Correlation of Maternal Age to their Children Born with Orofacial Cleft Treated at Wits Oral Health Centre

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Declaration

I, Dr Naseeba Ravat, declare that this research report is my own work. It is being submitted in fulfilment of a Master's Degree in Orthodontics at the University of Witwatersrand, Faculty of Health Sciences, Oral Health Centre. It has not been submitted before for any degree or as an examination at this university or any other institution.

Signed: ___ ___________________________

Dr Naseeba Ravat

0000232P

Date: ________13/06/2023_______________________

Dedication

For my father, Abdul Khalik Mohamed Ravat, who supported me, believed in me and encouraged me through all my academic endeavours.

08 July 1950 – 08 June 2021

“ *I will always miss you”*

Abstract

Objectives: Orofacial Clefts (OFC) are the most common congenital malformations of the craniofacial complex. OFC affects 1/700 live births globally with extensive variability across geographic origin, racial and ethnic populations. In South Africa the prevalence of OFC is 0.3 per 1000 live births. Advanced maternal age has been associated with cell division errors, that can predispose to the occurrence of OFC. This study evaluated the correlation of advanced maternal age with the occurrence of OFC and the types of OFC.

Methods: A retrospective cross-sectional study was conducted to review records of 105 children with OFC from 1 January 2013 to 31 December 2019. The clinical information reviewed included the child's sex, race and type and laterality of OFC. The mother's demographic information including the age at birth to the child with OFC, race, marital status, education level, number of children that the mother gave birth to as well as the familial history of OFC was documented. Stata version 17.0 was used to analyse the data and statistical tests were conducted at a 5% significance level.

Results: The mean maternal age at the birth of a child with OFC was 26 years, with a range of 17-46 years. The majority of the mothers were Black (64.76%) followed by White (14.29%), Indian (11.43%) and Coloured (9.52%). There were more female children with OFC (51.43%) compared to male children (48.57%). Most of the children were Black (63.81%) followed by White (12.38%), Indian (11.43%) and Coloured (10.48%). The predominant type of OFC was the unilateral cleft lip and palate (CLP) occurring on the left side of the face (39.05%) compared to the right side (23.81%). Bilateral CLP was observed in 28.67% of children, and 0.57% of children had a cleft palate only. Mothers who gave birth to children with right CLP were older (over 30 years old) compared to those who gave birth to children with left CLP (under 25 years old). No statistical significance was found between maternal age and OFC.

Conclusions

The clinical appearance of OFC highlighted the maternal age differences to type of OFC in our study sample. Children with right CLP were born to older mothers whilst the youngest mothers had children with CP. The majority of the mothers were unemployed, single parents with low level of education. This signified the burden of care these single parents have in caring for their children with OFC.

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List of abbreviation/Acronyms

AI	Artificial Intelligence
ANOVA	Analysis of Variance
BC	Bilateral Cleft Lip
BCLP	Bilateral Cleft Lip and Palate
CA	Cleft of Alveolus
CEO	Chief Executive Officer
CL	Cleft Lip
CLP	Cleft Lip and Palate
CP	Cleft Palate
DNA	Deoxyribonucleic Acid
DS	Downs Syndrome
FAS	Foetal Alcohol Syndrome
GSTIT1	Glutathione S-transferase-theta 1
HOS	Head of School
HREC	Human Research Ethics Committee
LCL	Left Cleft Lip
LCLP	Left Cleft Lip and Palate
MDT	Multi-disciplinary Team
NR	Naseeba Ravat
OFC	Orofacial Clefts
PI	Principal Investigator
POPI	Protection of Personal Information
RCL	Right Cleft Lip
RCLP	Right Cleft Lip and Palate
SA	South Africa
SATB2	Special AT-Rich Sequence-Binding Protein 2
SUMO1	Small Ubiquitin-Related Modifier 1
TDD	Tetrachloro Dibenzo-P-dioxin
WOHC	Wits Oral Health Centre

CHAPTER 1

Introduction and Literature Review

1.1 Orofacial Clefts

Orofacial Clefts (OFC) are congenital structural malformations that affect the craniofacial complex. These malformations are present from birth and are major contributors to disabilities, morbidity and mortality in children (Worley *et al.*, 2018). Congenital abnormalities feature in the top 20 causes of global burden of disease and account for 9.4% of under five mortality (Murray et al., 1996; Paulson, 2021). Data presented in a global report estimated 6% of infants are born with birth defects that are genetic or partially genetic in origin. This is an estimated 7.9 million children, worldwide. At least 3.3 million children will die before the age of five, and the rest will suffer severe disabilities and complications for life (Tsehay *et al*., 2019).

OFC can be divided into 3 types: a) Cleft lip (CL) b) Cleft palate (CP) and c) Cleft lip and palate (CLP). Clinical appearance of CL can be on the right side or the left side of the lip, or on both sides, which is termed as bilateral CL. Similarly, CLP can be on the right or left side, termed unilateral CLP or on both sides, termed as bilateral CLP. Figure 1.1 shows the clinical appearance of OFC. Clefts can further be subdivided into: i) isolated clefts, and ii) non- isolated clefts. Isolated clefts occur alone or sometimes with minor defects; and non-isolated clefts, usually occur with major defects which may include congenital heart defects or hydrocephalus (Stanier and Moore, 2004). OFC can also occur as part of a syndrome such as Pierre Robins Syndrome or Velocardiofacial Syndrome (Venkatesh, 2009).

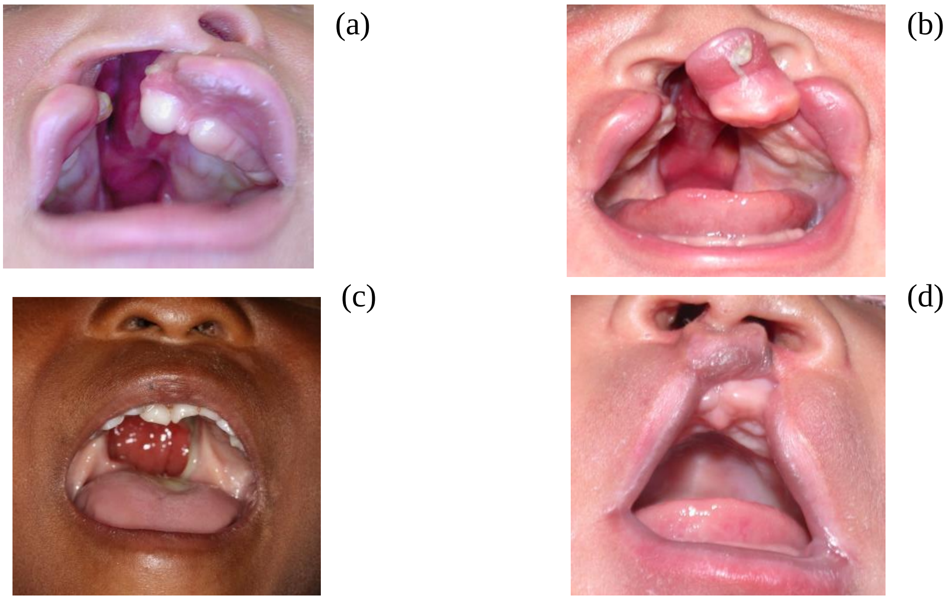

Figure 1: Clinical appearance of OFC

(a) unilateral CLP, occurring on the right side, (b) bilateral CLP, (c) CP, (d) unilateral CL occurring on the left side[Pictures reproduced through courtesy of Professor P. Hlongwa, Department of Orthodontics, School of Dentistry, Faculty of Health Sciences, University of Pretoria, South Africa.]

