk DS dan beberapa tambahan data subjek normal. Data rujukan ini diharapkan dapat memudahkan pengurusan subjek DS Malay. Maklumat ini juga dapat membantu maksilofasial, ortognatik , pakar bedah plastic dan rekonstruktif, pakar ortodontik, pakar medico-legal dan ahli sains forensik dalam menjalankan pengurusan yang objektif dan kuantitatif, menentukan strategi rawatan dan menilai hasil rawatan. Sebagai tambahan, saiz struktur kraniofasial menunjukkan dimorfisme dalam kebanyakan kategori umur. Pertimbangan jantina juga patut diambilkira ketika membuat penilaian klinikal dan prosedur yang mungkin mempengaruhi kompleks kraniofasial. Lagi pula, pengkuantitian berdasarkan data 3D memberikan pemahaman baru terhadap perubahan pertumbuhan kraniofasial dan morfologi berbanding pendekatan konvensional 2D.

3D-CT CRANIOFACIAL MORPHOMETRY AMONG MALAY DOWN SYNDROME AND NORMAL SUBJECTS IN HOSPITAL UNIVERSITI SAINS MALAYSIA

ABSTRACT

This is the first three-dimensional computed tomography (3D-CT) study, as far as the author is aware, of the craniofacial morphology of Malay Down Syndrome (DS) subjects. This information is intended to increase understanding of the DS quantitative phenotypic features in order to improve the strategies required with the management of these patients. To study the size of the craniofacial structure which include linear and angular measurements in Malay DS subjects and to compare them with normal subjects. Additionally, sexual dimorphism and age differences of both the DS and normal subjects were also investigated. This is a cross sectional 3D-CT study on 240 Malays (Normal = 180, DS = 60) aged between 0 to 35 years old. The initial 2D formats were then reconstructed into 3D utilising Mimics V17.0 software. Linear and angular dimensions of craniofacial structures were measured for each subject using consistent landmark definitions. Comparisons were made between DS and normal subjects, between the males and females and among selected age groups. The selected ages are children (0 to 6 years), pre-adolescents (7 to 12 years), adolescents (13 to 20 years) and adults (21 to 35 years). High reliability of the examiner in carrying out the measurements was detected with ICC values in the range of 0.8 to 1. Generally, each craniofacial region showed unique growth pattern as observed from infancy to adulthood. Most of craniofacial measurements in DS subjects showed smaller values than normal from infancy to adulthood. Sexual

dimorphism was observed for linear and angular variables in both DS and normal subjects. The number of variables that showed sexual dimorphism increased from infancy to adulthood. Differences in size between males and females were not obvious during infancy as only a few variables showed significant differences. During preadolescent and adolescent stages, only some of the variables showed significant size differences between the sexes. Sexual dimorphism in the craniofacial region was most evident during adulthood with more variables displaying significant differences between the sexes. The majority of the craniofacial variables increased in size significantly from birth to 35 years of age in both DS and normal subjects. This study provides comprehensive description of the craniofacial dimensions of Malay DS subjects. This study has generated craniofacial reference data for DS and some additional normal data. These reference data is hoped to facilitate management of Malay DS subjects. Moreover, these information could also help maxillofacial, orthognathic, plastic and reconstructive surgeons, orthodontists, medico-legal experts and forensic scientists to perform objective and quantitative management, decide treatment strategies and assessment of treatment outcome. In addition, the size of craniofacial structures showed sexual dimorphism at most age categories. Gender consideration should be taken into account in clinical assessment and procedures which may affect the craniofacial complex. Furthermore, quantification based on 3D data provides new insights into craniofacial growth changes and morphology compared with conventional 2D approaches.

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Craniofacial morphology depends on the hard tissue (bone) and the soft tissue structure which cover it. Quantitative assessment is widely used to estimate the size and the shape of craniofacial morphology. Several medical fields like orthodontics, clinical genetics, maxillofacial and plastic surgery utilize quantitative assessment to facilitate diagnosis, effective treatment planning and postoperative assessment of patients (Ferrario *et al.*, 2000; Fariaby *et al.*, 2006; Baik *et al.*, 2007; Miloševic *et al.*, 2008). The analysis of the human craniofacial structures is a science and art, utilizing both aesthetic and anthropologic tools.

The appearance of the craniofacial morphology particularly the face, being the most variable part of the human body, is influenced by age, sex and ethnicity (Porter and Olson, 2001; Fernández-Riveiro *et al.*, 2002; Porter, 2004). Literature search emphasizes that in addition to sex, age and ethnicity also influences the craniofacial morphology (Choe *et al.*, 2004; Porter, 2004). Among various cultures certain morphological features which are considered as aesthetically pleasing in one culture might not aesthetically appeal the others (Choe *et al.*, 2004). Because of this, normal reference values of craniofacial morphology in various populations, ages and sexes are needed, especially during the assessment, diagnosis, and management of patients with craniofacial deformities (Nagle *et al.*, 2005).

