Phenotypic and genetic attributes of congenital and hereditary anomalies ascertained at a tertiary care hospital, Rawalpindi

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بسم الله الرحمن الرحيم

DECLARATION

I hereby declare that the work accomplished in this thesis is the result of my own research work carried out in Human Genetics Lab, Department of Zoology, Quaid-i-Azam University Islamabad. The epidemiological study was carried out at the tertiary care hospital, Rawalpindi. This thesis has neither published previously nor does it contain any material from the published resources that can be considered as the violation of the international copyright law.

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CERTIFICATE

This dissertation "Phenotypic and genetic attributes of congenital and hereditary anomalies ascertained at a Tertiary Care Hospital, Rawalpindi" submitted by **Ms. Fatima Shaheen** is accepted in its present form by the Department of Zoology, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad as satisfying the thesis requirement for the degree of Master of Philosophy in Human Genetics.

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Dedication

This dissertation is dedicated to

My beloved Mother

For her endless support, love, encouragement and all prayers in the accomplishment of this study

To my beloved Father

For all his support, encouragement, love, prayers, investments throughout my educational career

To my beloved Grandmother

For all her love, prayers and sacrifices

And

To my honorable Teachers

For being source of inspiration and enlightenment

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Abbreviations

CA	Congenital anomalies
CHD	Congenital heart defects
CLD	Congenital limb defects
CNS	Central nervous system
CP	Cerebral palsy
CVS	Chorionic villus sampling
DS	Down syndrome
FAS	Fetal alcohol syndrome
GDM	Gestational diabetes mellitus
ICD	International classification of disease
MMC	Myelomeningocele
MRI	Magnetic resonance imaging
NMDs	Neuromuscular diseases
NTD	Neural tube defects
OMIM	Online Mendelian Inheritance in Man
WHO	World Health Organization

Abstract

Congenital anomalies (CA) also known as birth defects are functional, structural, and metabolic defects that occur during the period of organogenesis and observed at birth or later in life. Birth defects are caused by mutation in a gene, chromosomal aberrations, environmental factors, micronutrient deficiencies and multifactorial effects. CA cause significant mortality and morbidity among children both in developing and developed countries. The main objective of this study was to investigate the prevalence pattern of hereditary and congenital anomalies in the multiethnic population of Rawalpindi, and the elucidation of their phenotypic and genetic attributes and associated disorders. During this epidemiological study, a total of 517 independent cases with CA were recruited from Holy Family Hospital, which is the main tertiary care hospital in Rawalpindi. All the anomalies were diagnosed by expert physicians and pediatricians and were classified into eight major categories. In this cohort, the prevalence of the birth defects was in the following order: neurological disorders (39.1%), neuromuscular disorders (21.1%), limb defects (13.5%), musculoskeletal defects (7.4%), blood disorders (4.3%), orofacial defects (3.9%), metabolic disorders (3.7%), cardiovascular defects (2.1%). The ratio of affected males (56%) was high as compared to affected females (44%). The sporadic cases (n=375, 73%) were abundant in comparison with familial cases (n=142, 27%). There were more syndromic cases (63%) than isolated cases (37%). Parental consanguinity was found in 70% of the cases. The current study provides useful information about the prevalence pattern of birth defects and will be helpful in making policies for the prevention of CA. As most of the CA were of preventable nature, certain health care measures should be taken to reduce the prevalence of such anomalies. Immunization, vaccination, antenatal care, proper medications and improved nutrition can minimize the number of these anomalies.

Chapter 1

Introduction

1.1 Congenital and hereditary anomalies

A congenital anomaly (CA) also known as a birth defect or congenital anomaly, can be defined as any defect in the structure, function and metabolism that occur during the developmental period and detected at birth or later in life. Congenital means acquired in the womb. Congenital anomalies are one of the leading causes of stillbirths and neonatal deaths worldwide. These anomalies are detected in 3% of all newborns and 7% of neonatal deaths occur due to these anomalies worldwide (Mekonnen et al., 2020). At least 3.3 million children with birth defects die before age 5 each year and about 3.2 million of those who survive will be mentally and physically disabled for life (Alexander et al., 2016). Annually, birth defects affect 3-7% of children globally. CA is caused by defects in embryogenesis or intrinsic abnormalities in the development process. If not managed properly, CA can cause long-term physical, visual, mental and auditory disabilities (Abdou et al., 2019). Birth defects usually occur during organogenesis (between 3rd and 8th week of pregnancy) (Raza et al., 2012).

Some of CA can be treated for example cleft lip or palate, some are life-threatening for example bilateral renal agenesis and some CA can be survived long term but cause severe disabilities like brain defects (Devi et al., 2007). The symptoms and signs of birth defects may range from mild, moderate and severe to lethal. CA is one of the major causes of impairment in developing and developed countries and represents a major childhood health issue (Taboo, 2012). In developed and developing countries, the birth prevalence of CA is similar; however, because of the lack of proper services for the care of affected newborns, the health impact of birth defects is higher in developing countries (Penchaszadeh, 2002).

An estimated 10-30% of all CA occur due to genetic factors, environmental factors account for 5-10%, multifactorial inheritance are responsible for 20-35% and unknown causes are responsible for 30-45% of congenital anomalies. So most of congenital anomalies are the result of the interaction of environmental factors and genes (Shawky and Sadik, 2011).

CA can be classified into two categories on the basis of the involvement of different organs or parts of the body: a single primary defect (isolated) and multiple malformation syndromes (syndromic). The cause is unknown for most of the single primary defects; they are explained on the basis of multifactorial inheritance. Teratogens, chromosomal abnormalities and single gene defects are responsible for syndromic malformations (Tootoonchi, 2003).

Congenital anomalies can be developmental or structural. Structural anomalies affect the body parts. Developmental CA affect the body's working, person's learning or senses. In some cases, CA affects both the development and the structure for example down syndrome, and spina bifida (Kay and Sissons, 2020). Structural CA can be categorized into two groups: minor and major anomalies. Those that do not cause severe health problems in the neonatal period or have less implications fall in the Minor category. Those that greatly affect the health of the neonates and the future life of the individual and need medical treatments and surgeries in most cases, come in the major category (Anele et al., 2022). An estimated 2–3% of live births are affected by major CAs and are responsible for 20–30% of still births. Major CA occurs as a result of interaction between genes and environmental factors and varies with geographical location and time (Ajao and Adeoye, 2019). Congenital

heart disease (CHD), neural tube defects (NTDs), cleft lip and palate, and limb reduction anomalies are major congenital anomalies (Kishimba et al., 2015).

Hospitalization and treatment of children with CA pose financial problems to their families and it is usually impossible to recover completely (Tayebi et al., 2010). It is a stressful situation not only for parents but also for the community when a child is born with an anomaly. Communities usually attributed it to sin or God's anger, when a mother gives birth to a child with an anomaly and as a consequence, parents are likely to feel guilty (Mekonnen et al., 2021).

1.2 Worldwide prevalence of congenital anomalies

CA cause significant infant mortality. Worldwide prevalence studies revealed that the birth prevalence of CA varies significantly from country to country (Francine et al., 2014). According to World Health Organization (WHO), every year around 240,000 newborns die worldwide within 28 days of birth due to congenital anomalies and are responsible for 170,000 deaths of children between the ages of 1 month and 5 years. In Egypt, birth defects are responsible for about 15% of all infant deaths (Shawky and Sadik, 2011). In developed countries, the dominant cause of infant morbidity and mortality is CA (Taboo, 2012).

The prevalence of CA was reported to be 1.7% in Brazil, 3.63% in Iraq, 2.22% in Eastern India. Ethiopia is one such country with high rates of CA. The proportion of CA was 199 per 10,000 children who visited the hospitals in central and north-west Ethiopia. The rates of birth defects were high in low and middle-income countries due to environmental teratogenic risk factors as compared to high-income countries (Mekonnen et

al., 2020). More than 90% of congenital anomalies are reported in low and middle-income countries (Ajao and Adeoye, 2019).

The birth prevalence is 1.07% in Japan and 4.3% in Taiwan. In the US, the rate of in congenital anomalies is 2-3%. In England, the birth prevalence is 2% and it is 1.49% in South Africa. The prevalence of major CA is 1.64 % in Lebanon (Francine et al., 2014). The prevalence of birth defects is 3.3% in UK. The prevalence of CA ranges between 0.4-11.1 % according to hospital-based studies in Nigeria (Ajao and Adeoye, 2019). In Sudan, the birth prevalence of CAs was 82/1000 live births and in France, the birth prevalence was 39.7/1000 live births. The birth prevalence ranged between 20 and 30/1000 live births in Uganda, Nigeria, Kenya, Saudi Arabia and Pakistan (Abdou et al., 2019). In Nepal, the prevalence of CAs was reported in 52 per 10000 children and responsible for 7% of all neonatal deaths (Khanal et al., 2019). In Korea, among babies with birth defects, the infant mortality rate was 6.8 per 10,000 live births (Kurdi et al., 2019). The estimated prevalence of birth defects is 60.5 per 1000 live births in Tanzania (Kishimba et al., 2015). The type and frequency of birth defects may vary in different populations due to variations in socio-economic status, ethnicity, environmental factors, nutrition, life style and maternal age among different countries (Kumar et al., 2021).

1.3 Prevalence and mortality in Pakistan

In Pakistan, the prevalence of CA is very high because of large sib ships, low socio-economic status, the usual practice of consanguineous marriages and maternal factors. CA account for 6-9% of prenatal deaths (Bibi et al., 2022). 2% of total death counts in Pakistan occur due to congenital anomalies (Bhatti et al., 2019). Studies conducted in Pakistan show

the birth prevalence of congenital anomalies as low as 1.4% to as high as 7% (Anbreen et al., 2021).

1.4 Causes and risk factors

Identifying the cause and recognition of risk factors are important for minimizing the prevalence of preventable anomalies (Tootoonchi, 2003). Identification of risk factors helps in the prevention of congenital anomalies. Indeed, the identification of modifiable risk factors of CA provide the basis for primary prevention which include promoting healthy dietary habit, prevention of sexually transmitted infections, preventing maternal infections during the periconceptional period and fortification of foods with folic acid (Mekonnen et al., 2020). The cause for 40 to 60% of CA is unknown. Genetic causes account for 30-40%, environmental causes contribute 5-10%. Among the genetic causes, single gene disorders contribute 25%, chromosomal abnormalities contribute 6% and multifactorial contribute 20-30%. However, for 50% of CA the cause is known (Francine et al., 2014).

Large sib ships, exposure to radiation, chemical compounds, infectious agents, prematurity, use of medication, maternal illnesses, and occupational exposures are some of the risk factors associated with the increased prevalence of congenital disorders. Environmental exposure can have a preconceptional mutagenic action or a postconceptional teratogenic action. Maternal nutritional deficiencies (e.g. vitamin B1, iodine and foliate deficiency) in the periconceptional period are established risk factors for neural tube defects (Taboo, 2012). In low and middle-income countries, maternal infections with syphilis and rubella are also reported as risk factors of CA (Mekonnen et al., 2020). The prevalence studies worldwide indicate that there is a high ratio of congenital anomalies in the offspring of consanguineous couples. Consanguineous marriages account for increased incidences of

abortion and stillbirth (Riaz et al., 2016). History of inheritable congenital diseases, previous miscarriages, and stillbirths are important factors in the etiology of birth defects (Ajao and Adeoye, 2019).

1.5 Causes of CA

1.5.1 Single gene disorders

` Congenital anomalies arise due to single gene disorders, chromosomal aberrations and multifactorial disorders. Genetically determined disorders contribute significantly to stillbirths and child mortality and future disability. Single gene disorders result from mutation in the gene. Single gene disorders can affect any aspect of structure or function as the mutation can occur in any gene so they are heterogenic. Single-gene disorders follow the Mendelian pattern of inheritance. They include autosomal dominant, autosomal recessive and X-linked disorders (Blencowe et al., 2018). Examples include sickle cell anemia, cystic fibrosis, Tay-sachs disease, hemophilia, color blindness etc.

1.5.2 Multifactorial disorders

Multifactorial disorders are those that result from the interaction of genes and environment and its expression requires the interaction of both. Examples are asthma, cardiovascular diseases, various neurological disorders and autoimmune disorders (Kere et al., 2010). Multifactorial disorders contribute to 20-35% of CA (Shawky and Sadik, 2011).

1.5.3 Chromosomal abnormalities

Chromosomal abnormalities contribute for 6% of CA (Francine et al., 2014). There are two types of chromosomal abnormalities that are structural abnormalities and chromosomal number abnormalities (aneuploidy, polyploidy). Polyploidy results from errors in meiosis 1

or failure of chromosomal segregation in meiosis 2. Chromosomal number abnormalities are more common (Asgari and Kamrani, 2013). Down syndrome caused by an extra chromosome and called trisomy 21, is a familiar example of chromosomal abnormalities. Other examples are Edward's syndrome and Patau syndrome (Aliyu, 2021). Down syndrome occurs in all populations; however, the number of live births is varied by differences in the age of mothers at the time of conception (Antonarakis et al, 2021).

1.6 Risk factors

1.6.1 Folic acid deficiency

A deficiency of folic acid increases the chance of some disorders related to the brain and spine. The improper development of these organs in the womb results in spina bifida. Taking folic acid supplements before conception and during pregnancy minimizes the risk of neural tube defects. Pregnant women need 400 μg of folic acid per day (Kay and Sissons, 2020).

1.6.2 Advanced maternal age

The risk of congenital anomalies is high in old women pregnancies (Taboo, 2012). For all human autosomal trisomies like trisomy 21, advanced maternal age at conception is a major risk factor. This is due to the non-disjunction of homologous chromosomes or chromatids that happens during the meiotic divisions that occur in the formation of oocytes (Antonarakis et al., 2021). According to WHO, advanced maternal age is a risk factor for abnormal intrauterine fetal development.

1.6.3 Intrauterine infections

Some common viral infections associated with congenital anomalies include; Rubella virus, Zika virus**,** and Cytomegalovirus. Infection with Rubella during pregnancy leads to serious complications in the fetus. Approximately 25% of the infant born to mothers who contract rubella in the first trimester of pregnancy have congenital rubella syndrome (Aliyu, 2021). Congenital syphilis accounts for a significant burden in developing countries (Penchaszadeh, 2002). During pregnancy exposure to high temperatures can enhance the probability of certain birth defects, especially those that involve the development of the brain and spinal cord (Kay and Sissons, 2020).

1.6.4 Use of un-prescribed medicines

In developing countries, the use of medicines without prescription and the usual practice of home remedies of unknown composition during pregnancy are associated risk factors of congenital anomalies. Some antiepileptics, antibiotics and antidepressants are prescription medications that are known to increase the risk of birth defects in the exposed offspring (Penchaszadeh, 2002).

1.6.5 Use of alcohol

During pregnancy exposure of the fetus to alcohol is a risk factor of congenital anomalies and mental retardation (Penchaszadeh, 2020). During pregnancy, the use of alcohol increases the chance of fetal alcohol syndrome (FAS). It affects growth and development and causes brain damage (Kay and Sissons, 2020). Among the environmental factors, prenatal ethanol exposure is the most common cause of congenital anomalies. The prevalence of FAS is approximately 2 to 7 of 1,000 live births. Maternal alcohol consumption is an associated risk factor of NTDs. The mother's drinking frequency and

developmental stages of alcohol exposure along with genetic susceptibility determine the variability of birth defects (Sarmah, 2016).

1.6.6 Consanguinity

There is an increased risk of genetic disorders among the offspring of consanguineous unions because of the combination of autosomal recessive gene mutations that is inherited from a common forefather. The probability of the combination of detrimental recessive genes increases when the parents have a closer biological relationship (Shawky et al., 2013). A consanguineous union is characterized by the degree of relatedness between the couple: first cousins, half-first cousins, double first cousins, second cousins, first cousins once removed second cousins once removed and third cousins (Tayebi et al., 2010). The practice of consanguineous marriages is common in many communities throughout the world, especially in South Asia, the Middle East and North Africa. The difference in the prevalence of consanguineous marriages is usually related to religion, race, sociocultural factors, and ethnicity (Bener et al., 2006).

There are significantly increased incidences of abortion and stillbirth among consanguineous unions. Further, there is a high incidence of reproductive losses (i.e. Post-neonatal, neonatal, infant, and pre-reproductive mortalities) in the consanguineous communities compared to the non-consanguineous marriages (Riaz et al., 2016).

1.7 Neurological disorders

Worldwide studies indicate that central nervous system disorders are frequently encountered CA. The most common CA that affects the central nervous system (CNS) is spina bifida and is often represented as the most complex CA consistent with survival

(Bowman et al., 2001). Myelomeningocele (MMC) is a birth defect in which the spinal cord does not develop properly due to incomplete closure of the neural tube at approximately 28 days of gestation. The prevalence of Spina bifida is 1–10 per 1000 live births worldwide (Phillips et al., 2017). Myelomeningocele is the most common form of spina bifida, which affect the brain and mainly involves cognition, behavior, and adaptation. Myelomeningocele accounts for 80-90% of all spina bifida births. Because of its intricacy, the diagnosis and treatment of newborns with spina bifida start before birth and through adulthood. The etiology of spina bifida involves genetic and environmental factors as it is a neurogenetic disorder. Spina bifida is usually associated with congenital anomalies of the brain and hydrocephalus (Fletcher and Brei, 2010). There are some sensory and motor neurological problems below the level of the lesion in individuals with MMC. This may disturb lower limb functions or cause paralysis that disturbs or prevents walking (Copp et al., 2016). Environmental factors that have been linked to spina bifida include insufficient folic acid intake, environmental exposures, hyperthermia, obesity, pregestational maternal diabetes mellitus and maternal anticonvulsant therapy (valproic acid and carbamazepine). Folic acid intake before conception and in the first trimester reduces the risk of neural tube defects including spina bifida. Nutrient sources of folic acid include whole grains and legumes (dried beans, soy beans, etc.) and dark leafy green vegetables (Philips et al., 2017).

Hydrocephalus is one of the complicated and multifactorial neurological disorders. In hydrocephalus, there are abnormalities in the flow or resorption of cerebrospinal fluid (CSF), which causes ventricular dilatation. Congenital and acquired are the two clinical forms of hydrocephalus. An estimated incidence of hydrocephalus is 1 in 1500 births. Congenital hydrocephalus may occur in isolation (non-syndromic) or associated with other anomalies (syndromic). About 40% of hydrocephalus cases occur due to some genetic causes. Along with genetic factors, some environmental factors contribute to the

development of congenital hydrocephalus, such as intracerebral hemorrhage, congenital anomalies, use of alcohol during pregnancy, exposure to X-ray radiations and infections during pregnancy (Zhang et al., 2006). To maintain intracranial pressure in the normal range, the ventricular shunt is required in 70–85% of children with spina bifida and hydrocephalus. The signs and symptoms of shunt failure vary with age (Phillips et al., 2017).