Approximately 70% of all OFC cases are non-syndromic, while 30% are associated with a syndrome (Dixon *et al*., 2011) A study conducted in 2003 reported that isolated CP can be associated with other congenital malformations like congenital heart defects, polydactyly and hydrocephalus, more frequently than CL and CLP (Mossey and Catilla, 2003). Patients born with CLP or CP have a higher risk of mortality than patients with CL alone (Christensen *et al.*, 2004). Adult patients with OFC have an increased incidence of structural brain abnormalities, which may lead to mild cognitive impairment and seizures (Nopoulos *et al*., 2002). A Statistics South Africa (2018) report on mortality and causes of death from findings of death notifications revealed that the number of deaths due to congenital abnormalities were 438, of which 15 were due to CLP.

1.2 Prevalence of OFC

Orofacial clefts affect 1 per 700 live births globally (Dixon *et al.*, 2011). There is an extensive variability of OFC across geographic origin, racial and ethnic populations (Dixon *et al*., 2011). A systematic review conducted by Panamonta *et al*. (2015) reported the prevalence rate of OFC in the Asian continent to be approximately 1.57 per 1000 births. The prevalence rate of OFC in North America was reported to be 1.56 per 1000 births , including Whites, Blacks, Asians and Hispanics. European populations have a prevalence rate of approximately 1.55 per 1000 births, which includes Whites and Arabs (Panamonta *et al.*, 2015). The prevalence rate of OFC in Africa ranged from 0.3 to 1.65 per 1000 births. The highest rate in Africa, was recorded in Kenya at 1.65 per 1000 births, and the lowest rate in Nigeria at 0.3 per 1000 births (Panamonta *et al.*, 2015). The prevalence rate of OFC in Africa was comparatively low when compared to Europe, Asia and America. According to Butali and Mossey (2009), the possible reason for the low prevalence rate in Africa, could be due to numerous births occurring out of a hospital setting, and hence unregistered.

The highest prevalence rate has been reported in the Indian and American-Asian communities at 1 per 500 births (Kalaskar *et al*., 2013). This was followed by higher prevalence rates in the Japanese (1.73 per 1000 live births) and Chinese races (1.56 per 1000 births) compared to Whites (1.55 per 1000 births), and the lowest prevalence rates were found in Blacks (0.15 per 1000 births) (Panamonta et al., 2015).

The prevalence of CLP in South Africa has been reported at 0.3 per 1000 live births (Hlongwa *et al*., 2019). Provincial variations were reported, with the highest prevalence rate in the Free State at 1.2 per 1000 live births, and the lowest prevalence rate recorded in the Northern Cape, with 0.1 per 1000 live births. The Western Cape reported 1.0 per 1000 births, and KwaZulu Natal and Mpumalanga at 0.2 and 0.3 per 1000 births respectively. The Gauteng province recorded a prevalence rate of 0.5 per 1000 births, with the majority of cases being CL and CP. CLP is predominant in males compared to females, whereas females had a greater occurrence of CP only. Unilateral clefts occurred more commonly in males, with a predominance on the left side for both genders. According to a study by Hlongwa and co-workers, OFC cases in South Africa were predominantly found amongst Black people (Hlongwa *et al*., 2019), however, research investigating ethnic differences amongst South Africans is necessary for future perspectives.

1.3 Development of OFC

During the embryological development of the foetus, the facial region arises from the first branchial arch. There are five facial processes that appear from which the facial structures develop: the frontonasal process, two maxillary processes and two mandibular processes (Allan, 2010).

Development of the upper lip begins as early as the 4th gestational week. The lower portion of the frontonasal process differentiates into nasal placodes, which are paired medial and lateral nasal processes. The two medial nasal processes merge with one another and with the maxillary process on each side to form the upper lip and premaxilla or primary palate. Failure of fusion between the maxillary process and median nasal process can result in a unilateral or bilateral cleft lip (Allan, 2010).

Embryological development of the palate occurs between the 6th and 7th week of gestation. The medial nasal processes for the upper lip form a triangular mass posterior to the upper lip, which is called the median palatal process. The maxillary prominence forms projections called the lateral palatal processes. The palatal processes initially lie vertically, then elevate to form the lateral palatal shelves. The palate develops from the fusion of the lateral palatal shelves with the median palatal process. The fusion occurs along a Y-shaped line, from anterior to posterior Lack of growth of shelves, failure of elevation of the shelves and failure of fusion of the shelves result in CP (Allan, 2010).

Clefts of the palate can occur along the lines of fusion and may be cleft of hard palate, soft palate, both hard and soft palate, unilateral or bilateral (Allan, 2010). Failure of closure of the palatal shelves may occur in the following possibilities: reduction of the growth of the palatal shelves, caused by reduced cell proliferation (Enomoto *et al*., 2010), inability of the midline epithelium to fuse efficiently, and degeneration of the mid-line fusion of the palate due to some degenerative processes (Dudas *et al.*, 2007). Sakuma *et al.* (2018) identified 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) as a highly toxic environmental dioxin that causes CP in mice. TCDD caused disruption of the development of the lateral palatal shelves as well inhibition of the fusion of the palatal shelves, thus leading to the formation of a CP (Sakuma *et al*., 2018).

1.4 Aetiology of OFC

Numerous causative factors of OFC have been identified, all of which are complex. OFC can occur as a result of the association between behavioural, environmental and genetic factors of the parents of the OFC affected child (Murray, 2002).

Maternal smoking and foetal alcohol syndrome (FAS) have been commonly identified as risk factors (Honein *et al*., 2007)). Increased exposure to smoking, alcohol consumption and toxins during pregnancy increases the possibility that genes in certain metabolic pathways are affected and result in the development of OFC (Dixon *et al*., 2011). In a study conducted by Martelli *et al.* (2015) the mechanisms by which maternal smoking increases the risk factor of OFC was studied. It was found that cigarette smoke contains toxins such as nicotine, tar, poly-carbons and carbon monoxide. Exposure of the embryonic tissues to these toxins affect the glutathione S-transferase-theta (GSTT1) gene, which in turn affects detoxification pathways in the developing foetus and leads to OFC formation (Shi et al., 2007; Shi et al., 2008; Zhu et al., 2009).

Alcohol consumption during pregnancy and during the periconceptual period affects the embryonic cells of the cranial neural crest cells, which leads to abnormalities in the facial structures, like OFC (Romitti *et al*., 2007). Alcohol consumption has also been associated with folate deficiency, which is a risk factor for OFC formation (Hernández-Díaz *et al*., 2000).
Exposure to pesticides, like dioxin, has been identified as an environmental risk factor for OFC (Garcia *et al*., 1999).

Gestational and pregestational diabetes have also been identified as risk factors for OFC (Correa *et al.*, 2008)). Gestational diabetes is a metabolic complication that occurs during pregnancy (Wu *et al*., 2020). A hyperglycaemic intrauterine environment leads to oxidative stress, which increases the risk of developing congenital abnormalities in the foetus (Yang et al., 2015; Yang et al., 2019). Stress experienced by the expectant mother, as well as certain infections may predispose the foetus to OFC formation (Carmichael *et al.*, 2007b). Stressful events and situations lead to an increase in corticosteroid production in the body (Wadhwa et al., 1996; Hobel et al., 1999). Elevated maternal corticosteroid levels in pregnancy cause an increased risk for OFC formation. Mothers taking corticosteroid medication during pregnancy also causes an increased risk of OFC formation (Källén, 2003; Carmichael et al., 2007a).