Down syndrome (DS) is a trisomy of chromosome 21 (partial or complete). DS is the most common autosomal aneuploidy among live-births. There are certain characteristic features of patients suffering from DS which involve functional and morphologic changes of body structures. The changes could range from cellular level to multiorgan involvement depending upon the affected individuals (Richtsmeier *et al.*, 2000; Tuxen *et al.*, 2003).

A distinctive craniofacial appearance and immediately recognizable craniofacial phenotype are present in almost all individuals affected with DS (Richtsmeier *et al.*, 2002; Tuxen *et al.*, 2003). In addition to other abnormalities, patients with DS often have a narrowed nasopharynx, a relatively larger and protuberant tongue and often accompanied with cleft lip/palate (Nargozian, 2004).

Subjects with DS present with reduced head size, shorter palpebral fissures and interorbital distance, flattened occiput, smaller maxilla and mandible sizes consequentially resulting in a small midface, small ear length and width and retrusive nasal bone (Richtsmeier *et al.*, 2000; Farkas *et al.*, 2002; Roizen and Patterson, 2003). A concave facial profile is commonly noticed with a shorter mid-face and a prominent mandible and forehead (Tuxen *et al.*, 2003). In addition, variations in the oral tissues, changes in morphology and size of the tongue and dental anomalies can be identified (Quintanilla *et al.*, 2002).

Traditionally anatomists and anthropologists have used dry skulls to evaluate various patterns of craniofacial structures (Rubin, 1997). Osteologic landmarks were the first variables to be measured in order to identify different craniofacial patterns

(Rubin, 1997). Recent advancements have allowed researchers to study living subjects by performing palpation techniques for superficial structures. Anthropometry allows a non-invasive approach to successfully study the craniofacial patterns in the three-dimensions.

For many years, Farkas (1994) applied direct anthropometry technique for studying craniofacial morphology. This approach has been implemented to study craniofacial growth and to compare patients' phenotype to the norms of the population (Farkas, 1996). Anthropometry is an inexpensive, non-invasive and simple tool to study craniofacial anomalies (Farkas, 1996; Allanson, 1997). However, subject's cooperation for data acquisition and capturing are the significant limitations of this approach (Guyot *et al.*, 2003).

Patients suffering from DS have also been examined using radiographic and anthropometric means (Farnas, 2001; Farkas *et al.*, 2002; Richtsmeier *et al.*, 2002; Bagić and Verzak, 2003). The invention of x-ray and cephalograph machines permits measurements on cephalometric radiographs (cephalometry) (Broadbent *et al.*, 1977) which allow an evaluation of the craniofacial morphology possible. The data of these investigations allow the comparison with healthy controls (Ward *et al.*, 2000; Ferrario *et al.*, 2003; Moore *et al.*, 2007).

The innovation of computed tomography (CT) has made it possible to capture and evaluate the entire craniofacial complex. Cormack and Hounsfield pioneered CT in the 1960-70s, for which Nobel prize in Medicine was awarded (Cormack, 1980; Hounsfield, 1980). It is currently among the most commonly utilized investigation

and imaging technique. CT allows sequentially layered imaging without the possibility of distortion and superimposition.

Advances in the CT technology came with the introduction of 3D reconstruction from axial slice data by Herman and Liu (1977). 3D reconstruction facilitates the surgical management plan and avoids the need for arbitrary surgical plans.

1.2 The Malays

The term "Malay Archipelago" refers to the region comprising the Malay Peninsula, Brunei, Singapore, Indonesia and the Philippines (Bellwood, 2013). The term Malay refers to the ethnic group of people living in the Malay Peninsula and East Sumatra (Bellwood, 2006; Bellwood, 2013). The Malays make up about 55% of the total Malaysian populations.

1.3 Problem statement

Studies of the craniofacial morphometry on the DS subjects have been performed in other parts of the world and different ethnicities such as in the United States, Europe, Africa and some Asian countries. However, these data would not be appropriate as a reference for diagnosis and treatment planning of DS patients from other populations. Also, most of the available DS craniofacial data were recorded by applying anthropometrical methods and 2D imaging techniques (Farkas *et al.*, 2001a; Bagić and Verzak, 2003; Ferrario *et al.*, 2004b; Sforza *et al.*, 2005; Korayem and AlKofide, 2014). Only a few studies utilized 3D approaches to obtain reference data for DS subjects (Sforza *et al.*, 2011; Sforza *et al.*, 2012).