Down syndrome (DS) is another neurological disorder that causes intellectual disability and is caused by trisomy of chromosome 21. Individuals with DS usually have short stature, intellectual disability, muscle hypotonia and congenital heart defects (CHDs). Down syndrome Individuals usually develop certain health conditions such as autoimmune diseases, hypothyroidism, hearing and vision problems, epilepsy, recurrent infections and blood disorders (including leukemia) (Antonarakis et al., 2021). The risk of birth of children with DS increases with advanced maternal age. DS is usually caused by a meiotic error named “nondisjunction" in the egg or the sperm that results in an embryo with three copies of chromosome 21 (Kazemi M et al., 2016).

1.8 Neuromuscular disorders

Inherited neuromuscular diseases (NMDs) are a diverse group of disorders that mainly affect the peripheral nerve, lower motor neurons, muscles or neuromuscular junctions. An estimated prevalence of inherited NMD is 1 in 3, 500 individuals. Cerebral palsy is a well-known neurodevelopmental disorder that begins in early childhood and continues throughout life. People with CP have certain psychiatric and behavioral problems like anxiety disorders, mood disorders, sleep disturbances and musculoskeletal defects such as hip displacement and spinal deformity (Rosenbaum et al., 2007).

Worldwide the prevalence of CP is 2 per 1000 live births. CP is a multifactorial disorder that is usually caused by injury to the brain before or at birth. CP itself is non-progressive, however, as the brain matures the clinical expression changes over time (Gulati and Sondhi, 2018).

1.9 Limb defects

Limb defects that are observable at the time of birth are congenital and it may arise when a part of or the entire limb do not develop normally during embryonic stages. Limb defects that cause disability are reduction defects. Polydactyly and syndactyly are less disabling limb defects. Prenatal exposure to different teratogens is the main cause of congenital limb defects (CLD), the well-known example of which is Thalidomide which causes a range of reduction defects (Vasluian et al., 2013). Congenital limb defects (CLD) cause psychological, physical and social impacts on the life of the individual. On the basis of the involvement of the limb and the nature of the defect, they may be categorized as preaxial or postaxial, upper or lower limb defects, isolated or syndromic, and the phenotypes range from mild to severe. CLD shows a prevalence of 5–21/10, 000 births (Riaz and Malik, 2021).

Depending upon the involvement of skeletal and associated soft tissue parts of the limbs, classification of congenital limb malformations include: (1) duplication (2) failure of separation of anatomical constituents, (3) failure of formation of anatomical constituents (4) under or overgrowth of various anatomical constituents, (5) congenital constriction band syndrome, and (6) skeletal defects (Alexander et al., 2016).

Congenital anomalies, traumas, tumors, diabetes, vascular diseases and malignancies are the primary causes of limb amputation (Jabeen and Malik, 2015). During the embryonic stages, disruptive events, such as vascular disruptions, and amniotic band constriction may

cause a reduction or hypo perfusion of the developing limbs (Vasluian et al., 2013). Maternal risk factors for structural abnormalities include harmful chemical exposure, advanced maternal age (≥35 years old) history of abnormal delivery, maternal gestational diseases (gestational diabetes mellitus (GDM) and hypertension), family history of congenital limb defects, and abnormal genetic examination, taking sedatives (Shi et al, 2018).

One of the most common congenital limb defects is clubfoot, the birth prevalence of which is 1 per 1000 live births. Clubfoot is associated with congenital myotonic dystrophy, amniotic band sequence, distal arthrogryposis, and myelomeningocele in 20% of cases (Dobbs and Gurnett, 2012). Polydactyly is among the most common congenital limb defect seen immediately at birth, and comes in a variety of forms. Its estimated prevalence is 0.3–3.6/1000 in live births and 1.6–10.7/1000 in the general population. Phenotypically, polydactyly is a heterogeneous group of defects in which the upper limbs are more affected than the lower and left foot more affected than the right. The right hand is more involved than the left hand. Post-axial polydactyly and preaxial polydactyly are the two most common forms of polydactyly. Males are often more affected than females (Umair et al., 2018). In the case of syndactyly, two or more digits are fused. It shows a birth prevalence of 1 in 2000 to 3000 live births. Syndactyly is due to the failure of separation of the toes or fingers into individual appendages, which usually happens between the sixth and seventh week of pregnancy (Ermito et al., 2009).

1.10 Diagnosis of CA

Prenatal detection of CA is essential to determine the prediction and consequence of birth defects. Present diagnostic techniques principally rely on imaging methods, such as fetal Magnetic Resonance Imaging (MRI) or ultrasound (US). Supportive invasive tests such as chorionic villus sampling (CVS) and amniocentesis are highly specific and sensitive for

the identification of genetic or chromosomal disorders in the fetus. However, Infection, miscarriage or amniotic fluid leakages are associated risk factors (Wagner et al., 2019). Prenatal identification of birth defects gives parents the chance to terminate the pregnancy when the fetus is suspected of having major birth defects (Stoll et al., 2001).

1.10.1 Ultrasonography

Ultrasonography is one of the most powerful techniques for antenatal diagnosis of congenital anomalies. Ultrasonography can diagnose at least 35 - 50% of major fetal anomalies with a specificity of 90- 100%. Ultrasonography is safe, noninvasive, fast, reproducible with real-time display, accurate and do not cause any harm to the patient at any time of pregnancy (Ali et al., 2021). Ultrasound screening for the detection of fetal structural abnormalities is generally performed at 19-21 weeks of pregnancy. Anencephaly, omphalocele, structural anomalies and urinary tract abnormalities can be detected through ultrasonography performed at a specific time of gestation (Todros et al., 2001).

1.10.2 Amniocentesis

It is the most commonly used method for diagnosing chromosomal abnormalities. In this procedure, amniotic fluid is extracted through the mother's abdominal wall. It is usually performed between the 15^{th} and 20^{th} week of gestation. If performed earlier it may cause fetal complications. The occurrence of early miscarriages after amniocentesis is a serious problem. About 1% incidence of spontaneous abortion is associated with amniocentesis (Tara et al., 2016). There are numerous benefits of amniocentesis. Some doubts raised by serum screening or ultrasound are confirmed by amniocentesis (Quinlan, 2008).

1.10.3 Chorionic villus sampling

Chorionic villus sampling (CVS) is usually performed between 10 to 13 weeks of pregnancy. In this procedure, a needle is inserted into the uterus to withdraw placental tissue for the diagnosis of a range of fetal anomalies. The primary advantage of chorionic villus sampling is earlier genetic results in pregnancy. Bleeding, pregnancy loss, rupture of membranes, infection and uncertain results are risk factors associated with chorionic villus sampling .limb reduction defects are risk factors associated with early CVS (before 10 weeks of pregnancy). However, these complications can be minimized with the advancement of ultrasound and a skilled provider (Jones and Montero, 2021).

1.10.4 Nuchal translucency screening

Nuchal translucency (NT) screening is a sensitive and excellent diagnostic test for the detection of fetal chromosomal abnormalities. Increased risk of abortion, fetal anomalies and fetal deaths are associated with NT thickness. A range of congenital anomalies including neurodevelopmental delay are associated with increasing NT (Roozbeh et al., 2017). NT screening is more sensitive in 11–12 weeks of pregnancy (Ceausu et al., 2018). First-trimester NT ultrasound is an important tool for the diagnosis of various congenital anomalies (Guraya, 2013).

1.11 Aims and objectives

The main objectives of the study were

- To find the prevalence pattern of congenital anomalies in the population of Rawalpindi at a tertiary care hospital
- To understand the inheritance pattern of these anomalies
- To understand the role of consanguinity in the prevalence of these anomalies
- To determine the occurrence of associated anomalies in index subjects
- To investigate the nature of occurrence of these anomalies (sporadic, familial; syndromic, nonsyndromic)

Chapter 2

Methodology

2.1 Study Duration

The current study was conducted from Oct. 2021- June 2022.

2.2 Study area

The current study was conducted in the Neonatal and Pediatric Department of Holy Family Hospital, Rawalpindi (Fig. 2.1-2.3). It is a tertiary care hospital that receives a large number of patients from joint cities Rawalpindi-Islamabad. All types of care are provided in this hospital and newborns are regularly screened for congenital anomalies before discharge from the maternity unit.

2.3 Sample size

A total of 517 cases with different congenital anomalies were recruited.

Fig. 2.1 External view of Holy Family Hospital, Rawalpindi

Fig. 2.2 Pediatric department (Holy Family Hospital)

Fig. 2.3 Neonatal Department (Holy Family Hospital)

2.4 Study design

A cross-sectional retrospective epidemiological study was carried out in Holy Family Hospital, Rawalpindi. A total of 517 cases were recruited during the current study. The main objective of the study was to find out the clinical and genetic attributes of congenital anomalies. Few of hospital-based studies were conducted to assess CA prevalence in the Pakistani population.

2.5 Study population and inclusion/exclusion criteria

The subjects recruited during the study belonged to different ethnic groups and were from different regions all over Pakistan. Mothers who delivered babies with CA within the study period and the patients admitted to the in-patient department were recruited and the subjects included neonates to children >9yrs. Minor defects were excluded from the study because of the difficulties in recruiting such defects. If multiple congenital anomalies were present, the primary major birth defect was taken (spina bifida with club foot, spina bifida was taken).

2.6 Questionnaire designing and filling

A standard questionnaire was designed according to the requirement of the study. All the data from the subject was recorded in written proforma which was divided into three sections: the first section included the demographic data i.e. age, gender, residence, origin, religion, socioeconomic status, occupation, family type, education, language, etc.

The second section consisted of various risk factors such as maternal pregnancy events, parental ages, consanguinity, and number of normal and affected siblings.

The third section covered the phenotypic details of anomalies and measurements like wait, height, head circumference, mode of delivery, gestation period etc. This proforma was

used for all types of anomalies. Parents or guardian of each index case was carefully interviewed and medical reports and photographs if available were taken.

2.7 Ethical approval

Due to ethical and moral limitations, while studying the human population, the study was approved by the Ethical Review Committee of Quaid-i-Azam University, Islamabad. The study was also approved by the Head of the Neonatal and Pediatric Department of Holy Family Hospital, Rawalpindi. Written consent was taken from the parents or guardians of the subject as the participant itself was below the legal age of providing consent or was incapable of providing it because of disability. The parents or guardian of the subjects was completely informed about the aim and objectives of this study. . They were assured before starting the study that their data will be kept confidential.

2.8 Data collection method

The mother or guardian of the index case was interviewed and all type of information mentioned in the proforma was obtained from them. Only those index cases whose parents or guardians were willing to be interviewed were recruited.

2.9 Pedigree construction

The information obtained by the parents or guardian of the patient was drawn in the form of a pedigree, which is the scientific representation of the family showing the ancestry, all family members, mode of inheritance of the disease, marriage types and affected, carriers and normal family members. Standard symbols were used in the pedigree to represent males, females, twins, marriage type, and affected and deceased family members.

2.10 Categorization of anomalies

All the anomalies were diagnosed and classified by the expert physician and obstetrician. Using different data bases and literature search, anomalies were classified into major groups. According to the criteria of Online Mendelian Inheritance In Man (OMIM), the major categories were further subdivided into minor categories. The anomalies were divided into familial and sporadic. On the basis of the involvement of multiple organ systems, the anomalies were divided into syndromic and non-syndromic. Neurological disorders were most common followed by neuromuscular and limb defects. The data was entered in the excel sheet for storage and different statistical tools were used for statistical analysis.

2.11 Socio-demographic attributes

Socio-demographic variables such as gender, age, caste, parental age at birth of the subject, family type, socio-economic status, family history of anomaly, parental consanguinity, education and living area were considered as risk factors.

Chapter 3

Results

During the current study, a total of 517 families/cases with different types of congenital anomalies were recruited from Holy Family Hospital, Rawalpindi. Among the index subjects, males were 56% and females were 44%.

The prevalence of congenital anomalies are in the following order: neurological disorders (39.1%), neuromuscular disorders (21.1%), limb defects (13.5%), musculoskeletal defects (7.4%), blood disorders (4.3%), orofacial defects (3.9%), metabolic disorders (3.7%), cardiovascular defects (2.1%) (Table 3.2). The sporadic occurrence of anomalies (n=375, 73%) was higher in comparison with familial occurrence (n=142, 27%). The majority of index cases originated from Punjab province (78%) and urban areas (56%). Syndromic presentations were 63% and isolated presentations were 37%. Parental consanguinity was found to be 70%.

3.1. Demographic attributes of index cases

3.1.1 Distribution of index subjects with respect to gender and familial/sporadic nature

The ascertained subjects were examined based on gender and nature of occurrence of the anomaly i.e. sporadic/familial. Male subjects showed higher representation 56%(n=291) as compared to female subjects 44% (n=226), and the sporadic occurrence of anomalies 73% (n=375) were higher in comparison with familial occurrence 27% (n=142) (Fig. 3.1; Table 3.1).

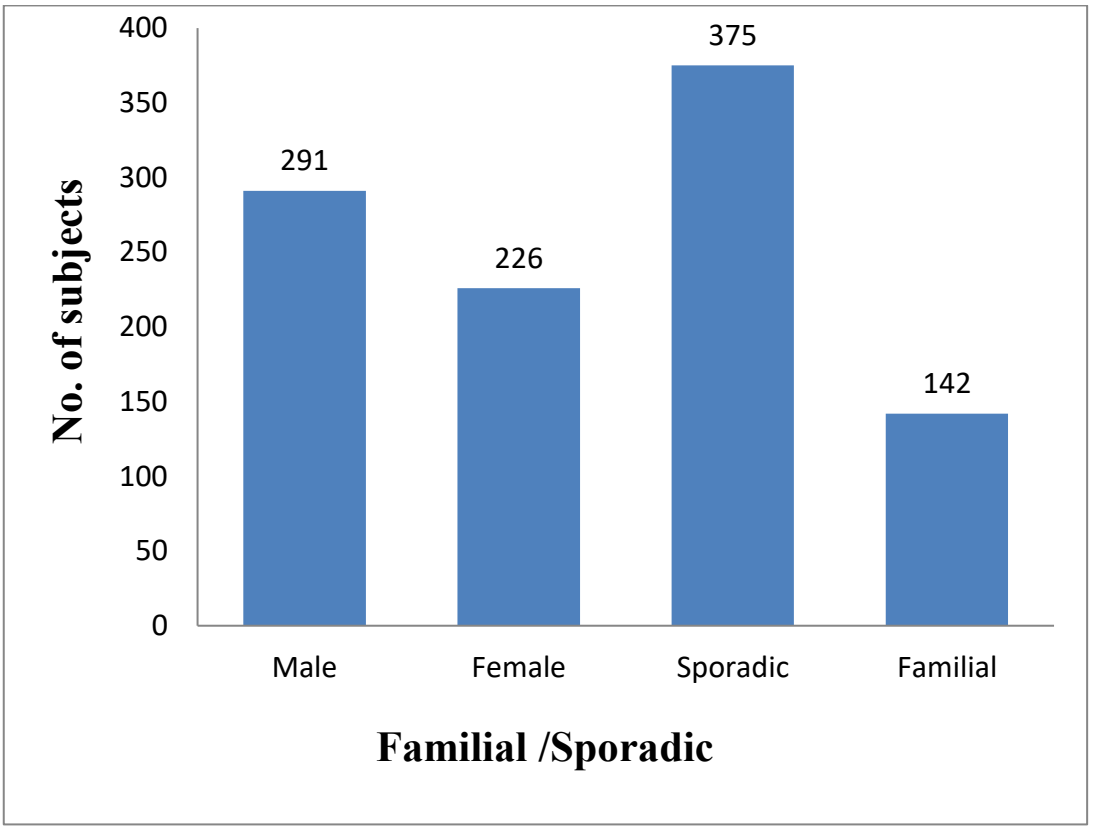


Fig. 3.1.1 Distribution of index subjects with respect to gender and nature of occurrence of anomaly

3.1.2 Distribution of index subjects with respect to the age range

Based on age the cases were classified into three age groups. The majority of the subjects fall in the age group up to 5 years. The distribution of gender-wise data with respect to age groups was statistically not significant (P=0.633) (Fig. 3.1.2; Table 3.1).

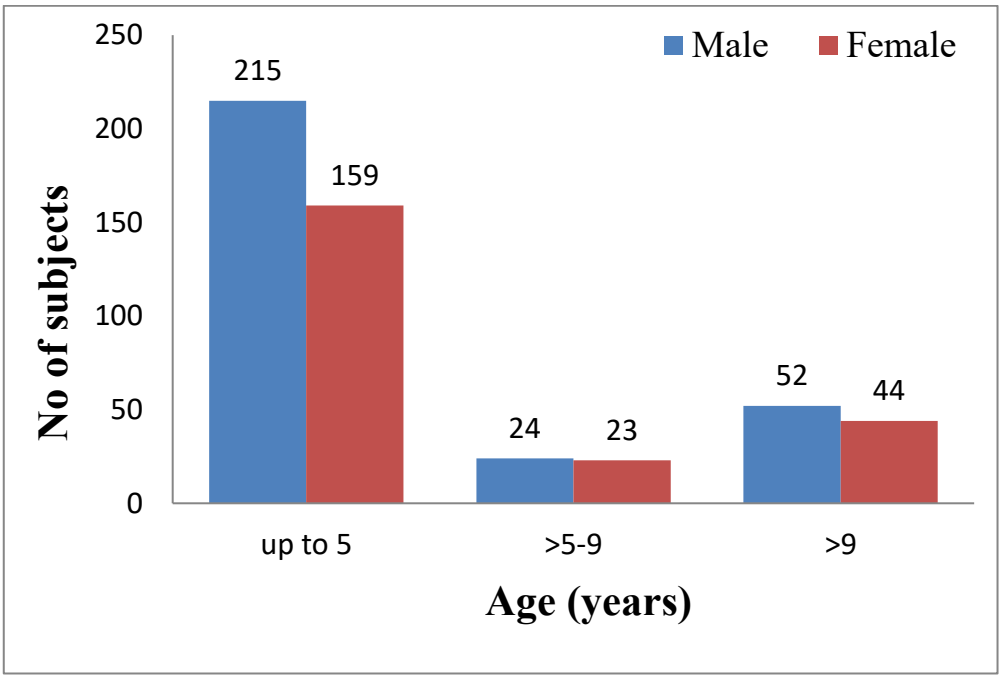


Fig. 3.1.2 Distribution of subjects with respect to age range

3.1.3 Distribution of index subjects with respect to origin

Based on the origin the index cases were categorized into two groups i.e. rural and urban. Most of the index cases belonged to the urban areas and the distribution of gender-wise data with respect to rural/urban status was statistically significant (P=0.037; Fig. 3.1.3; Table 3.1)

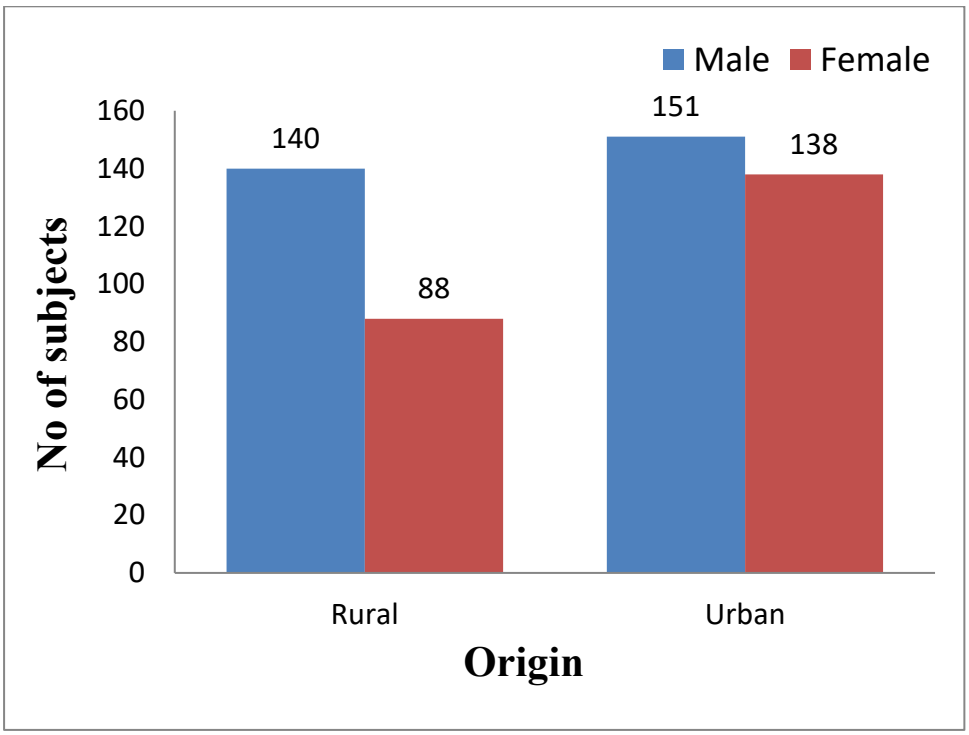


Fig. 3.1.3 Distribution of index subjects with respect to origin

3.1.4 Distribution of index subjects with respect to ethnicity

All the index subjects were categorized into five major ethnic groups. The ethnic group containing less number of subjects was grouped into “others “category. A large number of subjects recruited belonged to the ethnic group Pathan 14% (n=73) (Fig. 3.1.4; Table 3.1).