In a study conducted by Margulis *et al.* (2012) the use of topiramate, a drug used to treat epilepsy and convulsions, by pregnant mothers during the first trimester, was associated with an increased risk factor of OFCs. A study conducted by Bonaiti *et al*. (1982) found an increased frequency of epileptic mothers with children with OFC. Deficiencies in nutrition like folate, vitamin B6 and B12 have also been identified as causative factors in CL, CP and CLP (Ulrich et al., 1999; Wong et al., 1999 ; Mossey et al., 2009). Folate is important in the neural tube formation and a deficiency of folate can result in OFC formation. The embryonic tissues of the face are derived from neural crest cell (neural tube) and a folate deficiency will influence development of the facial structures (Munger *et al.,* 2004). Folate also has an indirect effect on OFC through homocysteine metabolism. Deficient folate levels are associated with elevated cysteine levels in the body. Mothers of children with OFC have had elevated cysteine levels (Johnson and Little, 2008).

Vitamin B6 and B12 are important constituents of different metabolic pathways of normal development. A deficiency in these essential vitamins have been implicated in OFC occurrence (Sauberlich *et al*., 1973). The use of multivitamins by expectant mothers has been reported to decrease the risk of cleft occurrence (Ingrid Goh *et al*., 2006).

Jamilian *et al*. (2017) reported that expectant mothers of consanguineous marriages had a higher risk of malformations like clefts. Birth weight as well as birth order have also been identified as possible risk factors,- the smaller the birth weight, the higher the risk of OFC (Cooper *et al*., 2000). Zeiger and Beaty (2002) stated that although birth order is a risk factor, the role of maternal age and other risk factors cannot be excluded in OFC cases. Many older mothers have an increase in homocysteine levels, which influence the occurrence of OFC and increase the risk of other anomalies such as cardiac, skeletal and neurological disorders. An increase in OFC cases were noted in 3^{rd} or 4^{th} pregnancies, due to folate deficiencies in mothers with multiple pregnancies, in close succession (Smits and Essed, 2001).

OFC that are associated with syndromes have a genetic aetiology and are caused by mutations of a single genetic locus, chromosomal abnormalities and teratogens. As a result of genetic testing, 75% of reported syndromes have an identified genetic cause. Chromosomes in a genome undergo deletions, duplications, inversion and translocation. This prevents the chromosomes from proper re-joining and causes a shift in DNA sequencing (Leslie and Marazita, 2013). Analysis of the breaking point has identified, amongst others, SUMO1 and SATB2 as the genes implicated in OFC formations (Fitzpatrick et al., 2003; Alkuraya et al., 2006). Genetic mutations, specifically in the vicinity of 19q13 have been investigated for the role in non-syndromic clefts (Warrington *et al.*, 2006). Clefts that occur in a syndrome have been linked to chromosomal disorders and Mendelian single gene disorders (Deshpande and

Goudy, 2019). Van der Woude Syndrome has been identified as the syndrome most associated with OFC (Burdick, 1986).

In a 2013 study, it was reported that CLP had twice the risk of occurrence in individuals with a positive family history of OFC (Goto *et al*., 2013). According to a study by Mitchell and Christensen (Mitchell and Christensen, 1996) multiple genetic loci were responsible for the genetic predisposition of OFC, especially CLP. CLP was found to have the highest percentage of familial occurrence and the subtype with most associated abnormalities (Silva *et al.*, 2022). Congenital heart defects and skeletal abnormalities were the most common malformations associated with OFC cases, CLP in particular (Jamilian *et al.*, 2017).

Gender also plays a role in the occurrence of OFC. Males tend to have a higher risk of CL and CLP, while females tend to have a higher risk of CP alone (Conway *et al*., 2015). It has been reported that in CL cases 75% are unilateral, with the left sided CL occurring twice as frequently as the right side (Gundlach and Christina, 2006).

1.5 Implications of OFC

Individuals with OFC experience several health challenges and complications from an early stage in life (Nackashi *et al*., 2002). Infants experience early feeding difficulties (Reid *et al.*, 2007). Infants with CL are able to breastfeed, but patients with CLP are unable to breastfeed (Martin and Greatrex-White, 2014). Mothers will need to consult with multiple medical personnel for advice regarding feeding and nutrition (Worley *et al.*, 2018). Children with OFC are at a higher risk for sensorineural hearing loss, conductive and mixed hearing loss . They are also at risk of acquiring middle ear disorders like chronic middle ear effusion and eustachian tube dysfunction (Doyle *et al*., 1980). Therefore, children with OFC will need to consult with speech and hearing pathologists for assessment of hearing and middle ear disease.

Lack of closure of the velopharynx affects speech of patients with OFC and they will require speech therapy during the developing years (Worley *et al.*, 2018). Children with OFC also experience a range of dental problems (Shah and Wong, 1980). They experience multiple dental complications such as teeth agenesis and anodontia, microdontia, gingival fibromatosis and periodontitis; retained teeth, rotated teeth, malocclusion and delay of tooth eruption. OFC affects the growth of the maxilla, which impacts on the physical appearance and which may add to the anxieties that these patients and parents present with (Shah and Wong, 1980; Wong Riff et al., 2018; Friedlander et al., 2019; Saltnes et al., 2019). Children with OFC may require orthodontic treatment, as well as prosthesis during different stages of their development. Orthodontic treatment can range from removable and fixed appliances, to orthopaedic and orthognathic surgery (Machos, 1996). Treatment of OFC requires multiple interventions and a multidisciplinary approach, from birth until adulthood (Farber *et al.*, 2019). Surgical management is required from early in life, throughout childhood and possibly into early adolescence (Worley *et al*., 2018).

Parents of children with OFC are often faced with the financial, emotional and social burden of caring for their children with OFC. They may also experience a plethora of emotions regarding their newborn with OFC (Hlongwa and Rispel, 2018). Patients with OFC from low socio-economic backgrounds and impoverished regions may not have access to appropriate treatment. The lack of access further impacts the burden of care, as well as adversely affecting their morbidity, mortality and quality of life (Wehby and Cassell, 2010). In order to meet the needs of the affected child, parents will need appropriate psychosocial support through counselling and access to appropriate resources. Early identification of risk factors will keep parents well informed and assist in providing needed support (Matthews *et al*., 1998). Studies focusing on OFC will further assist in identifying associated risk factors, which in turn can assist in educating expectant mothers, and in developing preventative strategies for the population at large (Sreejith *et al.*, 2018). Prenatal screening, genetic testing and an accurate prenatal diagnosis is essential in predicting a prognosis and determining a long-term treatment plan (Jones, 2002).

1.6 Effects of maternal age in OFC

The reproductive systems of older females have been reported to be prone to deteriorate, specifically the production of healthy oocytes. with an increase in infertility (Mikwar *et al*., 2020). Advanced maternal age has also been associated with cell division errors, especially during meiosis, leading to the production of oocytes with an abnormal number of chromosomes, known as aneuploidy. When an aneuploid oocyte is fertilized into an aneuploid embryo, the risk of congenital abnormalities are higher (Jones and Lane, 2013). Abnormal chromosomes are significant in the development of birth defects (Mikwar *et al*., 2020). Downs syndrome (DS), also known as trisomy 21, is a result of abnormal cell division which results in extra genetic material for chromosome 21 in older pregnant mothers (Morris et al., 2002; Coppedè, 2016). The abnormalities commonly associated with maternal age include congenital heart defects craniosynostosis and hypospadias (Reefhuis and Honein, 2004).