Malaysia is a multiracial country where Asian communities (Malay, Chinese, and Indian) form the bulk of the population. Only a few studies on quantitative craniofacial features have been performed on healthy subjects (Abdullah *et al.*, 2006; Yusof, 2007; Hussein *et al.*, 2009; Al-Khatib, 2010). Up to date, no craniofacial reference for DS subjects has been found. Therefore, a detailed quantitative analysis of the characteristics of the craniofacial morphometry in Malay subjects with DS using 3D technology would be useful to both clinicians and researchers.

1.4 Justification of study

The studies of craniofacial morphometry are not only of research interest, but also of clinical importance. Maxillofacial, orthognathic, plastic and reconstructive surgeries and orthodontics treatment procedures were performed on abnormal patients to achieve aesthetically accepted profile and balanced cranio-maxillofacial structures. These treatment methods can be better planned with the presence of a reference data specific for each population.

In this study, DS, in particular, was investigated as in Malaysia there is a relatively high incidence of DS with a reported incidence of 1 in every 950 live births. This could be due to a tendency towards more advanced maternal age which increased the prevalence of DS (Azman *et al.*, 2007). Moreover, the life expectancy of DS subjects has increased significantly in the last few years (Pennington *et al.*, 2003; Horbelt, 2007). With advanced medical care and advanced facilities, DS subjects are living longer and are being more socially compatible than before. With the decreased mortality rate and increased incidence, a trend of growth of DS population in Malaysia is noticed.

Therefore, from the craniofacial perspectives, there is a need for creating some form of reference data specific to age, sex and ethnicity, for a better understanding of their conditions and more importantly to plan effective management options ensuring better outcomes for the affected group of individuals. Ideal treatment outcome is the primary goal of cranio-maxillofacial surgeons and orthodontists. Thus, treatment of any malformations needs accurate data (Farkas *et al.*, 2005). This study has generated craniofacial reference data for DS and some additional normal data. These reference data is hoped to facilitate management of Malaysian DS subjects. Other potential use of these data would be in industrial fields such as construction of facial mask and helmet (Zhuang *et al.*, 2010).

Data from this research can be employed for the development of a computer software program which might predict post-treatment craniofacial appearance. Such computer program will involve complex algorithms for predicting and projecting the craniofacial appearance changes. This vision can be broadened into multi-disciplinary and multicentre research collaborations as it needs contributions and expert advice from mathematicians and computer program specialists.

1.5 Significance of the study

The assessment of craniofacial dimensions is of prime importance in the medical and dental fields for diagnosis, treatment planning and post-operative care. Orthodontists, maxillofacial surgeons, plastic, reconstructive, and orthognathic surgeons often require quantitative information during management of subjects with DS. Information and results of analyses derived from this study will benefit scientific professionals in other fields whom include medico-legal experts and forensic

scientists as this new data will supplement previous accessible information. In addition, these reference data will serve as a valuable resource for craniofacial researchers in the future.

1.6 Objectives

1.6.1 General objectives

To study the craniofacial morphometry in Malay DS and normal subjects in Hospital USM at selected age groups.

1.6.2 Specific objectives

1. To determine and compare the skeletal craniofacial morphometry between Malay DS and normal subjects.
2. To determine and compare the skeletal craniofacial morphometry between the males and females in Malay normal subjects.
3. To determine and compare the skeletal craniofacial morphometry between the males and females in Malay DS subjects.
4. To determine and compare the skeletal craniofacial morphometry among different age groups in Malay normal subjects.
5. To determine and compare the skeletal craniofacial morphometry among different age groups in Malay DS subjects.

1.7 Research questions

1. Is there any difference in the skeletal craniofacial morphometry between Malay normal and DS subjects?

2. Is there any difference in the skeletal craniofacial morphometry between males and females in Malay normal subjects?
3. Is there any difference in the skeletal craniofacial morphometry between males and females in Malay DS subjects?
4. Is there any difference in the skeletal craniofacial morphometry among different age groups in Malay normal subjects?
5. Is there any difference in the skeletal craniofacial morphometry among different age groups in Malay DS subjects?

1.8 Research hypotheses

1. There is significant difference in the skeletal craniofacial morphometry between Malay normal and DS subjects.
2. There is significant difference in the skeletal craniofacial morphometry between males and females in Malay normal subjects.
3. There is significant difference in the skeletal craniofacial morphometry between males and females in Malay DS subjects.
4. There is significant difference in the skeletal craniofacial morphometry among different age groups in Malay normal subjects.
5. There is significant difference in the skeletal craniofacial morphometry among different age groups in Malay DS subjects.