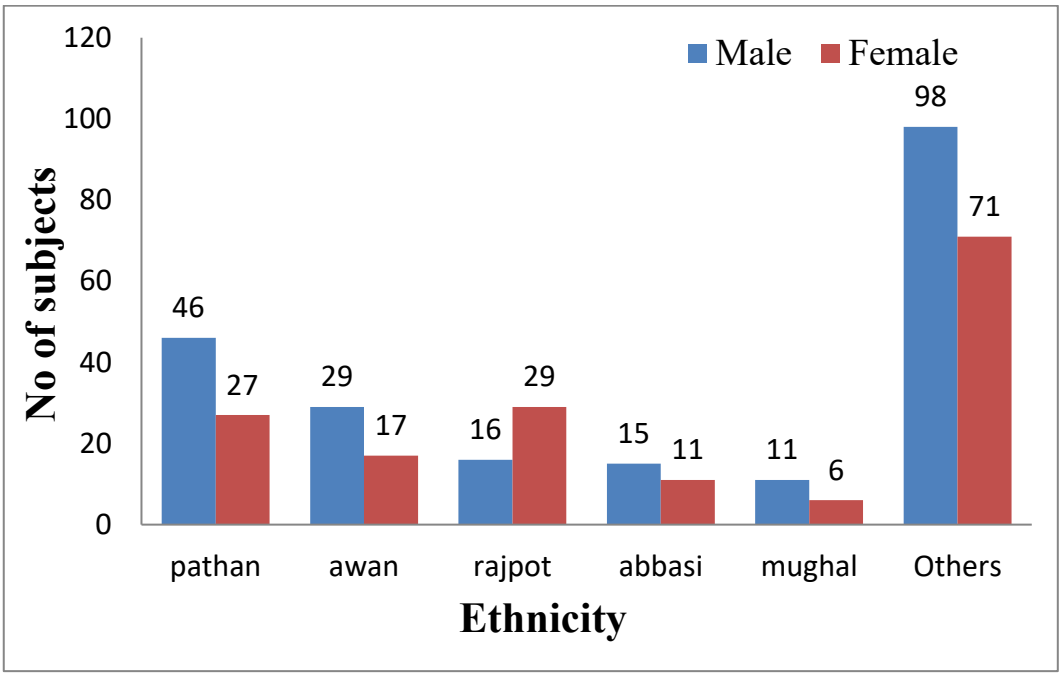


Fig. 3.1.4 Distribution of index subjects with respect to ethnicity

3.1.5 Distribution of index subjects with respect to socioeconomic rank

The index cases were analyzed on the basis of socioeconomic status and were classified as high, mid and low. The majority of the subjects fall in the low category 55% (n=288) and the distribution of gender-wise data with respect to socioeconomic status was statistically not significant (P=0.100) (Fig. 3.1.5; Table 3.1).

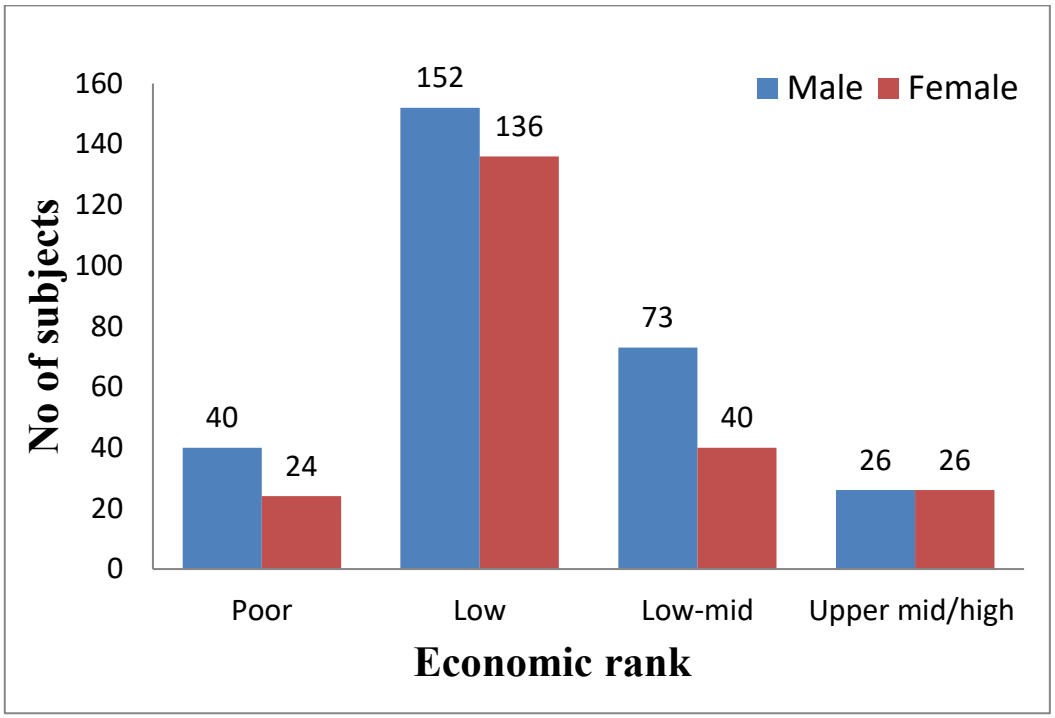


Fig. 3.1.5 Distribution of index subjects with respect to socio-economic rank

3.1.6 Distribution of index subjects on the basis of family type

Based on the family type the recruited subjects were categorized into two groups i.e. extended and nuclear. Index subjects with extended family type showed a higher percentage 58.8% (n=283) and the distribution of gender-wise data with respect to family type was statistically not significant (P=0.390) (Fig. 3.1.6; Table 3.1).

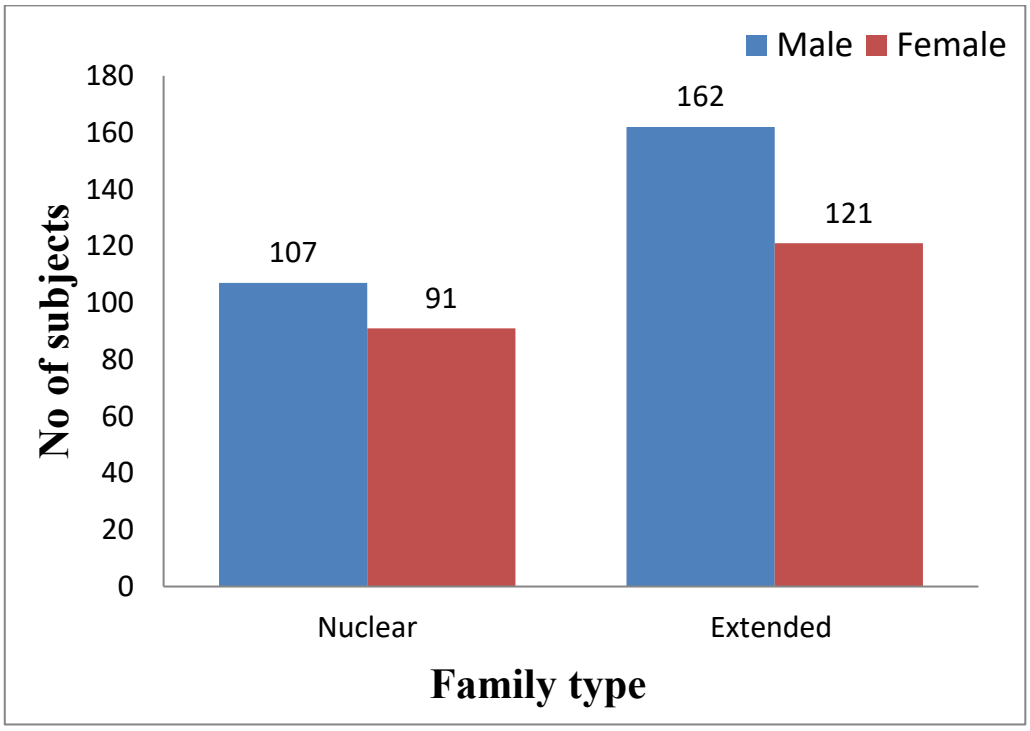


Fig. 3.1.6 Distribution of index subjects on the basis of family type

3.1.7 Distribution of index subjects with respect to province

On the basis of the province, the index cases were categorized into four major groups and the groups with less number of subjects were grouped into the 'others' category. Most of the index cases were from the province of Punjab 78% (n=405) and the distribution of gender-wise data with respect to province was statistically not significant (P=0.057) (Fig. 3.1.7; Table 3.1).

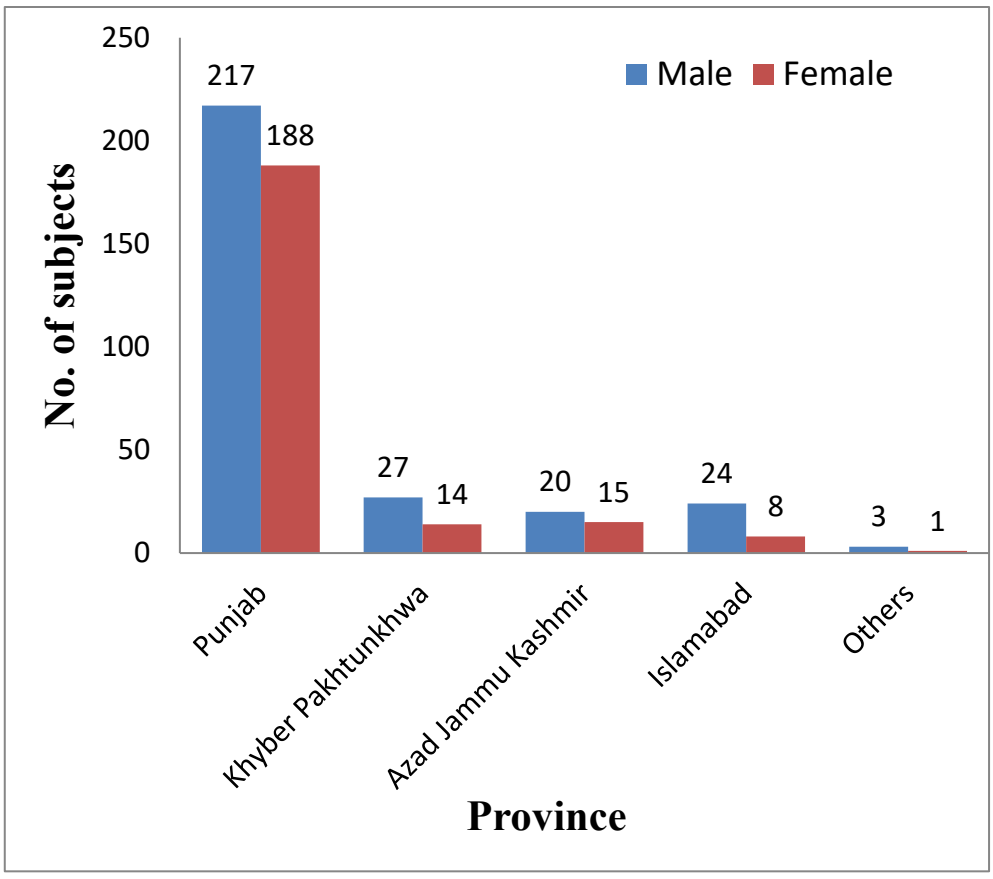


Fig. 3.1.7 Distribution of index subjects with respect to Province

3.1.8 Distribution of index subjects with respect to mode of delivery

On the basis of mode of delivery, index cases were categorized into two groups. Index subjects born through normal delivery were in major presentation of 66% and the distribution of gender-wise data with respect to mode of delivery was statistically not significant (P=0.587) (Fig. 3.1.8; Table 3.1).

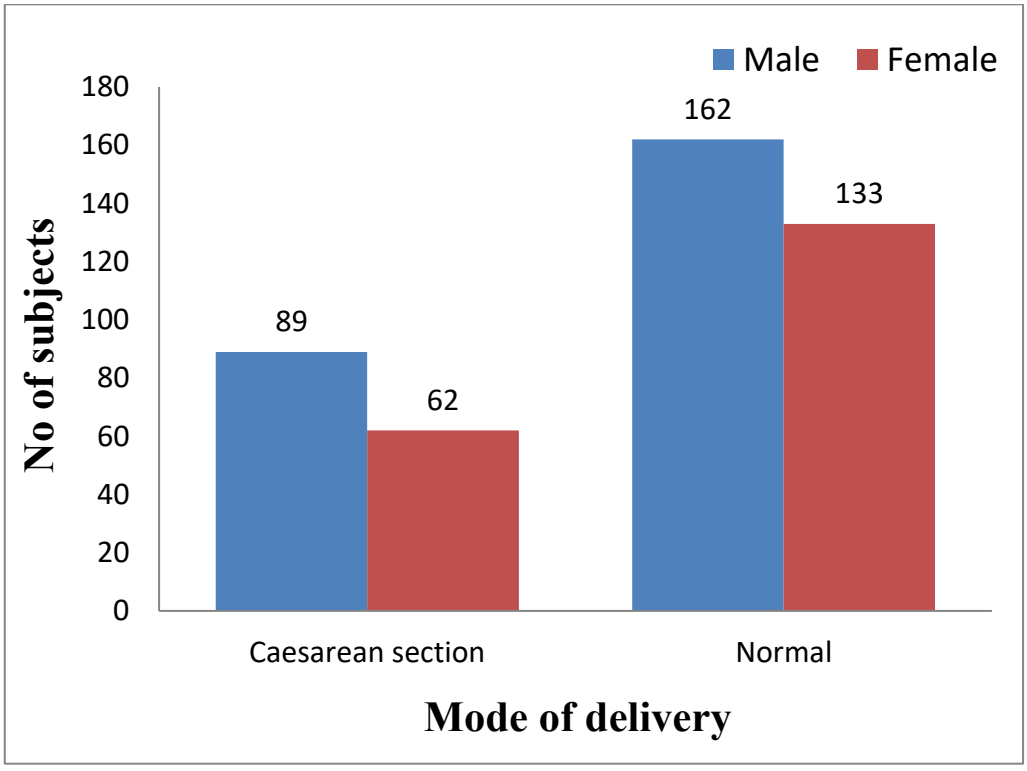


Fig. 3.1.8 Distribution of index subjects with respect to mode of delivery

3.1.9 Distribution of index subjects with respect to delivery spot

With respect to delivery spot, index cases were split into two groups. Hospital-based delivery showed high percentage of 86.7% and the distribution of gender-wise data with respect to delivery spot was statistically not significant (P=0.531) (Fig. 3.1.9; Table 3.1).