According to De Queiroz Herkrath *et al*. (2012), the older the mother's age, the higher the risk of having children with a CL, CP or CLP. Fathers over the age of 40 years have a 1.58 higher risk of having new-borns with cleft palate (De Queiroz Herkrath *et al*., 2012). This could be attributed to changes in the reproductive cells due to aging, environmental exposures or chromosome alterations. The uterus and placenta in older women become more permeable to teratogens and toxins (Mauk, 2010). Parents older in age seem to contribute to the severity of the cleft, with older parents having infants with severe forms of cleft lip and palate, as well as full cleft palate (Hermann *et al*., 2018). Once again, longer exposure to toxic elements due to advanced age was cited as a possible cause of OFC (Vieira *et al*., 2002). According to Vieira *et al.* (2002), increase in maternal age is greatly influenced by increase in paternal age (when both parents are older), the risk for abnormalities especially OFC, is much greater.

Mothers under the age of 20 years as well as those over the age of 40 have a higher risk of new-borns with congenital abnormalities (Croen and Shaw, 1995). Complications in pregnancies, in mothers under the age of 20 years have been linked to smoking, drug use and nutritional deficiencies; which lead to new-borns with low birth weight and increased risk of congenital malformations like OFC (Day et al., 1993; Croen and Shaw, 1995; Abma, 1997; Reefhuis and Honein, 2004). Younger women have lower awareness regarding the importance of periconceptual folic acid and a lower usage rate of folic acid and essential multivitamins (Mathews *et al.,* 2000). A study conducted in Washington State in 2003 reported a controversy in literature regarding maternal age. Mothers who were 20 years old and under, were twice as likely at risk of having an infant with an isolated CLP than older mothers (Deroo *et al*., 2003). A study conducted in the United States of America reported low birth weight in new-borns from pregnant teenage mothers, as they are still growing and developing themselves, thus there is a battle for nutrients that are essential for the developing foetuses (Scholl *et al*., 1994). Other associated factors with teenage pregnancies may be lack of knowledge, low socio-economic status, substance abuse and lack of prenatal vitamins (Croen and Shaw, 1995).

A study conducted in France found older paternal age to have an increased risk in OFC formation. Birth order, with the last born having the highest risk of OFC due to the father being older, was also established (Bonaiti, 1982). A study conducted in Poland in 2008 could not establish a positive association between advanced maternal age and isolated cleft lip and palate. Their results however found a positive relationship between advanced paternal age and CL formation – with or without CP (Materna-Kiryluk *et al*., 2009). A possible reason for birth defects (like CLP) being associated with advancing paternal age could be due to cytotoxic damage or alteration of DNA sequence during spermatogenesis, common in older men due to the accumulation of exposure to harmful environmental factors (Savitz *et al*., 1991). A study conducted in Nigeria in 2010 observed that fathers that were over the age of 40 years and mothers that were over the age of 35 years were of higher risk for the development of OFCs in new-borns. Advanced paternal age (over 40 years) was associated with isolated CP, whilst advanced maternal age (over 35 years) was associated with bilateral CLP and left-sided unilateral CLP (Omo-Aghoja *et al.*, 2010).

1.7 Rationale of the Study

The literature reviewed for the current study, within its imitations, has shown that there is still controversies regarding advanced maternal age and occurrence of OFC. There is scarcity of available data on the effects of advanced maternal age and occurrence of OFC in South Africa. Recent studies in South Africa (Carrim and Hlongwa, 2022, Moodley et al., 2018, Hlongwa et al., 2019) have reported on the prevalence of OFC, however did not focus on the correlation between maternal age and OFC. Therefore, the purpose of our study was to evaluate if advanced maternal age has an effect in the occurrence of OFC.

1.8 Aim of the study

The aim of this study was to correlate maternal age of mothers to their children born with OFC treated at Wits Oral Health Centre (WOHC).

1.9 Study Objectives

1. To describe the demographic profile of mothers of children with OFC treated at the WOHC.
2. To determine the types of OFC these children present with at WOHC.

3. To correlate maternal age of mothers to their children born with OFC and distribution of OFC in the sample.

CHAPTER 2
Materials and Methods

2.1 Study Design

This was a quantitative, retrospective, cross-sectional study from the period of 1 January 2013 to 13 December 2019.

2.2 Study setting

The study was conducted at WOHC Cleft Palate Clinic. This is a referral clinic for most of the patients with CLP in Johannesburg and surrounding areas including Soweto, Ekurhuleni, and other provinces such as Mpumalanga and North West.

2.3 Sample size

A convenient sample size of 133 was used, based on the number of available clinical records from the WOHC Cleft Palate Clinic (Carrim and Hlongwa, 2022). All clinical records for the selected study period, were reviewed for the mothers' maternal age and the child's type of OFC.

2.4 Sampling technique

The convenience sampling technique was used to collect data for this study. All available records were reviewed, and relevant information recorded on a data collection sheet.
Inclusion criteria for the sample was:

- All children with OFC treated at the WOHC
- Mothers of children with OFC, being treated at the WOHC

Exclusion criteria for the sample was:

- Incomplete patient records

2.5 Data collection

A data collection form (Appendix I) was used to review the demographic information of the mothers and the clinical information of the children with OFC. All records were assigned a unique number for confidentiality and anonymity of the participants. Any information that could identify the patient was not used and anonymity was maintained throughout the study.

Demographic information regarding the mother included age at the time of giving birth to child with OFC, race, marital status, education, number of children and the familial history of OFC. The clinical information of children with OFC was recorded in four categories: (1) type of OFC: (CL, CP, CLP); (2) laterality of OFC: bilateral cleft lip and palate (BCLP), left cleft lip and palate (LCLP), right cleft lip and palate (RCLP), bilateral cleft lip only (BCL), left cleft lip (LCL), right cleft lip (RCL), cleft palate only (CP) and cleft of alveolus (CA); (3) race; and (4) gender.

Data were recorded on MS Excel spreadsheets in a secure computer with a password protector that only the researcher and supervisors had access to. Statistical analysis was done using STATA version 17.0 and graphs plotted on Excel. All statistical tests were conducted at 5% significance level.

2.6 Statistical Analysis

The data were captured in an Excel spreadsheet and imported into STATA version 17.0 for analysis. The categorical variables (race, gender, types of OFC) were described using frequencies and percentages, while continuous variables (age) were summarized using the mean and standard deviation Normality of the continuous data was assessed using the Shapiro Wilk test and a p-value of above 0.05 indicate the satisfaction of the normal distribution. The histogram plot with a superimposed normal curve was also used to describe how the data deviated from the normal distribution. The box and whisker plots were used to give a pictorial view of continuous data, while the bar charts were used to give a pictorial view of the categorical data. The comparison between maternal age (continuous) and OFC type was done using the Kruskal Wallis test. Comparison between OFC types with other categorical variables (race, gender and age groups) were done using the Chi-square test. Significance was set at 5% and results were reported in tables following a structured study objective format.