CHAPTER TWO

LITERATURE REVIEW

2.1 Down Syndrome

2.1.1 Definition

Down Syndrome (DS) is a genetically inherited disorder which is characterized by an additional chromosome. It is a congenital autosomal (non–sex chromosomes) syndrome whereby in most cases, three copies of chromosome 21 are present, so the name "trisomy 21" has also been used to characterize this syndrome. This clinical entity was first described in 1866 (Minderer *et al.*, 2003). DS is also known as trisomy G, and mongolism and has been labelled as the most common cause of genetic intellectual disability among humans (Cohen *et al.*, 2003). DS affects approximately 1 in 600 to 1 in 2000 live births and currently affected 350,000 individuals in the United States (Cohen *et al.*, 2003). Males are slightly more affected by this syndrome than females and Hispanics are at higher risk than the rest of the population. Increased age of mother greatly poses a risk of conceiving a child with DS. At 30 years of age, the risk ratio is 1:1000 and increases to 9:1000 by age 40 years (Castilho and Marta, 2010; Areias *et al.*, 2011; Mathias *et al.*, 2011).

DS is characterized by a deficiency in general and mental development (Gorlin *et al.*, 2001). Individuals with DS present with several physical characteristics and systemic manifestations, with the craniofacial manifestations being the most distinctive (Desai, 1997).

2.1.2 Historical information about Down syndrome

Descriptions of DS dated back many centuries. In 1866, John Langdon Down first described the DS. Back in that era mentally retarded patients suffering from cognitive impairments were referred to as 'imbeciles" and "idiots" due to negligence and they were seldom categorized into categories based upon differential diagnoses. John Langdon Down studied the "hierarchical racial classification system" and concluded that individual norms of Mongolian descent were in close resemblance with the patients suffering from DS (Volpe, 1986; Down, 1995).

Down also described that when characteristic features of maternally non-related patients of DS were examined together they closely resembled each other as normally noticed among siblings (Down, 1995). Pertinent to these findings Down classified that patients suffering from DS were characteristically different from other patients suffering from cognitive impairment and therefore he termed these individuals as "mongoloids" or "Mongolian idiots" (Down, 1995). Although many authors before Downs' publication had described the patients with DS, Down is considered the first author to identify DS patients for their characteristic differences with the cognitive impairment patients (Pueschel, 2001; Kava *et al.*, 2004; Mégarbané *et al.*, 2009). While speculation and conjecture often attributed the cause of DS to alcoholism, syphilis, tuberculosis, occupational exposures, and even regression to a primitive human state (Pueschel, 2001), the true cause of DS was not proven until 1959 when Lejeune *et al.* (1959) revealed that a trisomy on the chromosome 21 was identified in DS individuals. This finding was subsequently confirmed by Jacobs *et al.* (1959).

However, after a thorough investigation of DS, Benda (1941) dismissed altogether the idea of a racial mutation or primitive regression to a previous human state as being the cause of DS.

In 1961, the World Health Organization informally suggested to not use the term “mongolism” and to name it Down's syndrome as a result of some medical specialty investigator were line of work to prevent the term “mongolism” and to explain individuals with DS as trisomy 21 anomaly (Howard-Jones, 1979).

2.1.3 Down syndrome and types of chromosomal aberrations

The presence of an additional copy of chromosome 21 is arguably the main reason behind intellectual and physical characteristics of DS. Cytogenetically DS is split into 3 sorts (Giraud and Mattei, 1975). These are:

1. Regular or free chromosomal trisomy 21: all cells have an additional chromosome 21. Around 90-95% of people with DS have free chromosomal aberrations for chromosome 21(Pangalos *et al.*, 1994; Mutton *et al.*, 1996; Savage *et al.*, 1998).

2. Translocation trisomy: the additional chromosome 21 is connected to a different chromosome. Translocation trisomies accounts for 2-4 DS cases encountered. In the majority cases of translocation trisomy, one among the parents is a translocation carrier (Pangalos *et al.*, 1994; Mutton *et al.*, 1996; Savage *et al.*, 1998).

3. Mosaic trisomy 21: it is a free trisomy 21 however just some cells have an additional chromosome 21. Mosaicism is outlined having 2 or a lot of genetically distinct cell lines. Just about 2-4% of DS patients are mosaics (Mutton *et al.,* 1996; Nguyen *et al.,* 2009).

2.1.4 Clinical features of Down syndrome

As previously mentioned, Dr John Langdon Down (1828-1896) was the primary investigator to explain the characteristic clinical signs of DS kids exactly (Ward, 1999). The data of clinical manifestations of DS compiled by physicians and supported by other health professionals are very important for an early identification, in order to reduce morbidity and mortality of those suffering DS kids (e.g. early operation of heart defects). Moreover, correct clinical identification of DS kids is very important to avoid traditional kids being investigated for DS supported solely upon certain clinical features (Devlin and Morrison, 2004).