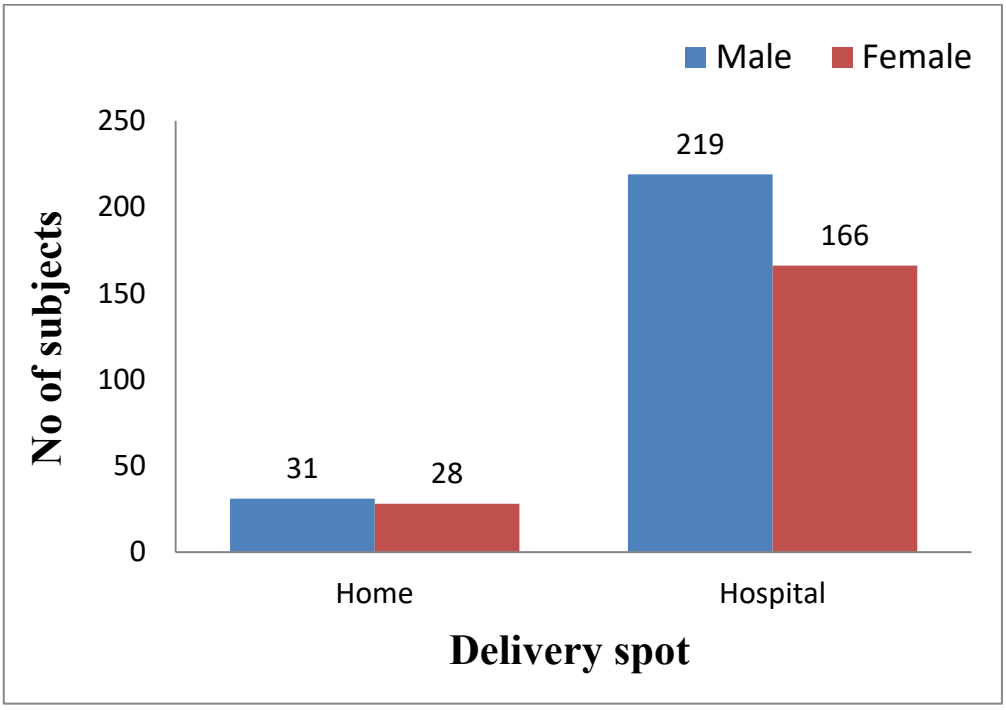


Fig. 3.1.9 Distribution of index subjects with respect to delivery spot

Table 3.1: Demographic attributes of patients with respect to gender and familial/sporadic nature

Variables	Gender		Familial/sporadic		Total	
	Male	Female	Sporadic	Familial	No.	%
Age categories (years)						
Up to 5	215	159	286	88	374	72.3
>5-9	24	23	29	18	47	9.1
>9	52	44	60	36	96	18.6
Total	**291**	**226**	**375**	**142**	**517**	100.0
	chi2= 0.915; P= 0.633		chi2=10.53; P=0.005			
Province						
Punjab	217	188	295	110	405	78.3
Khyber Pakhtunkhwa	27	14	25	16	41	7.9
Azad Jammu Kashmir	20	15	30	5	35	6.8
Islamabad	24	8	23	9	32	6.2
Others	3	1	2	2	4	0.8
	chi2=12.25; P=0.057		chi2=15.14; P=0.019			
Rural/urban origin						
Rural	140	88	159	69	228	44.1
Urban	151	138	216	73	289	55.9
	chi2=4.34; P=0.037		chi2=1.60; P=0.206			
Mother tongue						
Punjabi	145	132	200	77	277	53.6
Pashto	59	31	62	28	90	17.4
Urdu	19	22	31	10	41	7.9
Pahari	25	11	27	9	36	7.0
Hindko/Pothawari	22	19	31	10	41	7.9
Others	21	11	24	8	32	6.2
	chi2=21.59; P=0.119		chi2 =13.32; P=0.578			
Economic status						
Poor	40	24	50	14	64	12.4
Low	152	136	215	73	288	55.7
Low-mid	73	40	78	35	113	21.9
Upper mid/high	26	26	32	20	52	10.1
	chi2=10.64; P=0.100		chi2=10.55; P= 0.103			

Family structure						
Nuclear	107	91	139	59	198	41.2
Extended	162	121	212	71	283	58.8
Total	269	212	351	130	481	100.0
	chi2= 4.12; P= 0.390		chi2=5.94; P= 0.203			
Parental consanguinity						
No	90	66	123	33	156	30.2
Yes	201	160	252	109	361	69.8
	chi2=0.18; P=0.672		chi2=4.47; P= 0.035			
Delivery spot						
Home	31	28	40	19	59	13.3
Hospital	219	166	286	99	385	86.7
Total	250	194	326	118	444	100.0
	chi2(1) = 0.3918 P = 0.531		chi2=1.10; P= 0.293			
Delivery mode						
Cesarean section	89	62	111	40	151	33.9
Normal	162	133	216	79	295	66.1
Total	251	195	327	119	446	100.0
	chi2=1.93; P=0.587		chi2=5.17; P= 0.159			

3.2 Distribution of major and minor anomalies

A total of 517 cases with different hereditary and congenital anomalies were recruited and were classified into eight major categories. Out of these neurological disorders with 39.1% were most common followed by neuromuscular (21.1%), limb defects (13.5%), musculoskeletal defects (7.4%), blood disorders (4.3%), orofacial defects (3.9%), metabolic disorders (3.7%), cardiovascular defects (2.1%). Using OMIM and ICD-10 databases, major categories were further classified into minor categories. The 'Others 'category contains anomalies with less number of index cases (Fig. 3.2; Table 3.2).

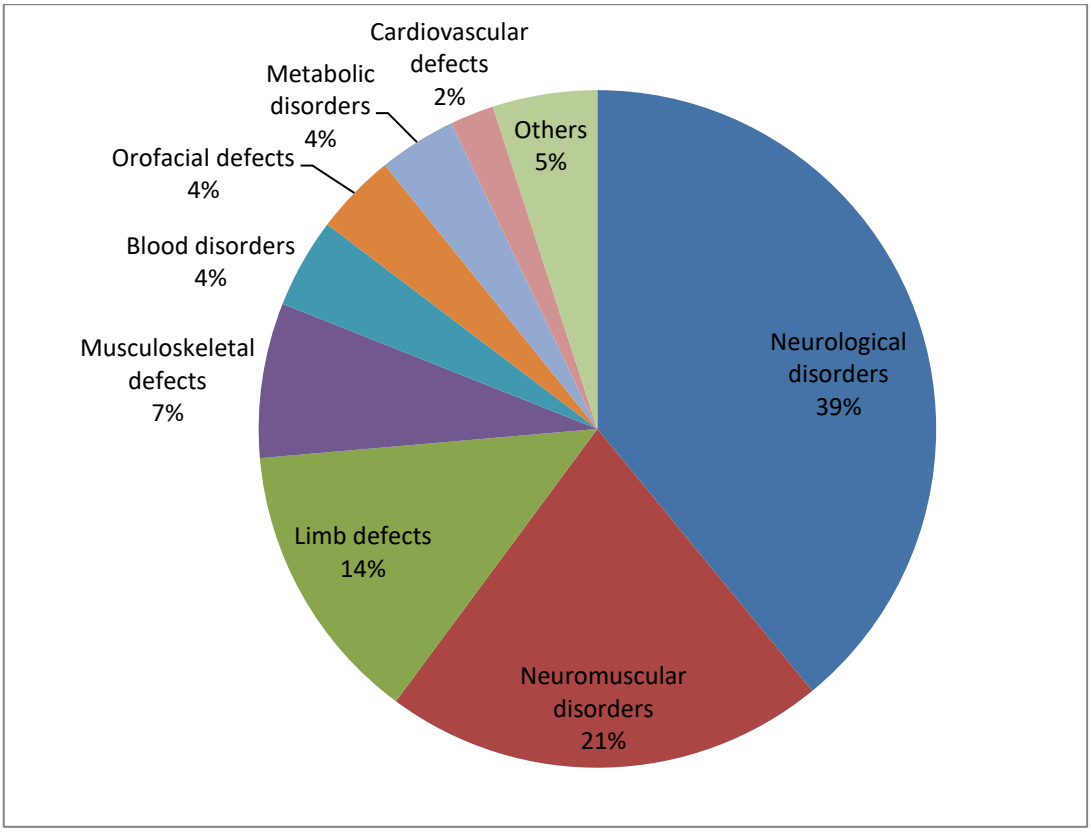


Fig. 3.2 Major categories of birth defects

Table 3.2: Distribution of major categories of congenital anomalies with respect to gender, familial/sporadic nature, and age categories

Major category	Gender		Familial/sporadic		Age categories (years)			Total	
	Male	Female	Sporadic	Familial	Up to 5	>5-9	>9	(No.)	(%)
Neurological disorders	117	85	155	47	167	13	22	202	39.1
Neuromuscular disorders	56	53	71	38	82	11	16	109	21.1
Limb defects	43	27	49	21	38	4	28	70	13.5
Musculoskeletal defects	20	18	29	9	22	7	9	38	7.4
Blood disorders	11	11	14	8	11	6	5	22	4.3
Orofacial defects	14	6	16	4	17	1	2	20	3.9
Metabolic disorders	10	9	18	1	15	3	1	19	3.7
Cardiovascular defects	6	5	10	1	10	0	1	11	2.1
Others	14	12	13	13	12	2	12	26	5.0
Total	**291**	**226**	**375**	**142**	**374**	**47**	**96**	**517**	**100.0**
	Chi2=4.31; P=0.828		Chi2=19.91; P=0.011		Chi2= 68.13; P<0.0001				

Table 3.3 Distribution of major and minor categories of congenital anomalies

Major/minor category	Frequency	Proportion	95% CI	ICD-10	OMIM
Neurological disorders	***202***	***0.391***	***0.349-0.433***		
Hydrocephaly	48	0.093	0.068-0.118	G91.9	236600
Spina bifida	48	0.093	0.068-0.118	Q05	182940
Down syndrome	35	0.068	0.046-0.089	Q90	190685
Developmental delay	22	0.043	0.025-0.060	Z13.42	618330
Intellectual disability	19	0.037	0.021-0.053	F03	300243
Epilepsy	11	0.021	0.009-0.034	G40	117100
Microcephaly	11	0.021	0.009-0.034	Q02	251200
Encephalocele	5	0.010	0.001-0.018	Q01.9	607132
Edwards syndrome	2	0.004	-0.001-0.009	Q91.3	601161
Leukodystrophy	1	0.002	-0.002-0.006		607694
Neuromuscular disorders	***109***	***0.211***	***0.176-0.246***		
Cerebral palsy (congenital)	79	0.153	0.122-0.184	G80	605388
Cerebral palsy (late onset)	30	0.058	0.038-0.078		
Limb defects	***70***	***0.135***	***0.106-0.165***		
Talipes (all types)	39	0.075	0.053-0.098	Q66.0	119800
Polydactyly (all types)	15	0.029	0.015-0.043	Q69.9	174200, 174400
Amputation (transverse)	5	0.010	0.001-0.018	Q73.0, Q72.0	217100
Brachydactyly (all types)	4	0.008	0.000-0.015	Q68.81	113000
Radial hemimelia	2	0.004	-0.001-0.009	Q73.8	
Syndactyly (all types)	2	0.004	-0.001-0.009	Q70	609815
Club hand	1	0.002	-0.002-0.006	Q71.4	
Fibular hemimelia	1	0.002	-0.002-0.006		
Split hand	1	0.002	-0.002-0.006	Q72.7	183600
Musculoskeletal defects	***38***	***0.074***	***0.051-0.096***		
Muscular Dystrophy	7	0.014	0.004-0.024	G71.0	310200
Arthrogryposis	5	0.010	0.001-0.018	Q74.3	108120
Osteopetrosis	5	0.010	0.001-0.018	Q78.2	259710
Dwarfism, skeletal dysplasia	4	0.008	0.000-0.015	E34.3	100800
Developmental dysplasia of hip	3	0.006	-0.001-0.012	Q65.8	142700

Multiple exostosis	3	0.006	-0.001-0.012	Q78.6	133700
Limb hypotonia	2	0.004	-0.001-0.009	P94.2	300868
Osteogenesis imperfecta	2	0.004	-0.001-0.009	Q78.0	166200
Achondroplasia	1	0.002	-0.002-0.006	Q77.4	100800
Apert syndrome	1	0.002	-0.002-0.006	Q87.0	101200
Crouzon syndrome	1	0.002	-0.002-0.006	Q75.1	123500
Dystrophic dwarfism	1	0.002	-0.002-0.006	E34.5	100800
Pfeiffer syndrome	1	0.002	-0.002-0.006	B27.0	101600
Rickets	1	0.002	-0.002-0.006	E34.4	100800
Scoliosis	1	0.002	-0.002-0.006	M41	181800
Blood disorders	***22***	***0.043***	***0.025-0.060***		
Thalassemia	12	0.023	0.010-0.036	D56	613985
Anemia	4	0.008	0.000-0.015	D64.9	
Hemophilia	2	0.004	-0.001-0.009	D66	306700
Pancytopenia	2	0.004	-0.001-0.009	D61.0	
Fanconi anemia	1	0.002	-0.002-0.006	D61.09	227650
Sickle cell anemia	1	0.002	-0.002-0.006	D57.1	603903
Orofacial	***20***	***0.039***	***0.022-0.055***		
Cleft lip and palate	8	0.015	0.005-0.026	Q37	119530
Cleft palate only	7	0.014	0.004-0.024	Q35	119540
Dysmorphic face	2	0.004	-0.001-0.009		
Choanal atresia	1	0.002	-0.002-0.006	Q30.0	608911
Cleft lip only	1	0.002	-0.002-0.006	Q36	600625
Pierre-Robin syndrome	1	0.002	-0.002-0.006	Q87.0	261800
Metabolic disorder	***19***	***0.037***	***0.021-0.053***		
Storage disorders	7	0.014	0.004-0.024		
Cystic fibrosis	4	0.008	0.000-0.015	E84.0	219700
Gaucher disease	3	0.006	-0.001-0.012	E75.2	230800
Hurler syndrome	2	0.004	-0.001-0.009	E76.0	607014
Wilson disease	1	0.002	-0.002-0.006	E83.0	277900
Mucopolysaccharidosis	1	0.002	-0.002-0.006	E76.3	252800
Niemann-Pick disease	1	0.002	-0.002-0.006	E75.2	257200
Others	***37***	***0.072***	***0.049-0.094***		
Congenital heart defects	11	0.021	0.009-0.034	Q23.4	614954
Deaf and Mute	6	0.012	0.002-0.021	H91.3	304500
Ichthyosis	6	0.012	0.002-0.021	L85.0	242300
Omphalocele	3	0.006	-0.001-0.012	Q79.2	164750
Albinism	1	0.002	-0.002-0.006	E70.3	203100

Ambiguous genitalia	1	0.002	-0.002-0.006	Q56.4	250790
Ectodermal dysplasia	1	0.002	-0.002-0.006	Q82.4	305100
Immunodeficiency	1	0.002	-0.002-0.006	D89.9	
Inflammatory bowel disease	1	0.002	-0.002-0.006	K50-52	612567
Keratoderma	1	0.002	-0.002-0.006	L40.3	144200
Pulmonary hypertension	1	0.002	-0.002-0.006	I27.0	178600
Retinitis pigmentosa	1	0.002	-0.002-0.006	H35.5	603937
Retinoblastoma	1	0.002	-0.002-0.006	C69.2	180200
Speech impairment	1	0.002	-0.002-0.006	R47.0	
Systemic lupus erythematosus	1	0.002	-0.002-0.006	M32	152700

3.2.1 Distribution of anomalies based on the sporadic and familial representations

All the index cases with a certain type of anomalies were analyzed into familial and sporadic groups. The sporadic occurrence of anomalies (n=375, 73%) was higher in comparison with familial occurrence (n=142, 27%).In neurological disorders 76% of index cases were sporadic and 24% were familial. Among neuromuscular disorders 65% of index cases were sporadic and 35% were familial. In limb defects, the percentage of sporadic cases was 70% and familial 30%. In musculoskeletal defects, sporadic cases were 76% and familial cases were 24%. In blood disorders, the percentage of sporadic was 63% and familial 37%. In orofacial defects, the percentage of sporadic cases was 80% and familial 20%. In metabolic disorders 94.7% of cases were sporadic. In cardiovascular disorders, 90% of cases were sporadic and in 'others' category 50% cases were sporadic and 50 % were familial. The distribution of anomalies with respect to nature of occurrence of anomalies was statistically significant (P=0.011) (Fig. 3.2.1; Table 3.2).

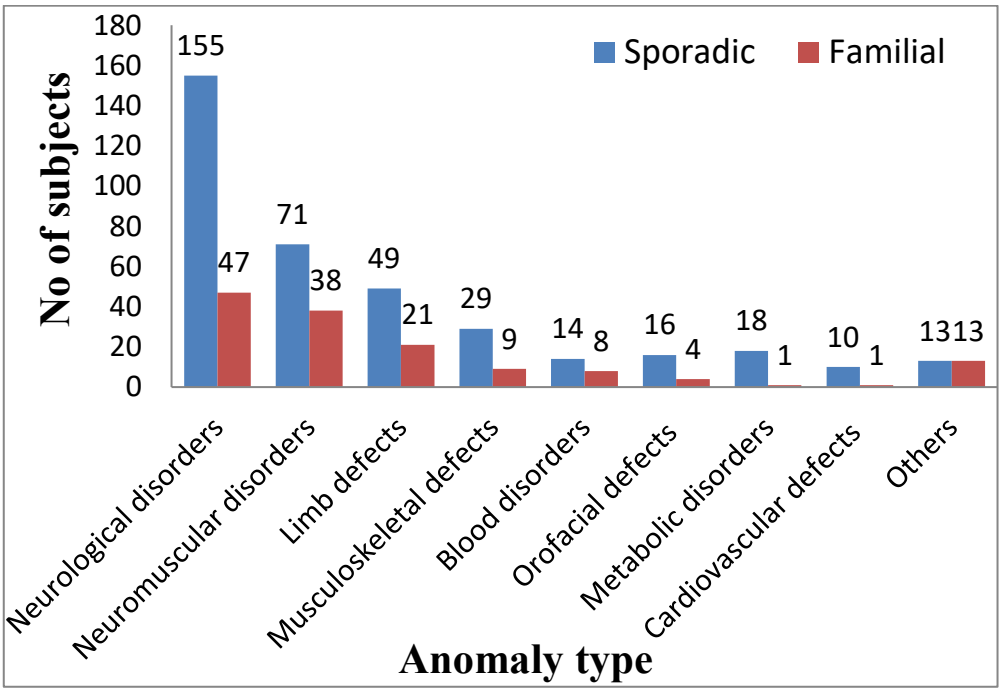


Fig. 3.2.1 Distribution of anomalies based on sporadic/familial nature

3.2.2 Distribution of anomalies based on the gender

All the index cases were analyzed on the basis of gender. The result showed that males were more affected than females in neurological, limb and orofacial defects while in neuromuscular, musculoskeletal, blood, metabolic and cardiovascular defects the ratio of affected males and females were almost the same and the distribution of anomalies with respect to gender was statistically not significant (P=0.828) (Fig. 3.2.2; Table 3.2).