2.7 Examiner reliability measurements

Repeat measurements of 10% of the records randomly selected were conducted by the principal investigator (NR) to determine intra-examiner reliability one week after the first measurements. One of the supervisors also measured 10% of these records to assess the inter-examiner reliability. Correlation coefficient closer to 1 indicated high reliability.

Intra-observer and inter-observer reliability was assessed using the Kappa correlation. The intra-examiner reliability and the inter-reliability tests showed a 100% agreement with Kappa scores for all types of findings analysed.

2.8 Ethical considerations

Ethical clearance was obtained from the Human Research Ethics Committee (HREC) Medical, of the University of the Witwatersrand, Johannesburg- M220220 (Appendix II). Permission to access data was obtained from the Acting CEO of WOHC (Appendix III) and from Acting Head of School (HOS) of WOHC (Appendix IV).

Patient records were reviewed by the principal investigator (PI), Naseeba Ravat (NR) only. All records were stored in a locked cupboard with access only given to PI. The PI is registered with the Health Professions Council of South Africa as a dentist in the category Independent Practice General Practitioner and is familiar with all the principles of patient confidentiality in medical records. By assigning a unique number to each patient record, no information that could identify the patient was used in the study. In this manner, it was ensured that confidentiality and anonymity was maintained throughout the study. The POPI act was taken into consideration and informed consent was waived as this was a record review study.

CHAPTER 3

The Results

The total sample size of 133 patients records with OFC was analysed. The sample size which was analysed for the study was 105 participants with complete records. A total of 28 records were excluded due to incompleteness. Of the 105 patients with OFC, 51.13% (*n* = 54) were females, while 48.87% (*n* = 51) were males. Nationality distribution revealed that most of the participants were from South Africa, 91.73% (*n* = 92), while 8.23% (*n* = 13) were non-South African. The demographic profiles of the participants are described below.

3.1 The demographic profile of mothers

Figure 2 below shows the maternal age distribution at the time of birth of a child with OFC.

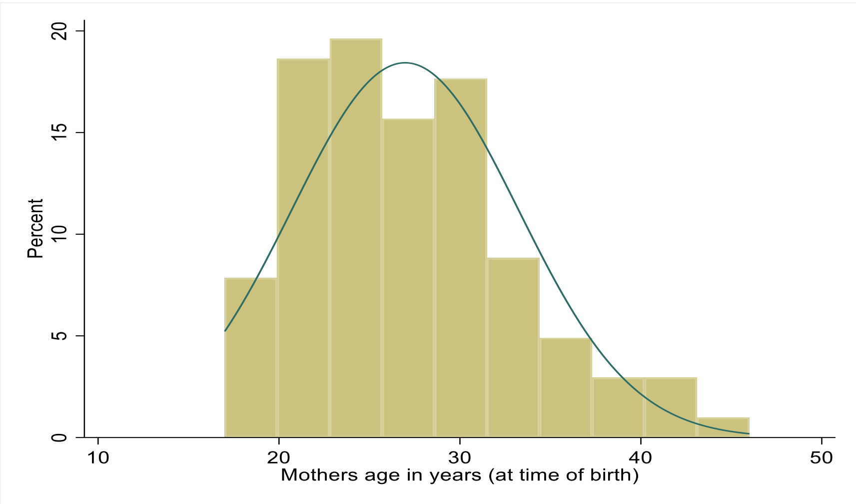

Figure 2: The maternal age distribution

The mean age (±SD) of the mothers at delivery was 26.97 (±6.28) years. The maternal age range at the time of delivery was 17 - 46 years. Since the maternal age was skewed to the right, the median and interquartile range (IQR) of the mothers was 26 (22 - 31) years.

Table 1 shows the socio-demographic profile of the mothers of children with OFC treated at the WOHC.

Table 1: The socio-demographic profile of the mother

Variable	Categories	Frequency	Percentages
Race	Black	68	64.76
	Coloured	10	9.52
	Indian	12	11.3
	White	15	14.29
Marital Status	Married	48	45.7
	Single	47	44.76
	Divorced	4	3.81
	Widowed	2	1.9
	Not specified	4	3.81
Level of education	High School	39	37.14
	Matric	32	30.48
	Tertiary Education	4	3.61
	Not specified	30	28.57
Employment Status	Employed	43	40.95
	Unemployed	59	56.19
	Not specified	3	2.86
Number of children	1	19	18.1
	2	21	20.0
	3	16	15.24
	4	10	9.52
	Not specified	39	37.14
Total		525	

The most predominant race was Black, followed in descending order by White, Indian and Coloured. Majority of mothers were married. Most mothers did not have tertiary education, were unemployed and single parents. The number of children amongst the mothers ranged from one to four.

3.2 The distribution of OFC in the sample

The demographic information of children treated with OFC is summarised in Table 2. There were more female children compared to male children. Most of the children were Black followed by White, Indian and then Coloured.

Table 2: Demographic and OFC profile of the children

Variables	Categories	Frequency	Percentages
Gender of child	Female	54	51.43
	Male	51	48.57
Race of the child	Black	67	63.81
	Coloured	11	10.48
	Indian	12	11.3
	White	13	12.38
	Non-specified	2	1.9
Child birth order of Children with OFC	First born	36	34.29
	Second born	13	12.38
	Third born	10	9.52
	Fourth born	6	5.71
	Last born	2	1.9
	Not specified	38	36.19
Family history of OFC	Don't know	58	55.24
	No	45	42.86
	Yes	2	1.9
Total		420	

Figure 3 shows the distribution of the OFC types among children treated at WOHC. The most common type of OFC was the left CLP 41(39.05%) followed by bilateral CLP 30 (28.67%), right CLP 25 (23.81%) and 9 (8.57%) CP.

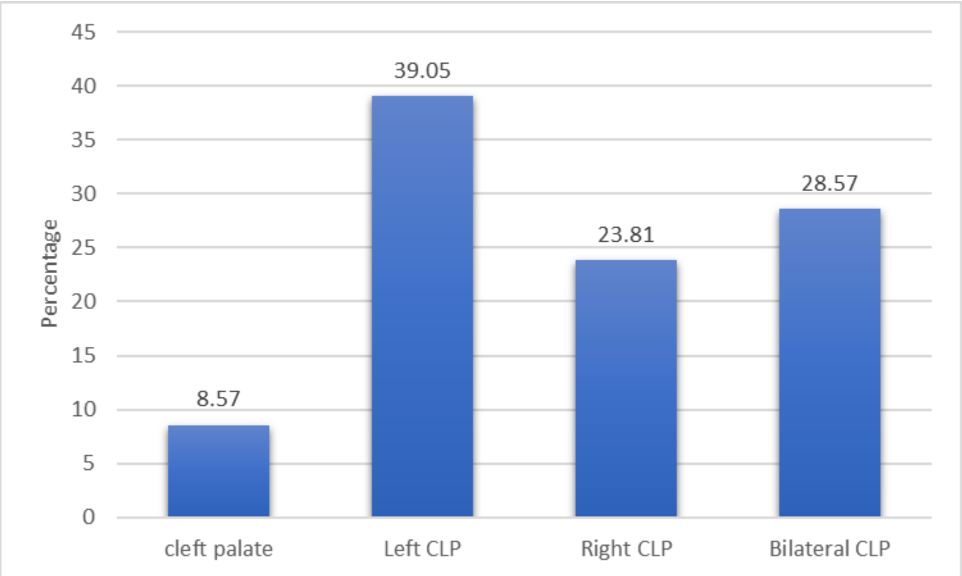

Figure 3: Types of OFC among children

3.3 The association between the maternal age and type of OFC

Figure 4 shows the maternal age distribution across the OFC types. Older mothers gave birth to children with the right CLP.