The most common characteristic clinical features of DS are the morphological differences in facial features, biological developmental delay, hearing and visual abnormalities, gastrointestinal anomalies, congenital heart defects, and malignant neoplastic disease significantly acute megakaryoblastic leukaemia. As DS is related to several inborn abnormalities and health issues, molecular mapping of the thus referred to as Down-critical region (DCR), of chromosome 21 was undertaken. The mapping provided proof that the DCR that spans 0.4 to 3 Mb on 21q22.2 is taking part in a job in pathologic process of DS (Delabar *et al.*, 1992; Sinet *et al.*, 1994). This interval is believed to be answerable for the expression of the many features contributory to retardation, short stature, muscular hypotonicity, joint hyper-

flexibility and different morphological signs i.e. flat nasal bridge, protruding tongue, extremely arched palate, narrow palate, folded ears, short and broad hands, incurved fifth finger and gap between first and second toes (Sinet *et al.*, 1994). In addition, locus D21S55-MX1 that is found in band 21q22.3 is believed to be answerable for the expression of different six morphological options i.e. epicanthus, oblique eye fissure, brushfield spots, transverse palmer crease, short stature and hypotonicity (Sinet *et al.*, 1994).

Moreover, DS is related to several complicated clinical features which could be situated outside the vital region of chromosome 21 indicating that over one region is accountable for the pathologic process of the DS phenotypes (Delabar *et al.*, 1992; Sinet *et al.*, 1994). With relation to the clinical features, it's necessary to emphasise that there's an excellent variability of the frequencies of phenotypical features in individual DS patients.

2.1.5 Genetic basis of Down syndrome

In DS, just about 95% of the cases are because of non-disjunction leading to an additional copy of a chromosome 21 (trisomy 21) as delineated by Lejeune *et al.* (1959). The remaining is because of translocations involving chromosome 21 and somatic mosaicism (Sherman *et al.*, 2005). Most Down's syndrome cases are because of a slip in maternal meiosis, whereby regarding 70 % originated throughout maternal meiosis I (MI) and regarding 20% throughout maternal meiosis II (MII). Defective paternal meiosis is found for up to 8-10% of all cases (Savage *et al.*, 1998; Petersen and Mikkelsen, 2001; Sherman *et al.*, 2005). Despite the fact that important progress has been made in recent years, the causes of the augmented non-disjunction

rate leading to DS are far away from understood. Maternal age, germ line mosaicism, and altered recombination stay the sole well-established risk factors for non-disjunction of chromosome 21 (Sherman *et al.*, 2005).

2.1.6 Risk factors for trisomy 21

2.1.6 (a) Advanced maternal age

Advanced maternal age at the time of conception is the most established and important risk issue for cellular division non-disjunction of chromosome 21 (Sherman *et al.*, 2005; Oliver *et al.*, 2008). Lionel Penrose 1933 was the first investigator from World Health Organization who noted the impact of advanced maternal age on the rate of DS (Penrose, 2009). Regarding approximately 2% of recognized pregnancies of ladies below the age of 25 years are trisomic, this will increase to 10% for ladies of 36 years and to 33% by the age of 42 years (Hassold and Sherman, 2000). The influence of maternal age has been determined altogether among the population studies in relation to race, geographical whereabouts or socioeconomic factors. However, the idea for the impact of increasing maternal age on the non-disjunction rate is basically unclear. In females, meiosis starts within the third month of fetal life and is inactive in prophase of MI from six months of fetal life ahead till ovulation that takes around ten to forty years (Hassold and Sherman, 2000; Warburton, 2005). At the time of ovulation, the oocytes complete MI and accomplish MII wherever they continue to be inactive till they're fertilised and afterward complete the cell division stage MII.

Warburton (2005) explained two hypotheses for the result of maternal age on the non-disjunction rate. One states that totally different variables have an effect on

the oocytes overtime like attenuated expression of checkpoint proteins that maintain sister chromatid adhesion or cell division checkpoint, that accumulated with increased maternal age leading to an increased non-disjunction rate (Jeffreys *et al.*, 2003; Vogt *et al.*, 2008). A second hypothesis is that biological aging of the oocytes is a very important issue which the frequency of trisomic conceptions can rely on the biological age of the women's oocytes, instead of upon the age.