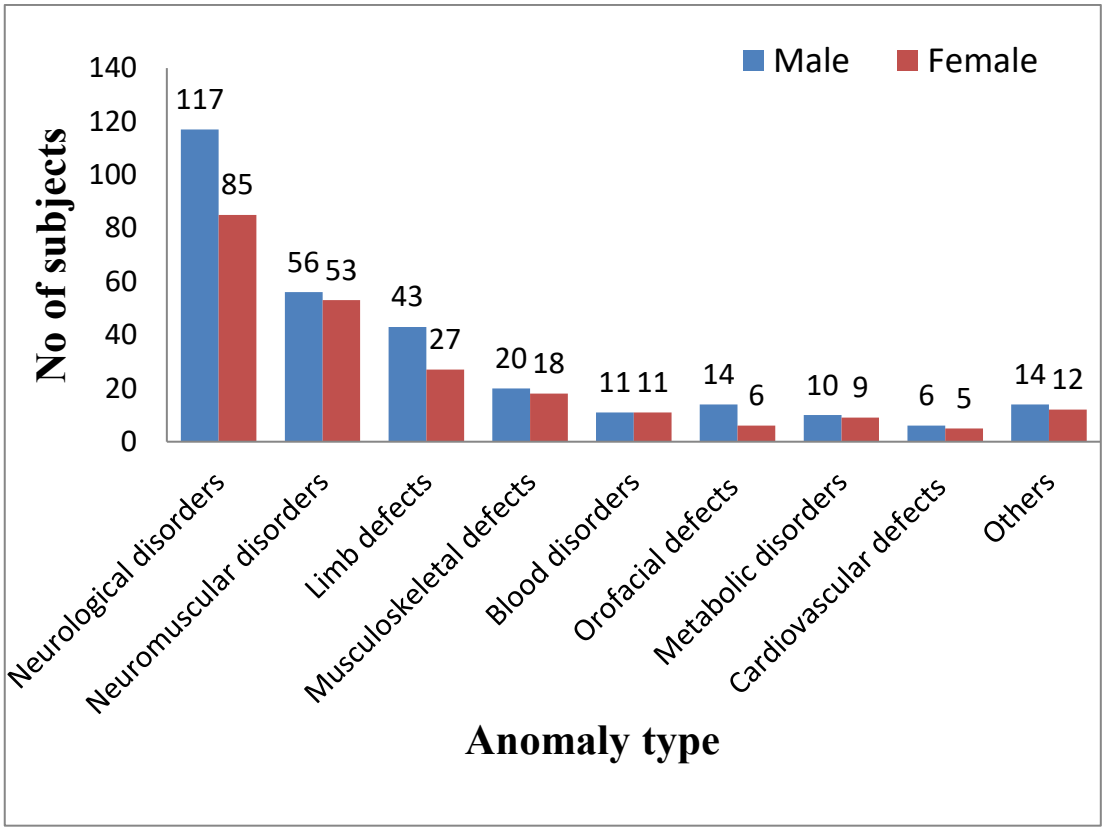


Fig. 3.2.2 Distribution of anomalies with respect to gender

3.2.3 Distribution of anomalies with respect to syndromic and isolated occurrence

The major anomalies were classified into isolated and syndromic groups. Anomalies with syndromic occurrence were 63% and with isolated occurrence were 37%. Syndromic presentations were more conspicuous among neuromuscular and neurological disorders (99% and 78%, respectively), whereas isolated presentations were evident in blood disorders, limb defects and metabolic defects (86%, 84% and 70%, respectively) (Fig. 3.2.3; Table 3.2).

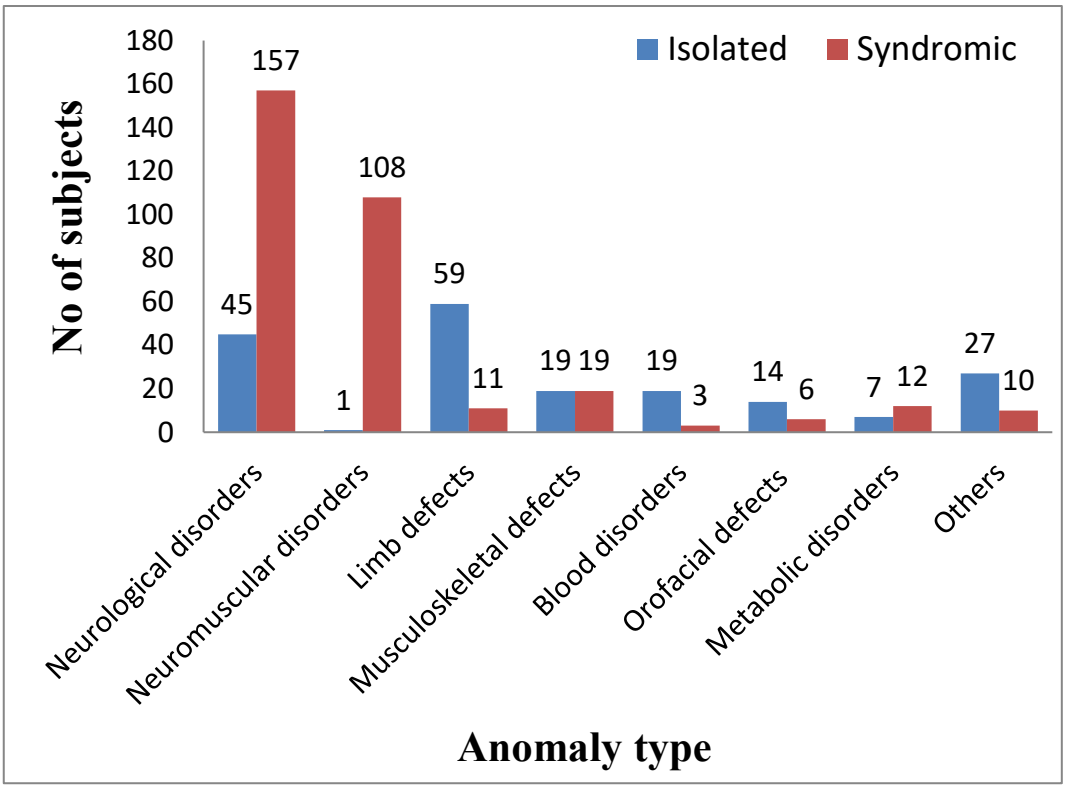


Fig. 3.2.3 Distribution of anomalies with respect to syndromic and isolated occurrence

3.2.4 Distribution of major anomalies with respect to parental consanguinity

In the current study, parental consanguinity played a significant role in all the anomalies.70% of the total cases had parental consanguinity. The highest consanguinity was observed in musculoskeletal defects and metabolic disorders (89%) followed by orofacial defects (85%), while the lowest rate of consanguinity was witnessed in neuromuscular disorders (63%) (Fig. 3.2.4).

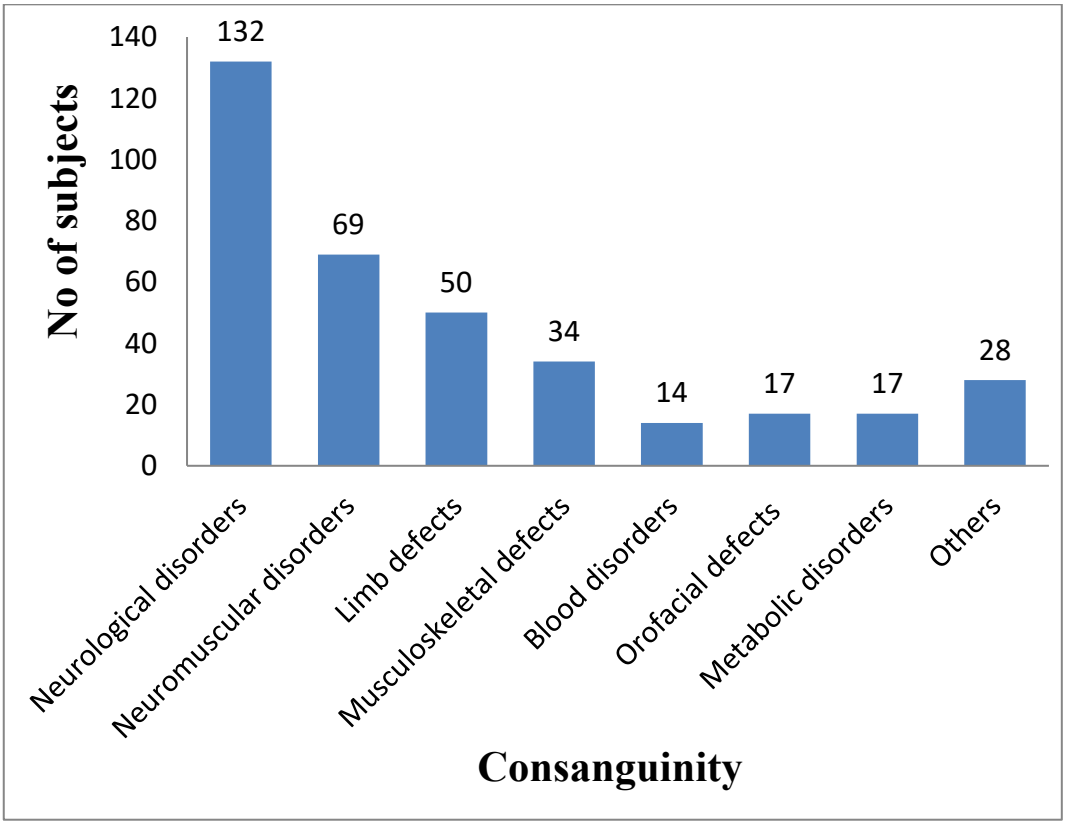
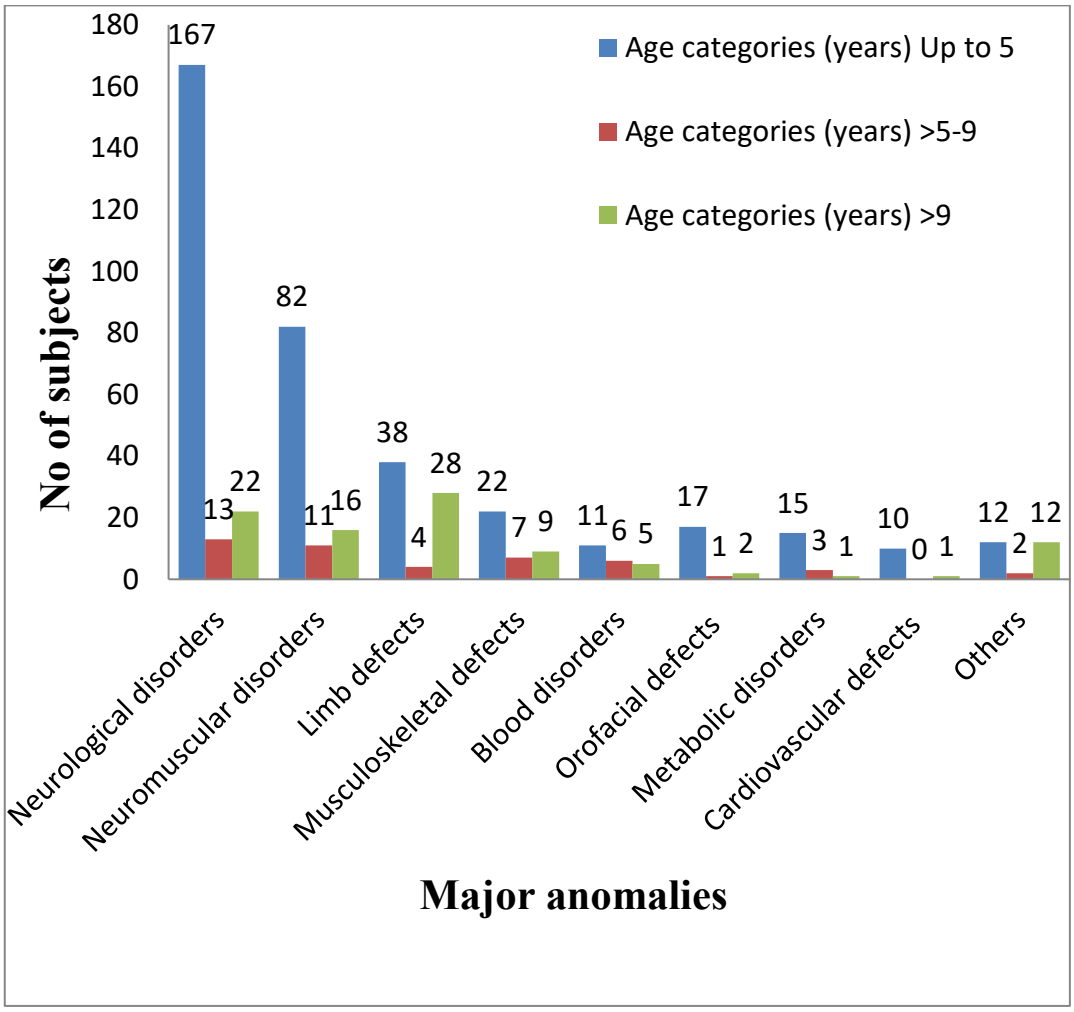


Fig. 3.2.4 Distribution of major anomalies with respect to parental consanguinity

3.2.5 Distribution of major anomalies with respect to age

Major groups of anomalies were analyzed with respect to age and were categorized into three groups. In all major categories, index cases with age up to 5 were more affected and the distribution of anomalies with respect to age groups was statistically significant (P<0.0001) (Fig. 3.2.5; Table 3.2).



3.2.5 Distribution of major anomalies with respect to age

3.2.6 Distribution of major anomalies with respect to total affected in all families

The data was analyzed on the basis of the number of affected members in all families and the results are shown in Table 3.4

Table 3.4 Distribution of major anomalies with respect to total affected in all families

Major category	Total affected in all families		
	Male	Female	Total
Neurological disorders	156	119	275
Neuromuscular disorders	83	83	166
Limb defects	66	42	108
Musculoskeletal defects	39	24	63
Blood disorders	19	17	36
Orofacial defects	17	7	24
Metabolic disorders	11	11	22
Others	42	29	71
Total	433	332	765

3.3 Associated disorders

3.3.1 Major Anomalies with associated disorders

All the hereditary and congenital anomalies were analyzed on the basis of associations with other disorders. Among all the anomalies, neuromuscular and neurological disorders were more commonly associated with other disorders (99%, 78%) respectively. Some of the most common associated defects were developmental delay (156), sensorineural/pinna defects (78), and epilepsy (51) (Table 3.5).

Table 3.5 Major Anomalies with associated disorders

Associations*	Major category (No.)			
	Neurological disorder (141)	Neuromuscular disorders (108)	Musculoskeletal defects (20)	Total (317)
Developmental delay	50	89	9	156
Sensorineural/pinna defects	9	63	1	78
Epilepsy	17	33	0	51
Visual anomalies	5	10	3	22
Talipes	17	3	0	21
Congenital heart defects	14	0	0	21
Intellectual disability	4	13	0	18
Skeletal defects	5	1	1	12
Microcephaly	2	7	0	10
Dysmorphic face	4	0	2	8

*Ten most common associations have been shown

3.3.2 Minor Anomalies with associated disorders

Among the minor anomalies, the cerebral palsy (n=107), hydrocephaly (n=45), spina bifida (n=25) and down syndrome (n=20) were more commonly associated with developmental delay, Sensorineural/pinna defects and epilepsy (Table 3.6).

Table 3.6 Minor Anomalies with associated disorders

Associations*	Minor category (No.)			
	Cerebral palsy (107)	Hydrocephaly (45)	Spina bifida (25)	Down syndrome (20)
Developmental delay	89	36	0	6
Sensorineural/pinna defects	63	4	0	0
Epilepsy	33	4	2	0
Visual anomalies	10	2	1	0
Talipes	3	1	15	0
Congenital heart defects	0	1	1	11
Intellectual disability	13	0	0	0
Skeletal defects	1	1	0	2
Microcephaly	7	0	0	0
Dysmorphic face	0	1	0	1

*Ten most common associations have been shown

3.3.3: Parity of index subjects in major categories

The data gathered during the current study was critically analyzed in order to find out the link between congenital anomalies and parity order. It was found that the incidence of anomalies was highest in subjects with 1st parity (28%), followed by 2nd parity (22%), 3rd parity (16%), 4th parity (14%), 5th parity (7.8%), 6th parity(5.1%) and so on (Table 3.7).

Table 3.7 Parity of index subjects in major categories

Major category	Parity								No of subjects
	1	2	3	4	5	6	7	≥8	Total
Neurological disorders	44	47	38	33	14	11	3	4	194
Neuromuscular disorders	34	25	13	12	9	7	6	2	108
Limb defects	21	12	9	11	2	2	3	2	62
Musculoskeletal defects	8	8	4	5	1	2	2	0	30
Blood disorders	7	6	3	1	2	0	2	1	22
Orofacial defects	6	4	3	3	3	0	0	0	19
Metabolic disorders	8	1	3	4	2	1	0	0	19
Others	11	6	6	3	5	2	0	0	33
Total	139	109	79	72	38	25	16	9	487

3.3.4 Disease segregating generations in familial cases

Of the total 517 recruited cases, only 142 cases were familial. These familial cases were analyzed on the basis of the number of generations segregating the disease. It was found that 83% cases were segregated only up to 1st generation, followed by 13% with 2nd generation and only 2.8% cases with 3rd generation (Table 3.8).

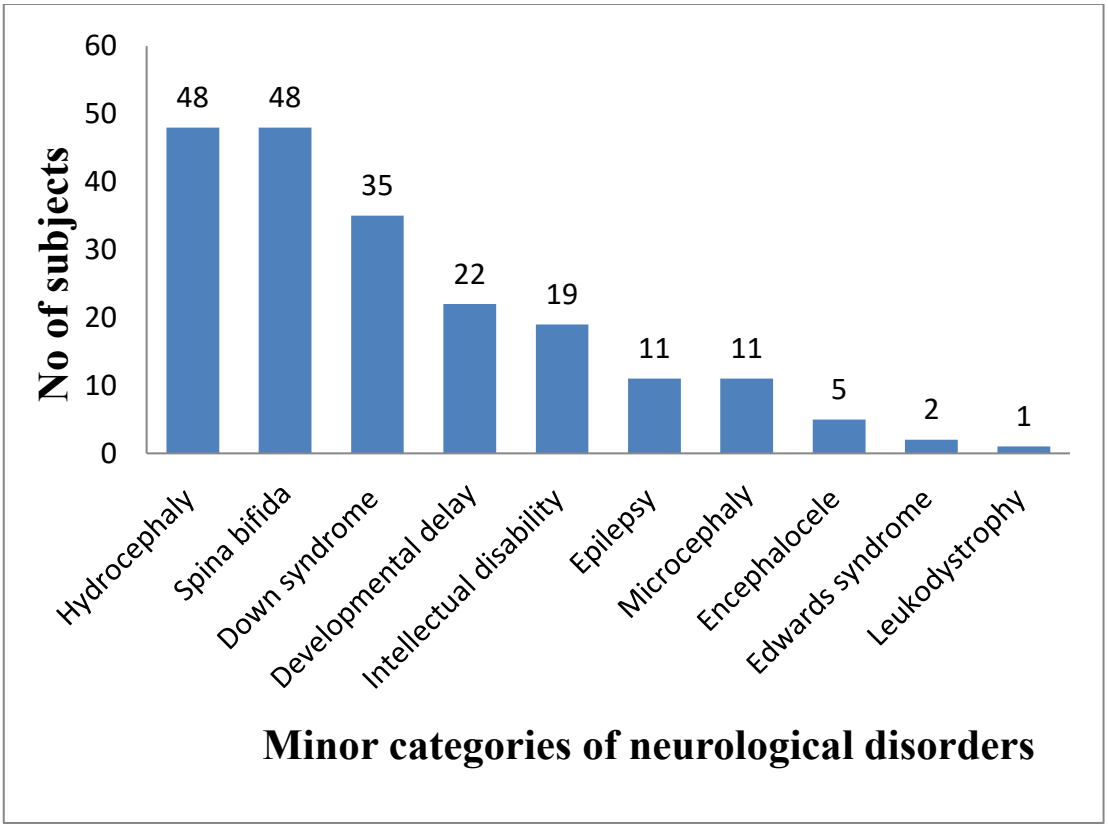
Table 3.8 Distribution of index cases based on disease segregating generations

Major category	Generations with disease….			
	1	2	3	Total
Neurological disorders	46	1	0	47
Neuromuscular disorders	35	3	0	38
Limb defects	9	10	2	21
Musculoskeletal defects	6	2	1	9
Blood disorders	8	0	0	8
Orofacial defects	3	1	0	4
Metabolic disorders	0	1	0	1
Others	12	1	1	14
Total	119	19	4	142

3.4 Neurological Disorders

3.4.1 Classification of Neurological Disorders

In the current study, of the total 517 index cases, neurological disorders (n=202, 39%) were most abundant. Neurological disorders were further classified into different minor groups. In these minor groups, the most prevalent was hydrocephaly (n=48) and spina bifida (n=48) followed by down syndrome (n=35), developmental delay (n=22), intellectual disability (n=19), epilepsy (n=11), microcephaly (n=11), encephalocele (n=5), Edward syndrome (2) and leukodystrophy (n=1) (Fig. 3.4.1, Table 3.9) .