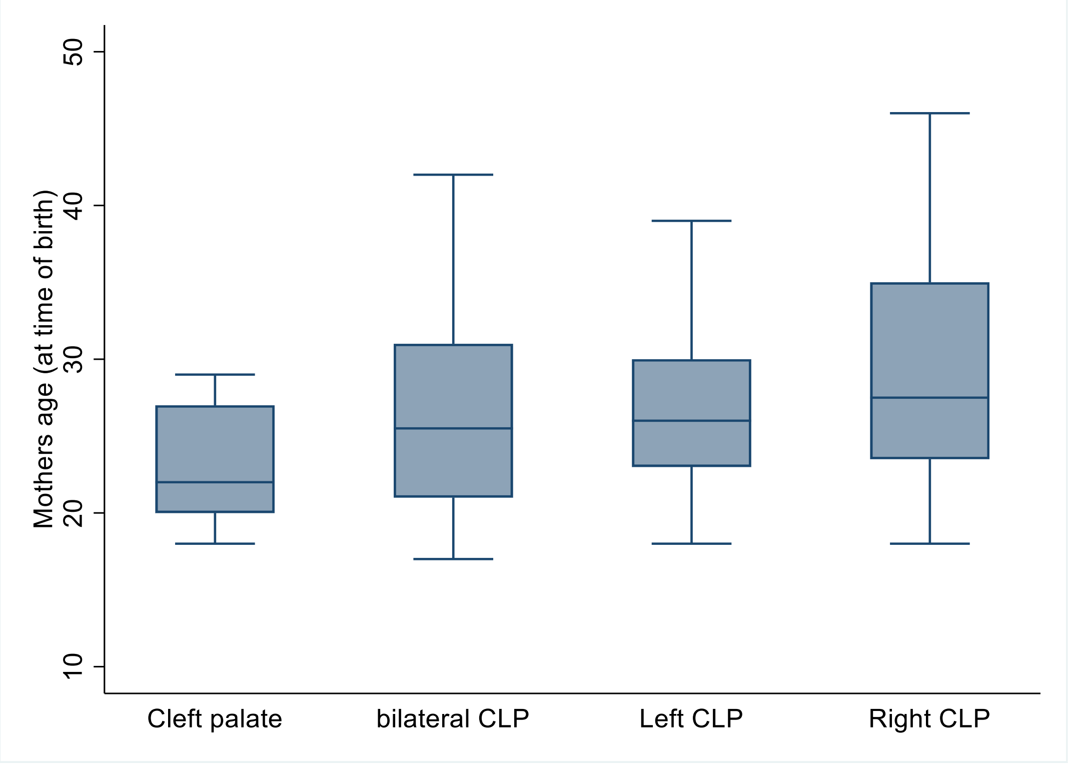

Figure 4: Maternal age in years across OFC types

Table 3 shows the median maternal age at the time of birth across the groups of OFC.

Table 3: The median maternal age distribution across the types of OFC

OFC types	**Maternal age (Assuming skewed data)**	
	Median(IQR)	**P-value**
Cleft palate	22(20-27)	0.1232
Left CLP	26(23-30)	
Right CLP	27.5(23.5-35)	
Bilateral CLP	25.5(21-31)	
Total	101	

Mothers who gave birth to children with right CLP were older compared to those who gave birth to children with left CLP. Mothers who gave birth to children with bilateral CLP were younger compare to those who had children with CLP (right or left), and the youngest group of mothers were those who gave birth to children with CP. The Kruskal Wallis test gives a clear non-significant maternal median age differences, p=0.1232. Table 4 shows the association between the maternal age categories with OFC types.

Table 4: The association between maternal age groups and OFC types

Maternal age groups	**Cleft palate n(%)**	**Left CLP n(%)**	**Right CLP n(%)**	**Bilateral CLP n(%)**	**P-value**
17-25 years	6(75.0)	17(42.5)	9(37.5)	15(50.0)	0.358
26-30 years	2(25.0)	14(35.0)	6(25.0)	7(23.43)	
>30 years	0	9(22.5)	9(37.5)	8(26.67)	
Total	8	40	24	30	

No significant association was observed between the maternal age categories at the time of childbirth and the OFC types using the Chi-square test (which was used to compare the categorical data), p=0.358.

3.4 Association between types of OFC and other variables

The association between OFC type and race of mothers is shown in Table 5. No statistically significant difference was found (p=0.115). No statistically significant difference was also observed between the OFC types and child's birth order p=0.183.

Table 5: The association between mothers' race, child birth order and OFC types

		Cleft Palate n(%)	**Left CLP n(%)**	**Right CLP n(%)**	**Bilateral CLP n(%)**	**P - value**
Mother's race	Black	5(7.35)	29(42.65)	12(17.65)	22(32.35)	0.115
	Coloured	0	5(50.0)	4(40.0)	1(10.0)	
	Indian	3(25.0)	4(33.33)	2(16.67)	3(25.0)	
	White	1(6.67)	3(20.0)	7(46.67)	4(26.67)	
Child's birth order	First	3(8.33)	11(30.56)	7(19.44)	15(41.67)	0.183
	Second or other	2(6.45)	10(32.26)	10(32.26)	9(29.03)	
	Not Specified	4(10.43)	10(52.63)	8(21.05)	6(15.79)	
Total		18	72	50	60	

Figure 5 shows the percentage distribution of OFC types and child's gender.

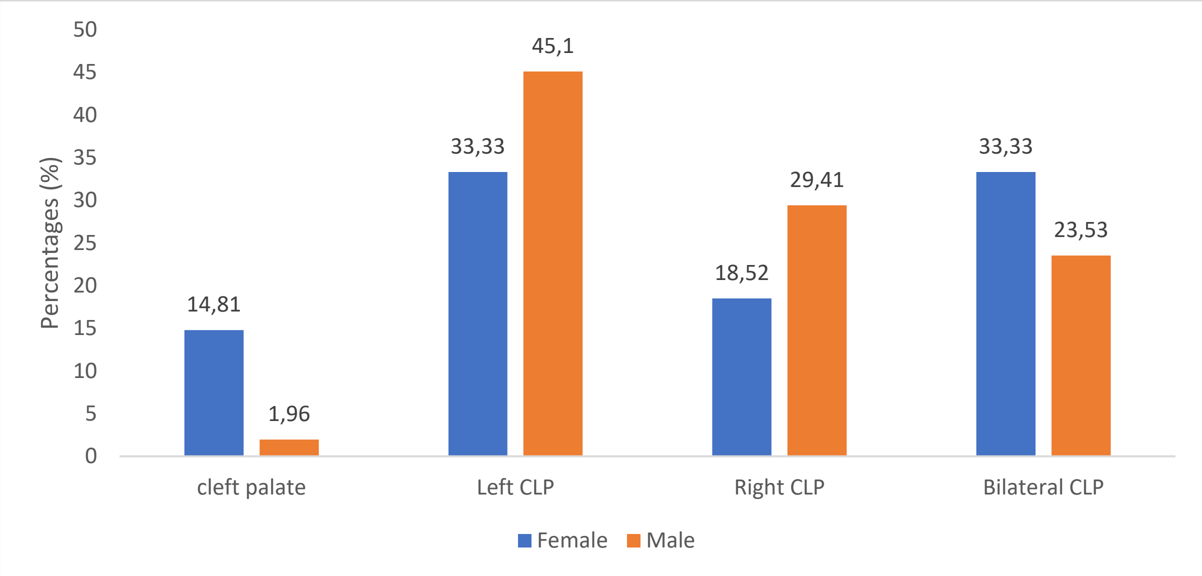

Figure 5 : The comparison of OFC types and child's gender

Female children had more CP and bilateral CLP compared to male children. Male children had predominant left and right CLP compared to the female children. These results showed a statistically significant association between the child's gender and the OFC type, p-value=0.043. Our study findings showed that CLP was common in both genders, with males reporting left and right CLP, and females having bilateral CLP. These results were statistically significant (P=0.043).