2.1.6 (b) Maternal recombination

Altered recombination is another vital issue after maternal age that is related to non-disjunction error. Warren *et al.* (1987) were the early investigators who provided proof that a proportion of maternal non-disjunction errors were related to reduced recombination on chromosome 21. Many studies relating to the aetiology of DS demonstrated a relationship between the non-disjunction and the altered recombination (Antonarakis *et al.*, 1993; Sherman *et al.*, 1994; Yoon *et al.*, 1996; Sherman *et al.*, 2005). Most of those studies approved that the placement of the recombination could be a risk issue for non-disjunction of trisomy 21(Yoon *et al.*, 1996; Savage *et al.*, 1998; Sherman *et al.*, 2005; Oliver *et al.*, 2008).

2.1.6 (c) Parental germline mosaicism

Parental gonadal mosaicism condition has been recommended by several studies as a risk issue for cases in families with multiple congenital anomaly conceptions(Cozzi *et al.*, 1999; Bruyère *et al.*, 2000). If parental gonadal mosaic is present, the repeat risk is higher and can rely upon the proportion of congenital anomaly cells present within the gonads. Therefore, in families with one affected kid

with free congenital anomaly, it's assumed that the repeat risk estimates to 1-2% on the premise of live births and diagnostic technique(Bruyère *et al.*, 2000).

2.1.6 (d) Mitochondrial (mtDNA) mutations

It has been hypothesised that mtDNA mutations might play a role within the aetiology of DS. the quantity of mitochondrial mutations will increase with age in numerous cells specifically in oocytes (Arbuzova *et al.*, 2002). The authors recommended as a potential clarification that mutations in mtDNA may reduce ATP levels and increase the generation of free radicals, that might successively have an effect on the synaptonemal complicated formation, chromosome segregation, the division spindle, and alter recombination (the enzymes collaborating in recombination and DNA repair are ATP dependent) resulting in abnormality (Arbuzova *et al.*, 2001).

2.1.6 (e) Consanguinity

Consanguineous marriages are historically common among Arab countries. This results in an augmented birth prevalence of infants with recessive diseases, inherent anomalies, morbidity and mortality. Rajab and Patton (2000) expressed that among 60,635 Omani couples, 24.1% were marriages between 1st cousins, 11.8% between second cousins, and 20.4% were among specific tribal groups. People who are closely akin have a better likelihood of carrying rare recessive alleles which might be transmitted to their offspring as homozygous sequence.

2.1.7 Systemic and oral manifestations of Down syndrome subjects

DS varies in severity and presentation, however there are many symptoms that are seen in an exceedingly majority of patients with DS. 80% of DS people have an intellectual quotient (IQ) between 25-50. Their height and weight levels at birth are usually below average, together with an associated growth delay (Cogulu *et al.*, 2006; Mathias *et al.*, 2011). These people have immune system deficiencies, thereby resulting in augmented susceptibility to infections of the gastrointestinal, respiratory and urinary tracts (Capute and Accardo, 1996). They're additionally at larger risk of developing leukaemia, hypothyroidism, and congenital heart diseases (Mathias *et al.*, 2011). Additionally, several people with DS have short stature, simian crease, and abnormal faces (small ears, eyes with a laterally directed upward slope, slim palpebral fissures, and a short, broad nose).

Affected people have many abnormalities of body organs and systems, with a variable phenotyping pattern (Desai, 1997; Richtsmeier *et al.*, 2000; Tuxen *et al.*, 2003). Among the foremost constant features, there's a particular and directly recognizable craniofacial phenotype (Richtsmeier *et al.*, 2000). The principal stigmata embody modifications in head size (overall reduction) and form (flattened occipital bone), a diminished anterior cranial base, reductions in maxillary and mandibular size, reduced interorbital distance along with small palpebral fissures, a small mid-face with reduced nasal protrusion, and small ear length and dimension (Desai, 1997; Farkas *et al.*, 2001b; Quintanilla *et al.*, 2002; Richtsmeier *et al.*, 2002; Bagić and Verzak, 2003).

The facial profile may generally be concave, with a distinguished forehead and lower jaw, and mid-facial dysplasia (Tuxen *et al.*, 2003). In addition, alterations in the oral mucosa, in the size and form of the tongue, and within the range, dimensions, form and arrangement of the teeth may be found (Peretz *et al.*, 1996; Desai, 1997; Quintanilla *et al.*, 2002). These modifications are clearly all interrelated: the anterior tongue position has been thought of an element explaining the increased prevalence of a category III malocclusion with crossbite and anterior open bite found in subjects with DS in comparison with the overall population (Quintanilla *et al.*, 2002). Identical mechanical issue might account for the proclination of the anterior mandibular teeth and reduced interincisal angle (Quintanilla *et al.*, 2002).