3.4.1 Minor categories of Neurological disorders

3.4.2 Distribution of Neurological Disorders with respect to gender, familial/sporadic and syndromic/isolated nature

Neurological disorders were analyzed on the basis of gender, syndromic/isolated, sporadic and familial nature. In neurological disorders males (n=117) were more affected than females (n=85). Sporadic cases were higher in number (n=155) and syndromic cases (n=156) were dominant (Table 3.9).

Table 3.9 Distribution of Neurological Disorders with respect to gender, familial/sporadic and syndromic/isolated nature

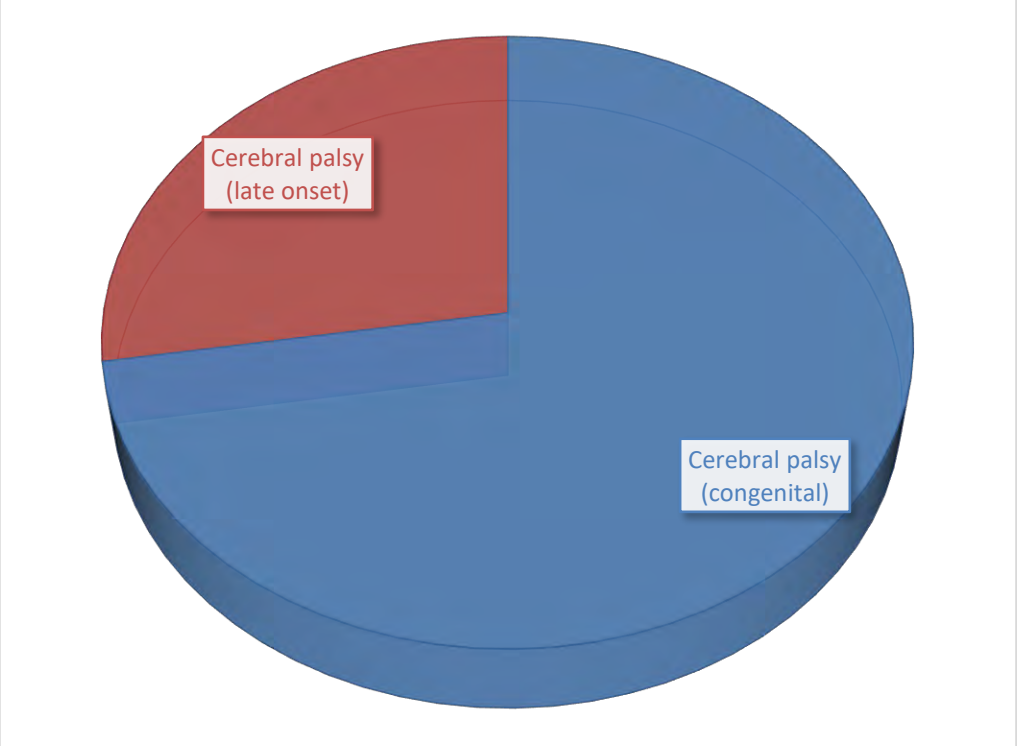
Anomaly	Gender		Sporadic/Familial		Syndromic/Isolated	
	Male	Female	Sporadic	Familial	Syndromic	Isolated
Hydrocephaly	27	21	35	13	44	4
Spina bifida	27	21	42	6	38	10
Down syndrome	19	16	33	2	34	1
Developmental delay	15	7	13	9	14	8
Intellectual disability	13	6	11	8	12	7
Epilepsy	7	4	8	3	4	7
Microcephaly	5	6	8	3	7	4
Encephalocele	1	4	5	0	0	5
Edwards syndrome	2	0	0	2	2	0
Leukodystrophy	1	0	0	1	1	0
Total	**117**	**85**	**155**	**47**	**156**	**46**
	$\chi2$=8.088, df= 9, P=0.5253: ns		$\chi2$=28.77, df= 9, P=0.0007: *** (S)		$\chi2$=47.58, df= 9, P<0.0001: *** (S)	

Ns= statistically non-significant, S= statistically significant

3.5 Neuromuscular Disorders

3.5.1 Classification of Neuromuscular Disorders

After the neurological disorders, the most prevalent was neuromuscular disorders (n=109), 21%). Neuromuscular disorders were further split into two groups' i.e. cerebral palsy (congenital) and cerebral palsy (late onset). Cerebral palsy (congenital, n=79) was more common than cerebral palsy (late onset, n=30) (Fig. 3.5.1, Table 3.10).



3.5.1 Classification of Neuromuscular disorders

3.5.2 Distribution of Neuromuscular Disorders on the basis of gender, familial/sporadic, syndromic and isolated nature

Neuromuscular disorders were second most prevalent disorders in the current study. All the cases of neuromuscular disorders were analyzed with respect to gender, familial/sporadic, syndromic and isolated nature. In this category males (n=56) and females (n=53) were almost equally affected. Sporadic cases (n=71) were in majority as compared to familial cases (n=38). Syndromic cases (n=108) dominated the isolated cases (n=1) (Table 3.10).

Table 3.10 Analysis of Neuromuscular Disorders on the basis of gender, familial/sporadic, syndromic and isolated nature

Anomaly	**Gender**		**Sporadic/Familial**		**Syndromic/Isolated**	
	Male	**Female**	**Sporadic**	**Familial**	**Syndromic**	**Isolated**
Cerebral palsy (congenital)	42	37	49	30	78	1
Cerebral palsy (late onset)	14	16	22	8	30	0
Total	**56**	**53**	**71**	**38**	**108**	**1**
	$\chi 2$=0.3675, df= 1, P=0.5444: ns		$\chi 2$=1.224, df= 1, P=0.2685: ns		$\chi 2$=0.383, df= 1, P=0.5359: ns	

Ns= statistically not significant

3.6 Limb Defects

3.6.1 Classification of Limb Defects

Limb defects were the third major category of anomalies in this study and represent a total of 70 cases. These were further split into 9 minor groups as follows: Tallipes (all types, n=39), Polydactyly (all types, n=15), Amputation (transverse, n=5), Brachydactyly (all types, n=4), Radial hemimelia (n=2), Syndactyly (all types, n=2), Club hand (n=1), Fibular hemimelia (n=1), Split hand (n=1) (Fig. 3.6.1, Table 3.11).

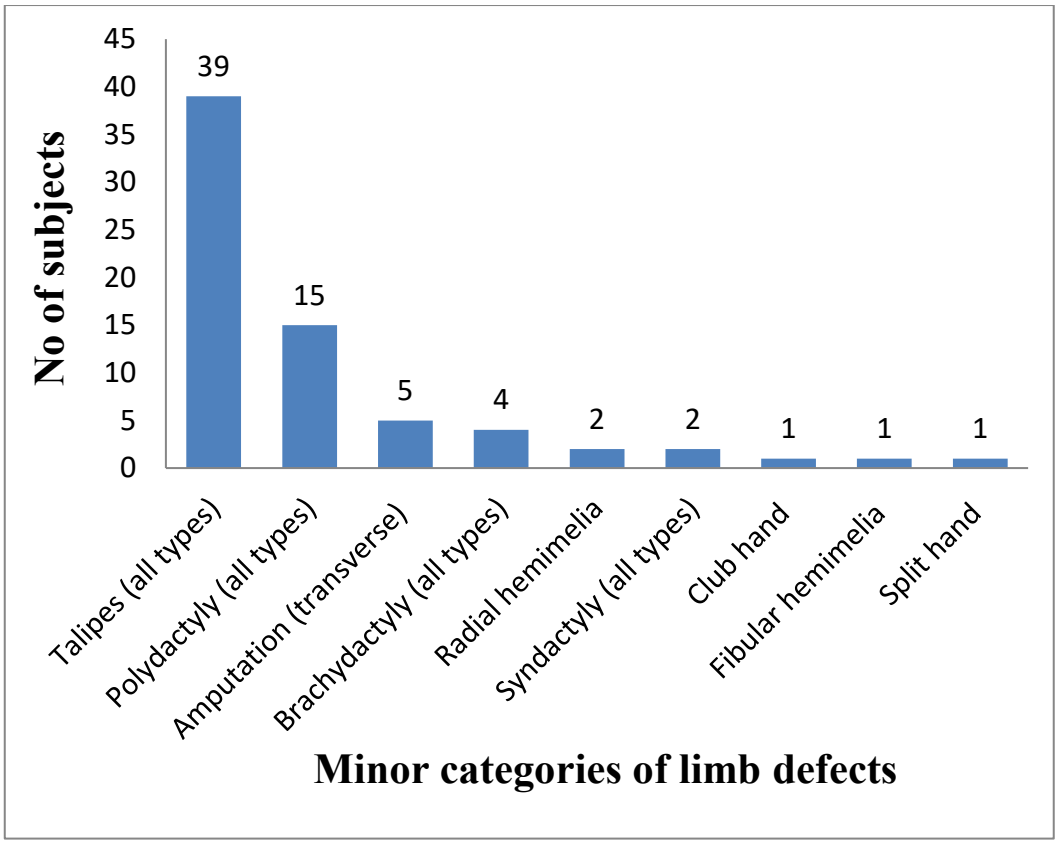


Fig. 3.6.1 Minor groups of Limb Defects

3.6.2 Distribution of Limb Defects on the basis of gender, syndromic and isolated nature

Limb defects were the third most prevalent disorder in this study. When we analyzed the limb defects with respect to gender, syndromic and isolated nature, it was found that number of males (n=43) were higher than number of females (n=27). There were more isolated cases (n=59) than syndromic cases (n=11) (Table 3.11).

Table 3.11 Distribution of Limb Defects

Anomaly	**Gender**		**Syndromic/Isolated**	
	Male	**Female**	**Syndromic**	**Isolated**
Talipes (all types)	27	12	6	33
Polydactyly (all types)	9	6	3	12
Amputation (transverse)	3	2	0	5
Brachydactyly (all types)	1	3	0	4
Radial hemimelia	2	0	0	2
Syndactyly (all types)	0	2	1	1
Club hand	1	0	1	0
Fibular hemimelia	0	1	0	1
Split hand	0	1	0	1
Total	**43**	**27**	**11**	**59**
	$\chi 2$=11.51, df= 8, P=0.1743: ns		$\chi 2$=9.774, df= 8, P=0.2813: ns	

Ns = statistically non-significant

3.7 Musculoskeletal Defects

3.7.1 Categorization of Musculoskeletal Defects

Musculoskeletal defects covered only 38 cases and include 15 minor groups. Muscular dystrophy (n=7) was the dominant minor group followed by arthrogryposis (n=5) and osteopetrosis (n=5) (Fig. 3.7.1, Table 3.12) .

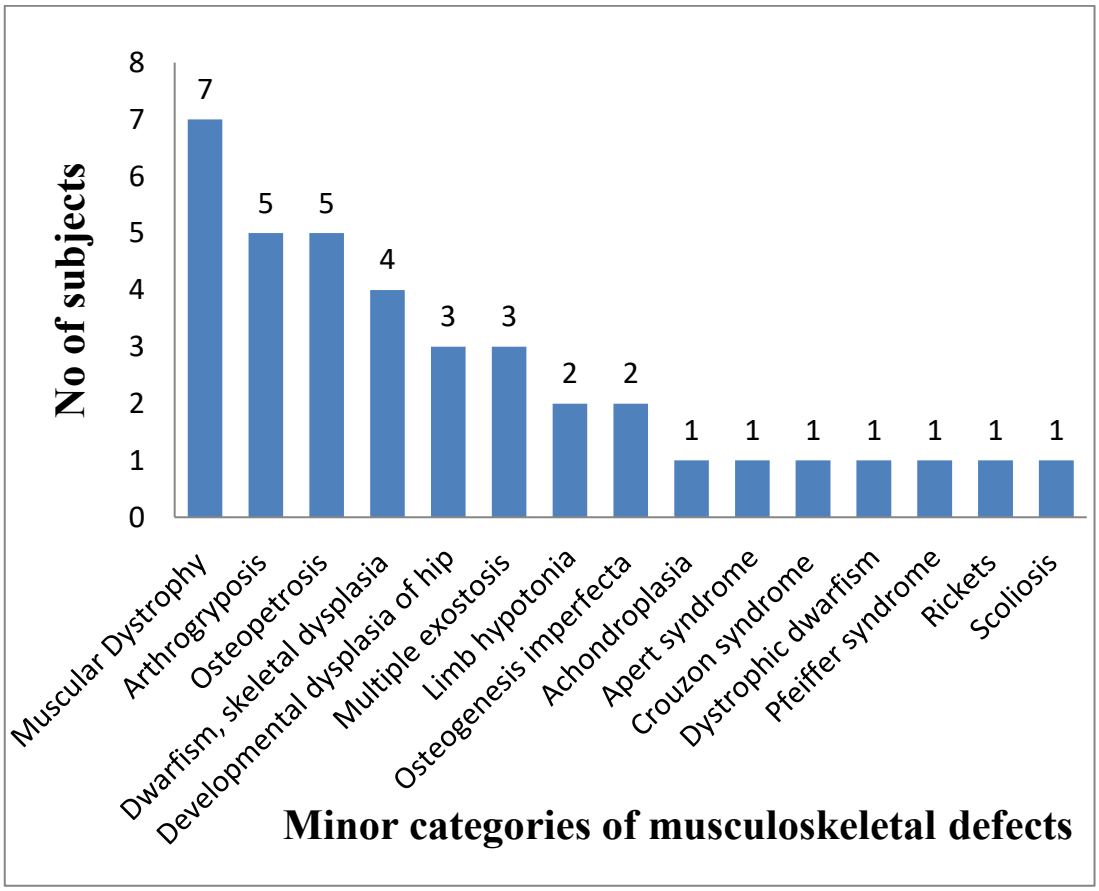


Fig. 3.7.1 Classification of Musculoskeletal Defects

3.7.2 Distribution of Musculoskeletal defects on the basis of gender, familial/sporadic, syndromic and isolated nature

Musculoskeletal defects were further analyzed with respect to gender, sporadic/familial, isolated and syndromic occurrence of anomalies. It was found that the ratio of affected males (n=21) and females (n=17) were almost equal. Sporadic cases (n=29) were dominant as compared to familial cases (n=9). The ratio of syndromic (n=20) and isolated cases (n=18) were almost same (Table 3.12).

Table 3.12 Analysis of Musculoskeletal defects

Anomaly	Gender		Sporadic/Familial		Syndromic/Isolated	
	Male	Female	Sporadic	Familial	Syndromic	Isolated
Muscular Dystrophy	5	2	4	3	2	5
Arthrogryposis	2	3	4	1	4	1
Osteopetrosis	2	3	4	1	5	0
Dwarfism, skeletal dysplasia	3	1	2	2	3	1
Developmental dysplasia of hip	0	3	2	1	1	2
Multiple exostosis	3	0	3	0	0	3
Limb hypotonia	1	1	2	0	0	2
Osteogenesis imperfecta	1	1	1	1	0	2
Achondroplasia	1	0	1	0	1	0
Apert syndrome	0	1	1	0	1	0
Crouzon syndrome	1	0	1	0	1	0
Dystrophic dwarfism	1	0	1	0	1	0
Pfeiffer syndrome	0	1	1	0	1	0
Rickets	1	0	1	0	0	1
Scoliosis	0	1	1	0	0	1
Total	**21**	**17**	**29**	**9**	**20**	**18**
	$\chi2$=15.44, df= 14, P=0.3490: ns		$\chi2$=7.676, df= 14, P=0.9055: ns		$\chi2$=23.38, df= 14, P=0.0544: ns	

Ns= statistically non-significant

3.8 Representation of some hereditary disorders

Some representative phenotypes encountered during the study are shown in figures 3.8.1-3.8.2 and pedigrees of some of the familial cases with 3 or more affected members are shown in figures 3.9.1-3.9.3.

3.8.1 Less severe phenotypic manifestation

Fig. 3.8.1 A: Microcephaly (small size head circumference), B: Down syndrome (presence of extra chromosome no 21), C: Cerebral palsy (mental retardation), D: Ichthyosis (pigmented skin)

3.8.2 Representation of Limb defects

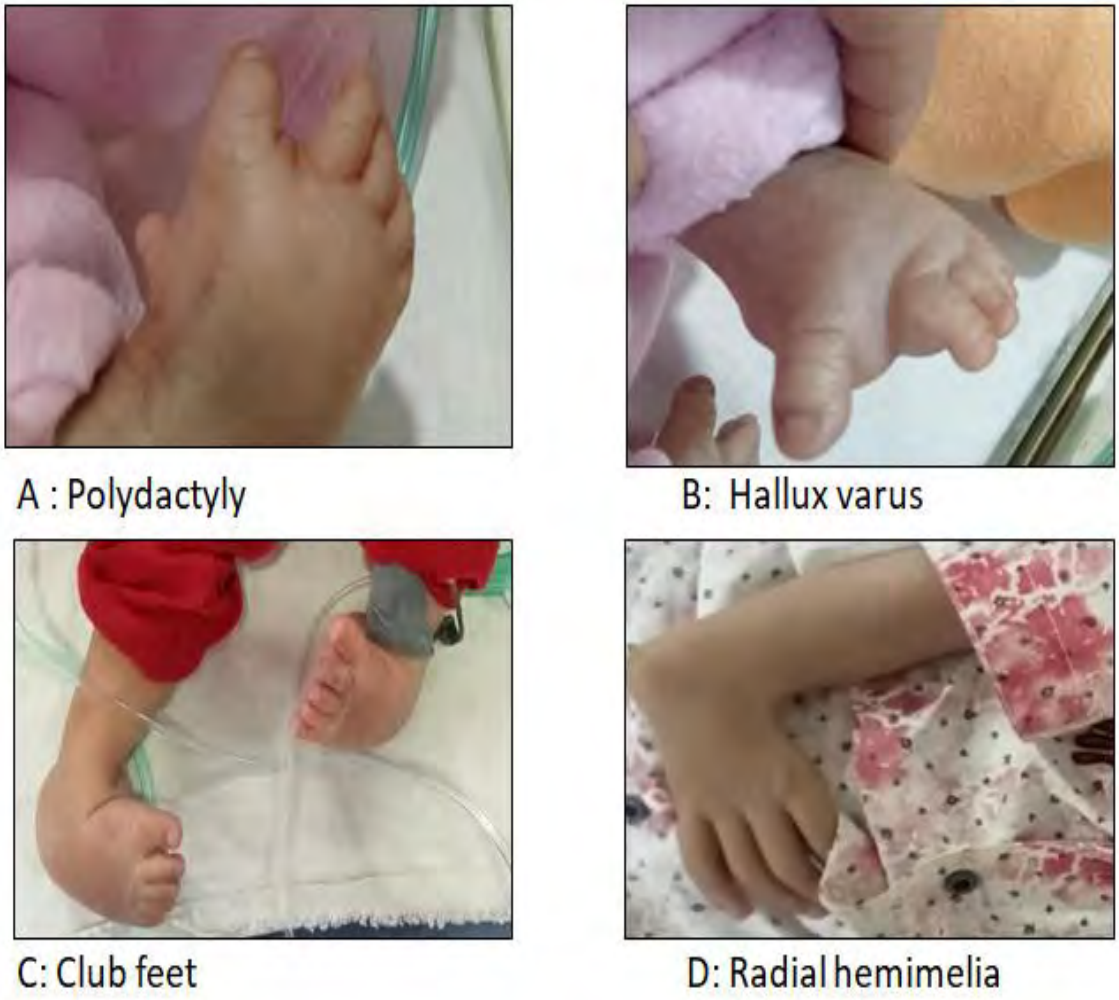


Fig. 3.8.2 A: Polydactyly (presence of extra digit), B: Hallux varus (space between the toe and first digit), C: Club feet (bending of feet), D: Radial hemimelia (absence of radius bone of forearm).