CHAPTER 4

Discussion

Our study is amongst the first in South Africa to report on the maternal age and children with orofacial clefts (OFC). We were able to evaluate 105 OFC patient records together with their mothers' information because the ascertainment of malformations depends on the completeness and accuracy of medical records.

Maternal findings

The mean maternal age of our study sample was found to be 26.97 years (SD:6.28). The median value was 26 years (IQR:22-33). Similar results were found in a study conducted at the University of Pittsburgh, with mean maternal age reported at 26.8 years (Abramowicz *et al.*, 2003). Other studies also reported similar results to our findings with mean maternal age range between 24.56 -26.62 years (Ács et al., 2020). Our study showed that women as young as 17 years also gave birth to children with OFC. The observed increase in malformation prevalence among women less than 20 years of age may be partially explained by diagnostic bias, as a result of the known association between young maternal age and poor pregnancy outcome (DeRoo *et al*., 2003). Our study did not evaluate this effect.

Our study results showed that older mothers gave birth to children with right CLP, whilst the youngest mothers had children with CP only. Mothers over 30 years of age had the least number of children born with OFC, with a predominance of right CLP cases. Mothers under 25 years had the majority of children born with OFC, with a predominance of CP and bilateral CLP cases. Mothers aged between 25 and 30 years had a predominance of left CLP cases. A study conducted by Hermann *et al*. (2018) reported an important association between parents over the age of 29 years, and the severity of both CP and CLP. Our study findings did not investigate the relationship between severity of the cleft and parental age, but we did not find any significant difference between the maternal age and the type of OFC (p=0.358).

A study conducted by DeRoo *et al.* (2003) reported that infants of mothers under 20 years of age had the greatest prevalence of CL, CLP, and CP. Mothers under the age of 20 years had a twice as greater risk of having a child with OFC compared with mothers aged 25 to 29 years (DeRoo *et al.*, 2003). Our study found similar results with mothers under the age of 25 years having the majority of children with OFC, and greatest predominance of left and bilateral CLP. A meta-analysis conducted by Vieira *et al*. (2002) concluded that there was no effect of advancing maternal age on OFC, whilst another meta-analysis conducted by De Queiroz *et al*. (2012) reported a positive association between advanced maternal age and OFC. Some studies have found a positive association between advancing maternal age and OFC (Croen and Shaw, 1995; Zeiger and Beaty, 2002). Others have found no association between maternal age and OFC (Baird et al., 1994; Vieira et al., 2002; Materna-Kiryluk et al., 2009). Our study did not evaluate the age of the father, but a study conducted in 1982 showed that the advanced age of the father has a significant role in OFC development, and both mother and father having advanced ages increases the risk factor, but having one parent younger in age decreases the risk factor (Bonaiti *et al.,* 1982). The controversies on the reported findings are due to differences in the cross-sectional time of the research and methodology variabilities.

Demographic profile of mothers

The race profile of the mothers in this study showed that majority of the mothers were African Black (64.76%). Our results are not perpetuating segregation, however, South Africa is still divided into race categories and the majority of the population (80%) is African Black (Statistics South Africa (2013). It is expected that most of the African Black individuals will seek treatment from public hospitals like WOHC since 90% of the black population is not covered by medical scheme (Jassat *et al*., 2022). More than 50% of the mothers in our study were single parents (unmarried, widow or divorced). Our study findings also showed most of mothers were unemployed with low education levels, 7.14% of mothers went to high school but did not matriculate, and 30.48% completed grade 12. Similar results regarding education levels were reported by (Alfwaress *et al.*, 2017) that 21.7% of parents of children with OFC had not graduated from high school and 23% completed grade 12. The challenges of these mothers for taking care of a child with OFC is underscored by our study, as they all relied on public sector hospital for the management of their children with OFC

Distribution of OFC

The findings of our study have shown that the most common cleft was CLP (92.43%), followed by CP (8.57%). Of the CLP cases, 39.05% were left CLP, 28.57% were bilateral, and 23.81% right CLP. A study conducted in Tanzania reported similar findings, with unilateral clefts more common than bilateral, and the left side being more commonly affected (Manyama *et al*., 2011). A study conducted by Shapira *et al*., in 1999 reported a similar order of frequency (Shapira *et al*., 1999) as our study findings.

Gender distribution of children with OFC

Males have been found to have a 2:1 greater ratio of OFC than females, with females having a greater predilection for CP while males have a predominance for CL and CLP (Conway *et al.,* 2015). Our study findings showed that majority of OFC cases were female (51.43%) and 48.57% were male. A study conducted by Hlongwa *et al*. (2019) in South Africa showed similar results, with female predominance of OFC cases. Similarly, Moodley *et al*. (2018) also reported a female predominance of OFC cases. However, some studies have shown a higher incidence of OFC in males than females (Manyama et al., 2011; Butali and Mossey, 2009).

Our study found that the most common occurring cleft in both males and females was left-CLP. Right CLP was found predominantly in males, with females having a higher predilection for bilateral CLP and CP. Similar results to our study findings were reported by Hlongwa *et al*. (2019) showing that CP was predominantly in females, whilst left CLP was found predominantly in males. A study conducted by Conway *et al*. (2015) also showed similar results to our study findings, with more females having CP. Our study results were also similar to findings of other studies (Shapira et al., 1999; Moodley et al., 2018).

Racial distribution of children with OFC

In a study conducted by Panamonta *et al*. (2015), in America, the highest prevalence rate for OFC worldwide was found amongst American Indians, followed by the Japanese, the Chinese, Whites and then Blacks, who had the lowest ratio. A study conducted by Hlongwa *et al*. (2019) in South Africa showed a predominance of OFC in Blacks. These results were concurrent with other recent studies conducted in South Africa (Carrim and Hlongwa, 2022; Moodley et al., 2018). Our study showed similar results, with the highest prevalence rate to be amongst Blacks (63.81%), then Coloureds (10.48%), Indians (11.43%) and Whites (12.38%). The majority of the population in South Africa is Blacks, and the results obtained could be a direct reflection of the population distribution. The predominant race that populates the specific region or geographic location where the research is being conducted will influence the number of cleft frequency counts (Carrim and Hlongwa, 2022).

Familial distribution in OFC

In our study, 1.9% of patients reported a positive family history of OFC. Of the 105 case reports, 42.86% reported negative for a family history, whilst 55.24% did not know if there was a history of OFC in the family. Goto *et al*. (2013) reported that OFC had a twofold higher risk of occurrence when associated with a positive family history. Kumar *et al*.(1991) reported a 28% frequency of OFC in a positive family history of clefts, whilst Aqrabawi reported no frequency with positive family history (Aqrabawi, 2008).