Midface dysplasia is common, resulting in a shortened palate antero-posteriorly. The small palate results in enlargement of the tongue, which consequently will increase pressure against the mandibular teeth. Midface dysplasia additionally leads to an open bite, which exacerbates the poor tonicity within the tongue. This might cause an open-mouth posture and tongue protrusion. Mouth breathing is the results of frequent upper respiratory tract infections and narrow nasal passages. The skeletal and soft tissue changes along all lead to increased drooling, angular cheilitis, dry mouth, and fissured lower lips and tongue (Shore *et al.*, 2010; Areias *et al.*, 2011).

The soft tissues of the subjects with DS that play a significant role in the analysis and recognition of the craniofacial structure, and relevant anthropometrical information are typically used for multiple diagnostic and forensic procedures. Among others, there are evaluations of traumas, chromosomal and single gene

alterations, teratogenic-induced conditions like the fetal alcohol syndrome, and facial reconstruction (Moore *et al.*, 2007; Mutsvangwa *et al.*, 2010; Weaver *et al.*, 2010). Moreover, age, sex, and race are factors that influence the soft tissue characteristics, each in healthy and in abnormal subjects (Kunjur *et al.*, 2006; Park *et al.*, 2008; Price *et al.*, 2009).

2.1.8 Down syndrome craniofacial measurements in other populations

Many studies have been performed on DS subjects utilising cephalometric radiographs. Among these Frostad *et al.* (1971) established that the overall measurements of the craniofacial complex were smaller in DS subjects as compared to normal subjects. Fink *et al.* (1975) in their study concluded that the sagittal portion of the endocranium, the midfacial region, and the mandible region of DS subjects was significantly smaller when compared to normal.

Sforza *et al.* (2011) measured the 3D nasolabial morphology in 64 North Sudanese DS subjects aging 4-34 years and compared with 682 sex and age matched controls. 3D facial measurements were recorded using a laser scan. Distances of selected areas, angles, and volumes were analysed. Subject data and reference data were statistically compared using z- scores and independent t-tests. Significantly smaller nose in subjects with DS was note when compared to reference subjects. In the DS subjects the nose had a different morphology (flatter angle of alar slope, acute angle of nasal tip). Overall reduced anteroposterior (nasal tip protrusion) and vertical dimensions (nasal height, nasal-bridge length), whereas the horizontal dimensions (inferior widths of the nostrils, alar base width) were increased. An increased nasolabial angle was noted. The cutaneous lip volume was considerably smaller,

whereas the vermilion lip area was larger within the subjects with DS. The mouth and philtrum widths were significantly reduced, whereas the vermilion height was significantly exaggerated.

Bagić and Verzak (2003) studied 104 Caucasian people with DS and 365 healthy controls, aged 7 to 57 years which were divided as 7 to 12, 13 to 18, 19 to 29 and 30 to 57 age ranges. Z-scores were calculated for every variable and therefore the variations within the craniofacial region were calculated by multivariate discriminative analysis. The results showed that head circumference (OFC), head length (g-op), and outer canthal distance (ex-ex) were accountable for 85.6% variability ($p<0.001$). The analysis of z-scores showed that majority of the variables were in subnormal (under –2 SD) and usually varied (from –2SD to +2SD), however none of the variables were within the supernormal ranges (over the +2SD). Some craniofacial characteristics have been found age-related.

Farkas *et al.* (2001a) conducted a study on 127 North American non-treated patients diagnosed with DS, aged between 7 months to 36 years. 23 linear and two angular measurements were taken from six selected craniofacial regions showed that 63.1% patients have measurements within traditional norms and 36.9% had unusual measurements. Among abnormal measurements, 90.8% were subnormal and 9.2% were in supernormal ranges. All statistical reports were supported by z-scores classified into smaller ranges to yield a simplified statistical distribution for every measurement. The aim of the study was to find the measurements nearest to the norms and the individuals indicating the most severe degrees of sub- or supernormality. About 1/4 of normal variables were classified as optimum, and half

of the subnormal or supernormal measurements were classified as severe. Highest frequency of optimal scores (93.7%, 119 of 127) were noted for Intercanthal dimensions and lowest frequency was noted for the head circumference (28.6%, 36 of 126). The data of the frequency of most abnormalities within the craniofacial regions can facilitate throughout visual examination of patients with DS.

Fischer-Brandies *et al.* (1986) revealed that both jaws in DS patients exhibit dysplasia at birth. It was noted in a later study the upper jaw was underdeveloped when compared to normal subjects. Quintanilla *et al.* (2002) added to the previous knowledge that DS subjects sometimes exhibit reduction of the anterior cranial base and protrusive lower incisors.