3.9 Pedigrees showing familial cases

3.9.1 A pedigree with Polydactyly

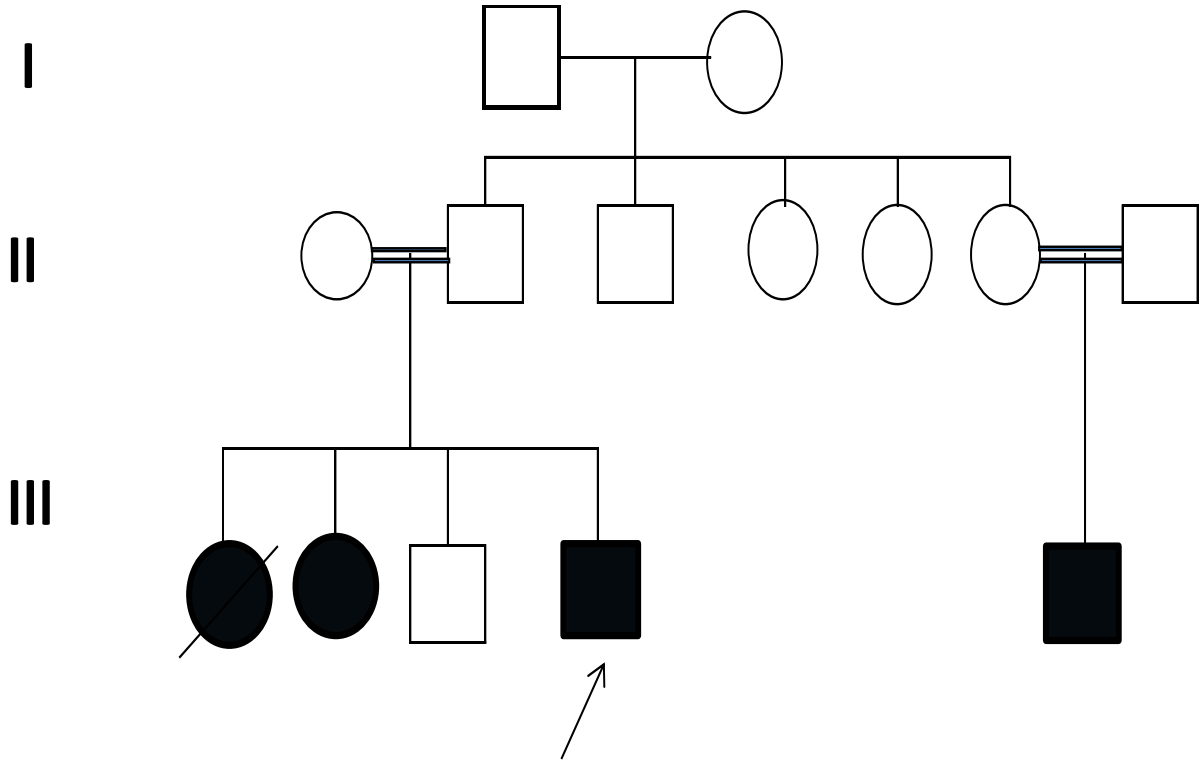


Fig. 3.9 Pedigree showing the affected family members with polydactyly, arrow indicate the index case.

3.9.2 A pedigree with Cerebral palsy

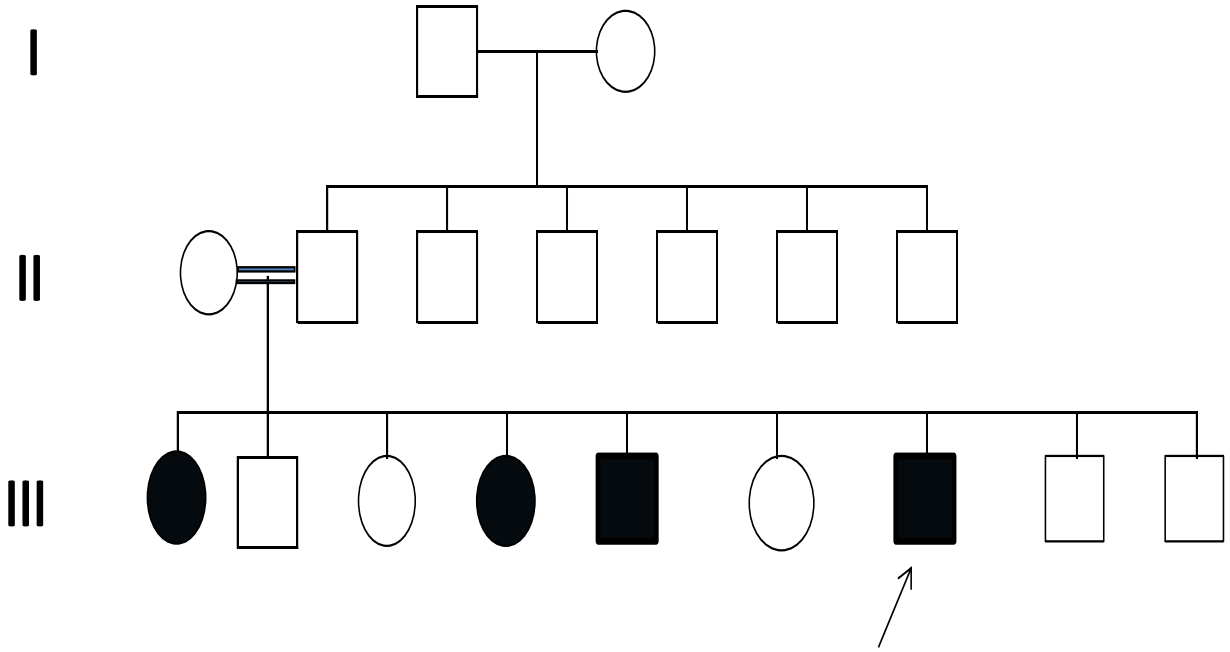


Fig. 3.9.2 Pedigree showing the affected family members with CP, arrow indicate the index case

3.9.3 A pedigree with Club feet

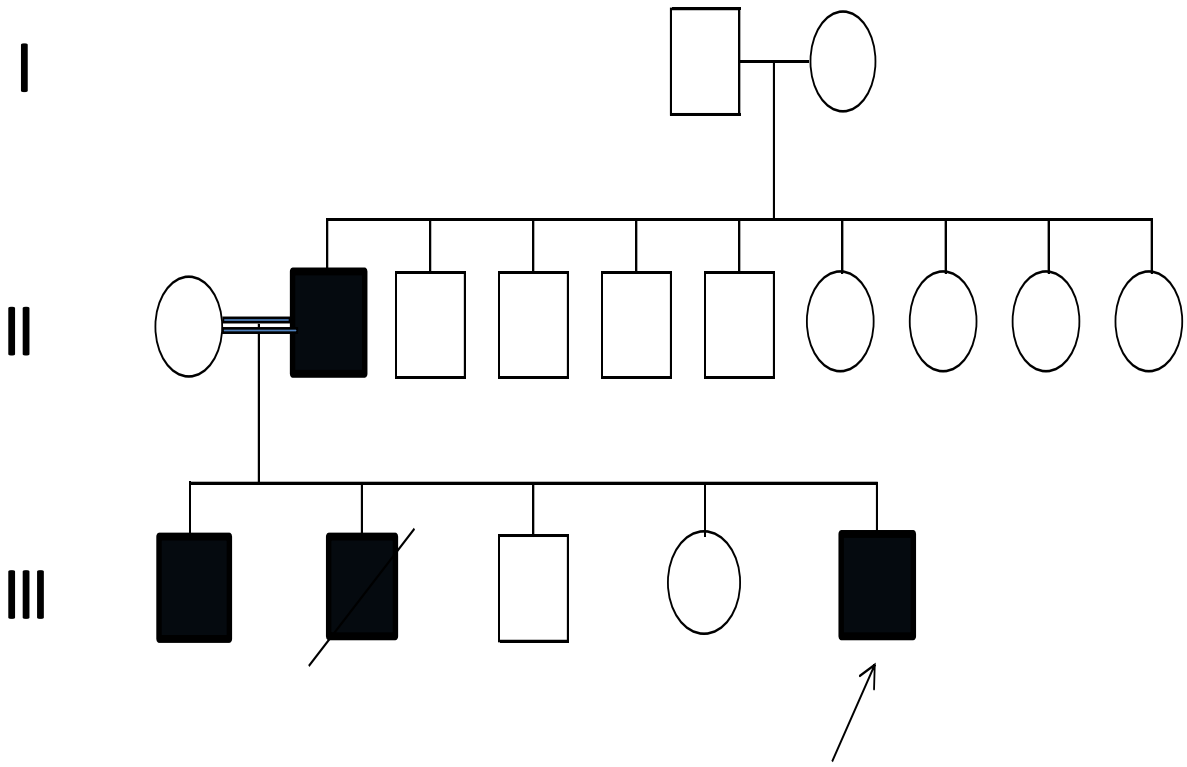


Fig. 3.9.3 Pedigree showing the affected family members with club feet, arrow indicate the index case

Chapter 4

Discussion

A literature search shows few of the hospitals-based studies that were launched to check CA prevalence in the Pakistani population. To assess the pattern, prevalence and associated disorders of CA, the current study was conducted at the Pediatric and Neonatal Section of Holy Family Hospital, Rawalpindi which is a tertiary care hospital receiving a large number of patients from joint-cities Rawalpindi-Islamabad. All types of care are provided in this hospital and newborns are routinely checked for CA before discharge from the maternity unit. Newborns with CA remain admitted to the hospital for further treatment. CA contributes to 6%-9% of prenatal deaths in Pakistan (Bibi et al., 2022).

The current study was carried out in order to better understand the prevalence-pattern of CA in a multi-ethnic Pakistani population. This study further explains the genetic and clinical attributes of CA which would help us in understanding the diversity among these disorders. The majority of the malformations could be prevented or minimized by medications, nutrition, immunization and prenatal care or by emphasizing the risk factors such as advanced maternal age, maternal illnesses and exposure to radiation. In addition, early diagnosis using amniocentesis, ultrasonography and chorionic villus sampling can manage at-risk pregnancies.

The results of the current study show that the most common category of CA was neurological disorders (39.1) followed by neuromuscular (21.1%), limb defects (13.5%), musculoskeletal defects (7.4%), blood disorders (4.3%), orofacial defects (3.9%), metabolic disorders (3.7%), cardiovascular defects (2.1%) and ‘others’ category accounted (5%).

Other studies conducted in Pakistan and studies conducted worldwide also showed a high prevalence of neurological disorders. The results are comparable to those of (Bibi et al., 2022) who found neurological disorders (40%) as the dominant group of anomalies in her study followed by limb defects (24.5%) and musculoskeletal defects (8.9%). The study conducted by (Zahra et al., 2016) also showed a high prevalence of neurological disorders (33.7%) followed by musculoskeletal (22.7%) and limb defects (21%). A study conducted in Iran by (Abdolahi et al., 2014) also reported a high prevalence of nervous system anomalies (24%).

In the current study, neurological disorders were further classified into spina bifida, hydrocephaly, Down syndrome, developmental delay, intellectual disability, epilepsy, microcephaly, anencephaly, Edward syndrome and leukodystrophy. The most common neurological disorders were hydrocephaly and Spina Bifida and Down syndrome was the next most common disorder in this sequence. A study carried out at a tertiary care hospital of Karachi showed that neurological disorders were abundant with 22% hydrocephalus cases and 20% anencephaly cases. However, renal malformations and gastrointestinal defects were the next most common birth defects (Anbreen et al., 2021) while in our study, limb defects and musculoskeletal disorders were more prevalent after neurological and neuromuscular disorders. These results are comparable with another study carried out in the tertiary care hospital of Mardan, Pakistan which showed that CNS anomalies were dominant and were followed by musculoskeletal defects. Another study carried out by (Taboo, 2012) also showed a high prevalence of anencephaly. The results of our study are also concordant with (Shawky and Sadik, 2011) which showed high prevalence of central nervous system disorders and chromosomal abnormalities.

In the current study, spina bifida was most commonly associated with clubfoot. This outcome is comparable with (Paton et al., 2010) which showed that a high percentage of syndromic cases of limb defects was due to myelomeningocele. Lack of maternal folic acid intake during the period of conception and first trimester could be the substantial cause of neural tube defects and Down syndrome caused by trisomy 21 is associated with advanced mother age (Shawky and Sadik, 2011).

In developing countries, the burden of neurological disorders remains high. Asphyxia, prematurity, neonatal infections, CNS infections and consanguinity could be the potential reasons (Sultan and Wasay, 2021). One possible reason for the apparent higher percentage of these types of anomalies may be because they are obvious at birth and are recorded more carefully than other defects (Tomatir et al., 2009).

Neural tube defect is one of the most encountered types of disorders and most of the fetal and infant mortality around the world are due to neural tube defects (Dastgiri et al., 2001). Ventricular shunt is required to maintain the intracranial pressure for 70-85% children with Spina Bifida and Hydrocephalus. However, sometimes shunt failure could occur and the symptoms of shunt failure vary with age (Philips et al., 2017).

Neuromuscular disorders (21%, n=109) were the second most dominant disorders in the current study. These disorders were further split into only two minor groups: cerebral palsy (congenital) and cerebral palsy (late onset). This pattern is concordant with the previous study carried out in Sialkot in Pakistan which shows a high incidence of CP after limb defects (Bhatti et al., 2019). A study carried out in the Hazara district of Pakistan (Bibi et al., 2022) also shows a high prevalence of CP. Cerebral palsy is the most dominant minor

group in terms of number of cases in all the minor groups of major groups of congenital anomalies. Neuromuscular disorders are a diverse group of diseases that mainly affect the peripheral nerve, lower motor neuron, neuromuscular junction, or muscles (Santos et al., 2013). Cerebral palsy (congenital) represents 72% of the neuromuscular disorders.

Birth asphyxia, meningitis, fits, hypoxic ischemia and traumas following the birth or during early ages lead to cerebral palsy in this study. Almost all the cases of cerebral palsy were associated with some other types of disorders like developmental delay, epilepsy, visual defects, sensorineural defects, microcephaly and intellectual disability (Gulati and Sondhi, 2017). Parental consanguinity was found to be 63% in neuromuscular disorders. The ratio of affected males and females was the same. 34.8% of the cases of neuromuscular disorders were familial which shows more involvement of environmental factors rather than genetic factors.

Limb defects with 13.5% (n=70) representations was the third major category of an anomaly in this study. Limb defects were further classified into 9 minor categories, of which talipes (55.7%), polydactyly (21.4%) and amputation (7.1%) were dominant. These results were comparable to the findings of (Francine et al., 2014) which show the high prevalence of limb defects followed by the genitourinary and nervous systems. A study carried out by (Bhatti et al., 2017) reported the highest prevalence of limb defects. Among the limb defects, talipes was more common which is in agreement with the hospital-based study carried out in India (Sarkar et al., 2013). Club feet if not treated, children with clubfoot will face many problems like they will walk on the sides and/or tops of their feet, will not be able to wear standard shoes, and problems in mobility and occupational opportunities (Dobbs et al., 2009), however, polydactyly do not affect the normal life and of milder nature.

59 cases of limb defects were isolated compared to the 11 syndromic cases; this finding is in disagreement with (Paton et al., 2010) which showed a high number of syndromic cases.

In contrast to current study, however, there is no such previous hospital-based study from Pakistan which reported the relatively high prevalence of limb defects. Syndactyly, polydactyly, brachydactyly and camptodactyly are usually not encountered in clinical practice due to their minor nature (Malik et al., 2014). Many studies showed that exposure to certain pesticides or pollutants increases the risk of having a fetus with limb defects, other factors may include maternal smoking (Paton et al., 2010). Sporadic cases were (n=49) more than familial cases (n=21) which showed that perhaps environmental factors played a role in etiology of limb defects.

Parental consanguinity was estimated to be 71.4% in limb defects. Consanguineous marriages are more common among Asian population. Consanguinity and hence genetic factors could be a major contributor to the prevalence of limb defects (Paton et al., 2010). Males (n=43) were more affected than females (n=27) in the case of limb defects. This finding was concordant with the study carried out by (Vasluian et al., 2013) in which more males than females were affected. Of the some of the known causes of club foot, nervous system disorders comprise the greatest number (Vasluian et al., 2013).

Musculoskeletal defects with 7.4% representation were the fourth prevalent disorders in this study.18.4% of the musculoskeletal defects were represented by muscular dystrophy. This finding was in disagreement with the study carried out by (Zahra et al., 2016) in which there was relatively high percentage of musculoskeletal defects (22.7%) as compared to our study. However, another study carried out by (Bibi et al., 2022) also showed low prevalence of musculoskeletal defects (8.9%).

The present study was analyzed on the basis of syndromic and isolated nature. Majority of the cases were of syndromic nature (n=326) and isolated cases were (n=191). This finding was inconsistent with the study conducted by (Zahra et al., 2016) who reported the maximum number of isolated cases (n=170). Our study also contradicts the study conducted in Tanzania (Kishimba et al, 2015) who reported high prevalence of isolated disorders (74%). Majority of the syndromic cases were from neuromuscular and neurological disorders (99% and 78%) respectively. Most common associated defects were developmental delay (n=176), sensorineural defects (n=78) and epilepsy (n=51). Among the minor categories, CP was most commonly associated with developmental delay (n=89) and spina bifida was most commonly associated with tallipes (n=15). (Zahra et al., 2016) and study conducted in India (Gulati and Sondhi, 2017) also showed CP is most commonly associated with developmental delay and intellectual disability and (Paton et al., 2010) showed association between tallipes and spina bifida.