Familial history has been shown to contribute to the occurrence of OFC, but due to lack of sufficient information in our study familial history was under reported compared to other studies.

Association between OFC and birth order

An increase in the number of previous births was found to have a definite association with OFC in a study conducted in Russia (Caseres and Prytkov, 1982). A study conducted by Vieira *et al*. (2002) found children with a higher birth order, especially child 3 and 4, to have a higher risk of OFC formation. Bonaiti *et al*. (1982) found OFC to be associated with increase in birth order, mostly due to the increase in parental age. Our study findings reported contrasting results, with first born children reporting bilateral CLP (41.67%) and children who were not first born had left CLP (52.63%).

Limitations of the study

Our study is hospital-based, utilising patients' records from WOHC, therefore, the findings cannot be generalised. The nature of the study being a cross-sectional means that records outside of the study period could not be utilised, hence their information could have been missed relating to our study. Poor records keeping affected our study as it was highlighted in a previous study by Carrim and Hlongwa (2022). In a study conducted by Wegner and Rhoda (2013), missing records, incorrect folder numbers and misplaced files were proven to be a hindrance in archival research (Wegner and Rhoda, 2013). Despite these challenges, our study has managed to analyse the maternal information and OFC types.

CHAPTER 5

Recommendations and Conclusions

Recommendations

Further investigations into parental age and OFC utilising a larger sample size is recommended. Establishing a data collection form, which will include paternal and maternal information will be beneficial for future studies. It is recommended that WOHC should have an electronic record keeping system, especially in this period of artificial intelligence (AI) and electronic age. Electronic data will ensure manageable information retrieval for future studies as well as completeness of the data.

Conclusions

Mothers under the age of 25 had the majority of children with OFC, with a predominance of CP and bilateral CLP. Mothers over the age of 30 years had the minimum of children with OFC, with a predominance of right CLP. A statistically significant association between maternal age and OFC was not established.

The results of our study highlights the importance of effective family planning services for older parents and teenage mothers as well as education and counselling regarding the risks involved in these pregnancies. The results may also contribute to implementation of suitable health policies for the provision of prenatal care and counselling for mothers at risk of having children with OFC.

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Appendix I: Data Collection Form

Correlation of maternal age to their children born with orofacial clefts treated at Wits Oral Health Centre
Patient Unique Number: …………………………………………………...

Part A: Demographic profile of the Mother of a Child with OFC

1.Maternal Age : ……………………………….
2. Age at the birth of the child with OFC:………………
2.Race:
- ☐ White
- ☐ Black
- ☐ Indian
- ☐ Coloured

3.Marital status:
- ☐ Married
- ☐ Single
- ☐ Divorced
- ☐ Widowed

4.Education Level:
- ☐ High school
- ☐ Matric
- ☐ Tertiary education
- ☐ Other ……………………………………………………………………………..

5.Employment status:
- ☐ Employed
- ☐ Unemployed……………………………………………………………………..

6.Number of Children: ………………………………………………………………………..
7.The child with OFC (1^{st}, 2^{nd}, 3^{rd} etc)? ……………………………………
8. Family history of cleft palate:
- ☐ Yes…….. specify:……………………………………………
- ☐ No
- ☐ Don't know

Part B:Demographic information of children with OFC

Gender: ☐ Male
☐ Female
Date of Birth: ………………………………………………………………………………
Race: ☐White
☐Black
☐Indian

☐Coloured

Type of Cleft:

☐ Cleft lip(CL):

☐ Bilateral CL

☐ Left CL

☐ Right CL

☐ Cleft palate:

☐ Hard palate

☐ Soft Palate

☐ Both

☐ Cleft alveolus(CA)

☐ Left

☐ Right

☐ Cleft lip and palate:

☐ Bilateral CLP

☐ Left CLP

☐ Right CLP

☐ Other deformities ?

☐Yes Specify:…………………………………………….

☐No

Appendix II: Ethics clearance

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R14/49 Dr Naseeba Ravat

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M220220

NAME: (Principal Investigator)	Dr Naseeba Ravat
DEPARTMENT:	Health Sciences Wits Oral Health Centre
PROJECT TITLE:	Correlation of maternal age to their children born with orofacial cleft treated at the Wits Oral Health Centre
DATE CONSIDERED:	25/02/2022
DECISION:	Approved unconditionally
CONDITIONS:	
NOTE:	If contact information regarding student study participants is required, please contact the University Registrar's office <Nicoleen.Potgieter@wits.ac.za>
SUPERVISOR:	Dr M Makofane and Prof P Hlongwa
APPROVED BY:	CB Penny Dr CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	06/05/2022

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary on the Third Floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions.Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report.** The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in **February** and will therefore be due in the month of **February** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

NRavat — Principal Investigator Signature

06/05/2022 — Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix III: Permission from Acting CEO

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

FACULTY OF HEALTH SCIENCES

School of Oral Health Science

Department of Oral Biological Sciences – Research, Unit 7 York Road, Parktown, 2193. Tel: 011 717 2110 Email: mervyn.turton@wits.ac.za

19 April 2022

Dr N. Ravat
Orthodontics Department
Faculty of Health Sciences
University of the Witwatersrand
Johannesburg

RE: PERMISSION TO CONDUCT RESEARCH

Your request for permission to conduct a research has been provisionally approved by the Committee.

Final approval will be granted after submission of the following information and documents:

1. Application is supported the applicant must sign her letter to the Acting CEO
2. The access of files should be communicated to Mr Ramoleta for monitoring.
3. The research need 133 patient's records which will require him/her to sigh records control register when collecting the records.
4. Ethics Clearance Certificate,

Regards,

Dr P.M. Molokomme

Acting CEO

Date: 21.4.2022.

Appendix IV: Permission from Acting HOS

Department of Orthodontics
16 March 2022

Prof J Shackleton
Acting CEO
Wits Oral Health Centre
School of Oral Health Sciences
University of the Witswatersrand

Dear Prof Shackleton

I, Dr Naseeba Ravat plan to conduct a retrospective qualitative study at the cleft palate clinic of Wits Oral Health Centre. The study forms part of a requirement to complete MSc Dent. My supervisors are Dr Makofane and Prof Hlongwa.

My Topic: Correlation of maternal age to their children born with orofacial clefts treated at the Wits Oral Health Centre
Student number: 0000232 P

The aim of the study is to determine whether there is a correlation between maternal age and OFC, as well determine the types these children present with at the Wits Oral Health Centre (WOHC).

I am writing to you, as the acting CEO, requesting permission to have access to clinical records of patients with OFC attending the Cleft Palate Clinic at the WOHC from Jan 2013 to Dec 2019.The records will be used onsite and will not be removed from the department. They will be handled with care and confidentiality and ensure the anonymity of the patients during data collection. I have applied for ethical approval from the Human Research Ethics Committee (HREC) Medical, of the University of Witwatersrand and will supply the ethical clearance certificate before commencing with data collection.

Yours sincerely,

Dr N Ravat

Permission Granted/~~Permission Not Granted~~

Joy Shackleton — 5 April 2022

Professor Shackleton — Date

Acting Head of School

Comments: Subject to receipt of an Ethics Clearance certificate prior to data collection.