Suri *et al.* (2010) in their study concluded that DS patients have a wider cranial base angle, reduced height of sella turcica from Frankfort horizontal plane, reduced anterior and posterior cranial base lengths and facial heights, smaller upper jaw with reduced anterior apical and basal dimensions, and smaller mandibular ramus and body. Anterior open bite was often noted with a pattern of forward rotation in both jaw planes. (Alió *et al.*, 2011) discussed that the upper jaw in DS subjects showed dysplasia in the horizontal and vertical planes, with a mean deficit of just about 10 mm within the latter.

Korayem and AlKofide (2014) studied the cephalometric radiographic characteristics of (60) DS subjects with (60) control subjects. He studied Saudi patients in which (27 were males and 33 were females) with an average age of 15.8 years (ranging from 12–22 years). According to which the variations between DS

subjects and controls were found by examining cephalometric radiographs. Anterior as well as posterior cranial base lengths were shorter, posterior cranial base appeared to be inclined backwards in DS subjects. DS subjects were found to have a retrognathic maxilla and shorter effective length, with an increased hyperdivergent mandible. Bimaxillary dental protrusion was estimated in DS subjects with a reduced nasolabial angle and prominent lips.

2.2 Methods of investigating the craniofacial morphology observations

Observations of craniofacial morphology are recorded for several centuries and most of the early studies of craniofacial morphology and growth were aimed toward describing patterns of normative variation (Finlay, 1980). Understanding the character and extent of normal variation of craniofacial complex, is important so as to research changes of morphology in patients with craniofacial abnormalities.

Normative references for craniofacial morphology are developed primarily by employing a few well-known methods, like anthropometry, craniometry, and cephalometric radiography. There are several alternative methods like photography, and laser surface scanning, as well as modifications to the above methods. Transient descriptions of those methods have been made in the following sections.

2.2.1 Craniometry

It can be described as physical anthropology in which we study dry human skulls from which soft tissues have been removed. Martin (1928) was the first scientist to produce craniometric references. Subsequently, the different South African tribal references were reported with a larger scale of sample size (De

Villiers, 1968). Howells (1989) compiled a comprehensive list of norms of various people among different geographical regions.

2.2.2 Anthropometry

Anthropometry is the science that deals with measurements taken directly from a subject; this method is also called direct anthropometry (Farkas, 1996; Farkas *et al.*, 2005; Nagle *et al.*, 2005; Roelofse, 2006). Facial measurement provides objective means to assess facial shape and notice shape changes over time, so as to diagnose genetic and acquired malformations, to assess and plan surgery, to review normal and abnormal growth and to differentiate between the results of treatment and normal growth (Douglas, 2004; Nagle *et al.*, 2005).

Facial anthropometry (measurement) and anthroposcopy (observation) play a key role in the diagnosis of several syndromes (Meintjes *et al.*, 2002; Zankl *et al.*, 2002; Douglas *et al.*, 2003; Guyot *et al.*, 2003; Zankl and Molinari, 2003). Characterization of facial tissues of DS patients has been widely performed using conventional anthropometry. (Quintanilla *et al.*, 2002; Roizen and Patterson, 2003).

Anthropometric information have been obtained directly on living subjects using several types of callipers, either analog or currently digital ones. The most advanced and widely known anthropometric references are provided by Farkas and Munro (1987), who obtained measurements from a large variety of subjects and made normative reference measurements of the craniofacial complex for North Americans (Western European Caucasian descendants) at yearly intervals from age six to eighteen years.

Any study carried out to assess the craniofacial variables need some considerations; these were reviewed by Farkas (1996) who indicated that the examiner's talent is the most significant demand for the accuracy of measurements. This relies on the quantity of subjects examined not for the length of conducting the measurements. The two alternative basic necessities for accuracy are the power to find the craniofacial landmarks and accessibility of high quality measurement tools. The accurate positioning of the head, length of examination and patient cooperation additionally influence the amount of accuracy.

2.2.3 Cephalometric radiography

With the appearance of cephalometric radiography (Broadbent, 1931), this technique has been used widely as analytical, descriptive, and diagnostic tool, especially in orthodontics and in research analysis. It's been used to review the craniofacial morphology of identical subjects throughout their growth periods. Cephalometric radiography has offered very helpful insights to orthodontists and surgeons regarding how growth changes could influence treatment for patients.

Since 1931, there has been a substantial quantity of research work that has investigated the utility and validity of cephalometric radiography analyses. Most of cephalometric radiography is predicated on the use of lateral cephalograms, in which landmarks and measurements represent the mid-sagittal plane. Many studies have created standards for craniofacial variables outlining changes (Riolo, 1974; Broadbent and Golden, 1975; Bhatia and Leighton, 1993). Landmarks identification is performed on the film or tracings, and a variety of linear and angular parameters are measured to calculate standards. Outline statistics have sometimes been given in