When the index cases were analyzed on the basis of sporadic and familial occurrence, it was found that sporadic cases were in majority as compare to the familial cases (72.5% and 27.5%). This percentage of sporadic cases was quiet higher than the previous studies carried out in Sialkot (Bhatti et al., 2019) and Hazara (Bibi et al., 2022) whose studies reported (65% and 60%, respectively) of sporadic cases than familial cases (35% and 40%, respectively). In case of neurological disorders, 76.7% cases were sporadic and 23.3% were familial. This finding is concordant with study conducted by (Zahra et al., 2016) whose study also reported more sporadic cases of neurological disorders (79.5%). In the case of limb defects, sporadic cases were 70% which were slightly higher than a study conducted by (Bibi et al., 2022) who reported sporadic cases of limb defects to be 65%.

The first reason for the sporadic occurrence of anomalies could be non-genetic factors like environmental factors including poor maternal nutrition, exposure to pesticides and

radiation during pregnancy, maternal illnesses etc. (Harris et al., 2017). Poor or no folic acid intake could also be the potential reason. Folic acid intake in the first trimester of pregnancy may help decrease the risk of congenital anomalies.

The second reason could be the traumas immediately after the child birth like meningitis, asphyxia, sepsis, fits, hypoxic ischemia, poor antenatal care etc. (Gulati and Sondhi, 2017). The third factor could be that most people do not want to disclose information about the number of affected family members.

In the presented study, male subjects were more affected than female subjects. Males were (56%) and females were (44%). A study carried out by (Paton et al., 2010) also shows high ratios of affected males than females. Studies carried out in Sialkot and Hazara, Pakistan also shows high ratio of affected males than females. The current study also correlates with other epidemiological studies carried out internationally. In a study conducted in India by (Sarkar et al., 2013) more males than females were affected. A study carried out in Turkey by (Tomatir et al., 2009) also shows that more males (54%) were affected than females (46%). In neurological and limb defects, the ratio of affected males was higher than females. This finding was consistent with a study carried out in the Netherlands by (Vasluian et al., 2013) who reported a high ratio of affected males in limb defects. However, the ratio of males and females was the same in blood disorders and metabolic disorders. A study conducted in Egypt by (Shawky et al., 2011) found that the ratio of affected males was greater than female subjects. However, a study conducted by (Tomatir et al., 2009) found that the prevalence of congenital anomalies was not affected by the gender of the subject.

The high ratio of affected males was due to the fact that females were afflicted with more severe congenital anomalies and could not be survive to be born with signs of life

(Sarkar et al., 2013). Another reason could be that recessive disorders need only one copy of defective gene to be expressed in males while females require two copies of the defective gene for the expression of recessive disorders.

In the present study, parental consanguinity was found to be 70%. The percentage of consanguinity was higher than the findings of previous studies conducted in Sialkot (17%), Hazara (66%) and Kurram agency (55.3%). A study conducted in Turkey by (Tomatir et al., 2009) reported 14.3% parental consanguinity. However, our finding is concordant with the study conducted by (Gul et al., 2021) who reported 68% parental consanguinity. In our study, the highest percentage of parental consanguinity was observed in musculoskeletal defects and metabolic disorders (89%) and the lowest percentage of consanguinity was observed in neuromuscular disorders (63%). Parental consanguinity was found in 76.7% of familial cases. (Shawky and Sadik, 2011) also reported that consanguinity played a major rule in the prevalence of congenital anomalies. A study conducted in Rahim Yar Khan District of Pakistan, (Riaz et al., 2016) also reported an association between consanguinity and the prevalence of congenital anomalies.

A study conducted in India (Sarkar et al., 2013) reported that the prevalence of congenitally malformed babies was more when born out of consanguineous marriages. The high consanguinity rate was due to the fact that many families prefer marriage among first cousins in order to preserve the family structure, links and provide social, economic and cultural benefits. It is a common view that there is a less chance of divorce between husband and wife in family marriages (Shawky et al., 2013).

All the recruited index cases were analyzed on the basis of the socio-economic status of families. The majority of the index cases (55.7%) fall in the low category, followed by low-mid (21.95), poor (12.4%) and a few fall in the high category (10%). This finding

contradicts the study conducted by (Zahra et al., 2016) where majority of the cases belonged to the low-mid category (n=113) and only few belonged to the low category (n=22).

All the index cases were also analyzed on the basis of age group. Most of the index cases fall in the age category newborn to up to 5 years (n=374), followed by age group >9 years (n=96) and then age group >5-9 (n=47). Our study contradicts the previous study conducted in Hazara, Pakistan by (Bibi et al., 2022) where majority of the cases fall in the age group >9 years. The present study is also in disagreement with (Bhatti et al., 2019) who showed high representation of index cases in age group of 9-19 years with congenital anomalies. However, our study is consistent with the study conducted in Nigeria by (Uju and Nneka, 2020) who also reported that the majority of cases fall in the age group 1-5 years.

All the familial cases of congenital anomalies were analyzed with respect to generations in which disease segregates. Most of the anomalies segregate in one generation (83.8%), followed by those segregating in two generations (13%) and then those segregating in three generations (2%). This finding is consistent with the findings of (Zahra et al., 2016) who found most of the cases segregating in the first generation.

The 517 Index cases were analyzed with respect to parity. Index cases with first parity were (28.5%), then come second parity (22.3%), then come third parity (16.2%). This finding is consistent with (Abdolahi et al., 2014) who found the majority of the cases in the first parity (50%) and then in the second parity (33%).

Chapter 5

References

Abdou, M. S. M., Sherif, A. A. R., & Wahdan, I. M. H. (2019). Pattern and risk factors of congenital anomalies in a pediatric university hospital, Alexandria, Egypt. *Journal of the Egyptian Public Health Association*, *94*(1), 1-9.

Ajao, A. E., & Adeoye, I. A. (2019). Prevalence, risk factors and outcome of congenital anomalies among neonatal admissions in OGBOMOSO, Nigeria. *BMC Pediatrics*, *19*(1), 1-10.

Alexander, P. G., Clark, K. L., & Tuan, R. S. (2016). Prenatal exposure to environmental factors and congenital limb defects. *Birth Defects Research Part C: Embryo Today: Reviews*, *108*(3), 243-273.

Ali, A. E. S., El-Sayed, G. A., Hamed, B. M., & Tumi, M. A. O. (2021). Prevalence Rate of Congenital Fetal Malformations in Second Trimester by Ultrasound Scanning in Zagazig University Outpatient Clinic. *The Egyptian Journal of Hospital Medicine*, *85*(2), 3889-3992.

Anele, C. R., Goldani, M. Z., Schüler-Faccini, L., & da Silva, C. H. (2022). Prevalence of congenital anomaly and its relationship with maternal education and age according to local development in the extreme south of Brazil. *International Journal of Environmental Research and Public Health*, *19*(13), 8079.

Antonarakis, S. E., Skotko, B. G., Rafii, M. S., Strydom, A., Pape, S. E., Bianchi, D. W., ... & Reeves, R. H. (2020). Down syndrome. *Nature Reviews Disease Primers*, *6*(1), 1-20.

Blencowe, H., Moorthie, S., Petrou, M., Hamamy, H., Povey, S., Bittles, A., ... & Modell, B. (2018). Rare single gene disorders: estimating baseline prevalence and outcomes worldwide. *Journal of community genetics*, *9*(4), 397-406.

Ceausu, I., Iliescu, D., Poalelungi, C., Posea, C., Bacalbasa, N., Dobritoiu, D., & Ples, L. (2018). The antenatal detection of fetal limb anomalies. *Congenital Anomalies:*

From the Embryo to the Neonate. Norderstedt, Germany: BoD–Books on Demand, 309.

Copp, A. J., Adzick, N. S., Chitty, L. S., Fletcher, J. M., Holmbeck, G. N., & Shaw, G. M. (2015). Spina bifida. *Nature reviews Disease primers*, *1*(1), 1-18.

Darin, N., Kimber, E., Kroksmark, A. K., & Tulinius, M. (2002). Multiple congenital contractures: birth prevalence, etiology, and outcome. *The Journal of pediatrics*, *140*(1), 61-67.

Dastgiri, S., Stone, D. H., Le-Ha, C., & Gilmour, W. (2002). Prevalence and secular trend of congenital anomalies in Glasgow, UK. *Archives of disease in childhood*, *86*(4), 257-263.

Dennis, M., & Barnes, M. A. (2010). The cognitive phenotype of spina bifida meningomyelocele. *Developmental disabilities research reviews*, *16*(1), 31-39.

Devi, R., Tilak, P., & Rajangam, S. (2007). Multiple congenital anomalies–an aetiological evaluation. *Bombay Hosp J [Review articles]. http://www. bhj. org/journal*.

Dobbs, M. B., & Gurnett, C. A. (2009). Update on clubfoot: etiology and treatment. *Clinical orthopaedics and related research*, *467*(5), 1146-1153.

Dobbs, M. B., & Gurnett, C. A. (2012). Genetics of clubfoot. *Journal of pediatric orthopaedics. Part B*, *21*(1), 7.

Ermito, S., Dinatale, A., Carrara, S., Cavaliere, A., Imbruglia, L., & Recupero, S. (2009). Prenatal diagnosis of limb abnormalities: role of fetal ultrasonography. *Journal of prenatal medicine*, *3*(2), 18.

Fletcher, J. M., & Brei, T. J. (2010). Introduction: Spina bifida—A multidisciplinary perspective. *Developmental disabilities research reviews*, *16*(1), 1.

Francine, R., Pascale, S., & Aline, H. (2014). Congenital anomalies: prevalence and risk factors. *Mortality*, *1*, 2.

Gul, M., Nazir, G., Saidal, A., & Bahadar, H. (2021). Parental consanguinity increases the risk of congenital malformations. *Rehman Journal of Health Sciences*, *3*(1), 48-51.

Gulati, S., & Sondhi, V. (2018). Cerebral palsy: an overview. *The Indian Journal of Pediatrics*, *85*(11), 1006-1016.

Guraya, S. S. (2013). The associations of nuchal translucency and fetal abnormalities; significance and implications. *Journal of clinical and diagnostic research: JCDR*, *7*(5), 936.

Jabeen, N., & Malik, S. (2015). Prevalence and pattern of traumatic limb amputations in female population of Bhimber District, Azad Jammu and Kashmir, Pakistan. *Pakistan Journal of Medical Sciences*, *31*(1), 54.

Jones, T. M., & Montero, F. J. (2021). Chorionic Villus Sampling. In *StatPearls [Internet]*. StatPearls Publishing.

Kazemi, M., Salehi, M., & Kheirollahi, M. (2016). Down syndrome: current status, challenges and future perspectives. *International journal of molecular and cellular medicine*, *5*(3), 125.

Khanal, G. P., Sharma, P., Kayastha, P., & Poudel, A. (2019). Prevalence and Spectrum of Fetal Congenital Anomalies: A Hospital Based Study from Mid-Western Part of Nepal. *Nepalese Journal of Radiology*, *9*(2), 2-9.

Kishimba, R. S., Mpembeni, R., Mghamba, J. M., Goodman, D., & Valencia, D. (2015). Birth prevalence of selected external structural birth defects at four hospitals in Dar es Salaam, Tanzania, 2011–2012. *Journal of global health*, *5*(2).

Kumar, J., Saini, S. S., Sundaram, V., Mukhopadhyay, K., Dutta, S., Kakkar, N., & Kumar, P. (2021). Prevalence & spectrum of congenital anomalies at a tertiary care centre in north India over 20 years (1998-2017). *Indian Journal of Medical Research*, *154*(3), 483-490.

Kurdi, A. M., Majeed-Saidan, M. A., Al Rakaf, M. S., AlHashem, A. M., Botto, L. D., Baaqeel, H. S., & Ammari, A. N. (2019). Congenital anomalies and associated risk factors in a Saudi population: a cohort study from pregnancy to age 2 years. *BMJ open*, *9*(9), e026351.

Mashhadi Abdolahi, H., Kargar Maher, M. H., Afsharnia, F., & Dastgiri, S. (2014). Prevalence of congenital anomalies: a community-based study in the Northwest of Iran. *International Scholarly Research Notices*, *2014*.

McNeese, M. L., Selwyn, B. J., Duong, H., Canfield, M., & Waller, D. K. (2015). The association between maternal parity and birth defects. *Birth Defects Research Part A: Clinical and Molecular Teratology*, *103*(2), 144-156.

Mekonnen, D., & Worku, W. (2021). Congenital anomalies among newborn babies in Felege-Hiwot Comprehensive Specialized Referral Hospital, Bahir Dar, Ethiopia. *Scientific Reports*, *11*(1), 1-8.

Nicolaides, K. H. (2004). Nuchal translucency and other first-trimester sonographic markers of chromosomal abnormalities. *American journal of obstetrics and gynecology*, *191*(1), 45-67.

Paton, R. W., Fox, A. E., Foster, A., & Fehily, M. (2010). Incidence and aetiology of talipes equino-varus with recent population changes. *Acta orthopaedica Belgica*, *76*(1), 86.

Penchaszadeh, V. B. (2002). Preventing congenital anomalies in developing countries. *Public Health Genomics*, *5*(1), 61-69.

Phillips, L. A., Burton, J. M., & Evans, S. H. (2017). Spina bifida management. *Current problems in pediatric and adolescent health care*, *47*(7), 173-177.

Quinlan, M. P. (2008). Amniocentesis: indications and risks. *AMA Journal of Ethics*, *10*(5), 304-306.

Raza, M. Z., Sheikh, A., Ahmed, S. S., Ali, S., & Naqvi, S. M. A. (2012). Risk factors associated with birth defects at a tertiary care center in Pakistan. *Italian journal of pediatrics*, *38*(1), 1-7.

Riaz, H. F., & Malik, S. (2021). Congenital limb defects in a married female population of the Rahim Yar Khan District in Pakistan. *Asian Biomedicine*, *15*(3), 137-144.

Riaz, H. F., Mannan, S., & Malik, S. (2016). Consanguinity and its socio-biological parameters in Rahim yar Khan district, Southern Punjab, Pakistan. *Journal of Health, Population and Nutrition*, *35*(1), 1-11.

Roozbeh, N., Azizi, M., & Darvish, L. (2017). Pregnancy outcome of abnormal nuchal translucency: a systematic review. *Journal of clinical and diagnostic research: JCDR*, *11*(3), QC12.

Rosenbaum, P., Paneth, N., Leviton, A., Goldstein, M., Bax, M., Damiano, D., ... & Jacobsson, B. (2007). A report: the definition and classification of cerebral palsy April 2006. *Dev Med Child Neurol Suppl*, *109*(suppl 109), 8-14.

Santos, S., da Silva Pequeno, A. A., Pessoa, A., Galvão, C. R. C., de Medeiros, J. L. A., Mathias, W., & Kok, F. (2014). Increased prevalence of inherited neuromuscular disorders due to endogamy in Northeast Brazil: the need of community genetics services. *Journal of community genetics*, *5*(3), 199-203.

Sarkar, S., Patra, C., Dasgupta, M. K., Nayek, K., & Karmakar, P. R. (2013). Prevalence of congenital anomalies in neonates and associated risk factors in a tertiary care hospital in eastern India. *Journal of clinical neonatology*, *2*(3), 131.

Sarmah, S., Muralidharan, P., & Marrs, J. A. (2016). Common congenital anomalies: Environmental causes and prevention with folic acid containing multivitamins. *Birth Defects Research Part C: Embryo Today: Reviews*, *108*(3), 274-286.

Shawky, R. M., & Sadik, D. I. (2011). Congenital malformations prevalent among Egyptian children and associated risk factors. *Egyptian Journal of Medical Human Genetics*, *12*(1).

Shawky, R. M., Elsayed, S. M., Zaki, M. E., El-Din, S. M. N., & Kamal, F. M. (2013). Consanguinity and its relevance to clinical genetics. *Egyptian Journal of Medical Human Genetics*, *14*(2).

Shi, Y., Zhang, B., Kong, F., & Li, X. (2018). Prenatal limb defects: Epidemiologic characteristics and an epidemiologic analysis of risk factors. *Medicine*, *97*(29).

Stoll, C., Tenconi, R., & Clementi, M. (2001). Detection of congenital anomalies by fetal ultrasonographic examination across Europe. *Public Health Genomics*, *4*(4), 225-232.

Taboo, Z. A. (2012). Prevalence and risk factors for congenital anomalies in Mosul city. *The Iraqi postgraduate medical journal*, *11*(4), 458-70.

Tara, F., Lotfalizadeh, M., & Moeindarbari, S. (2016). The effect of diagnostic amniocentesis and its complications on early spontaneous abortion. *Electronic physician*, *8*(8), 2787.

Tayebi, N., Yazdani, K., & Naghshin, N. (2010). The prevalence of congenital malformations and its correlation with consanguineous marriages. *Oman medical journal*, *25*(1), 37.

Todros, T., Capuzzo, E., & Gaglioti, P. (2001). Prenatal diagnosis of congenital anomalies. *Images in paediatric cardiology*, *3*(2), 3.

Tomatır, A. G., Demirhan, H., Sorkun, H. Ç., Köksal, A., Özerdem, F., & Cilengir, N. (2009). Major congenital anomalies: a five-year retrospective regional study in Turkey.

Toutounchi, P. (2003). Easily identifiable congental anomalies: Prevalence and risk factors.

Uju, I. M., & Nneka, G. J. (2020). Hearing among children with neurological disorders. *International Journal of Research in Medical Sciences*, *8*(8), 1.

Umair, M., Ahmad, F., Bilal, M., Ahmad, W., & Alfadhel, M. (2018). Clinical genetics of polydactyly: an updated review. *Frontiers in genetics*, *9*, 447.

Vasluian, E., van der Sluis, C. K., van Essen, A. J., Bergman, J. E., Dijkstra, P. U., Reinders-Messelink, H. A., & de Walle, H. E. (2013). Birth prevalence for congenital limb defects in the northern Netherlands: a 30-year population-based study. *BMC musculoskeletal disorders*, *14*(1), 1-14.

Wagner, R., Tse, W. H., Gosemann, J. H., Lacher, M., & Keijzer, R. (2019). Prenatal maternal biomarkers for the early diagnosis of congenital malformations: A review. *Pediatric Research*, *86*(5), 560-566.

Zahra, Q., Shuaib, M., & Malik, S. (2016). Epidemiology of congenital anomalies in the Kurram Tribal Agency, northwest Pakistan. *Asian Biomedicine*, *10*(6), 575-585.

Zhang, J., Williams, M. A., & Rigamonti, D. (2006). Genetics of human hydrocephalus. *Journal of neurology*, *253*(10), 1255-1266.

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