Clinical and genetic study of hereditary disorders in families ascertained from Layyah District

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Clinical and genetic study of hereditary disorders in families ascertained from Layyah District

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بِسْمِ ٱللَّهِ ٱلرَّحْمَٰنِ ٱلرَّحِيمِ

In the name of Allah, the Most Gracious, the Most Merciful

Declaration

I hereby declare that the research work on "**Clinical and genetic study of hereditary disorders in families ascertained from Layyah District"** is an independent effort, it is original and has not been submitted in current or similar form to any other institution.

Qanbar Abbas

CERTIFICATE

This dissertation "Clinical and genetic study of hereditary disorders in families ascertained from Layyah district" submitted by **Mr. Qanbar Abbas** 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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This study is heartedly dedicated to my beloved parents, teachers & all family members.

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List of Abbreviations

Abbreviations	Description
BMD	Becker's Muscular Dystrophy
CA	Congenital anomalies
CDC	Centers for disease control and prevention
CHA	Congenital and Hereditary anomalies
CNS	Central Nervous System
CP	Cerebral Palsy
DMD	Duchenne Muscular Dystrophy
DS	Down syndrome
ERG	Electroretinography
ICD	International Classification of Diseases
ID	Intellectual disability
IRB	Institutional Review Board
MD	Muscular Dystrophy
MRI	Magnetic Resonance Imaging
MSD	Musculoskeletal disorders
NGS	Next Generation Sequencing
NMD	Neuromuscular Disorders
OMIM	Online Mendelian Inheritance in Man
SD-OCT	Spectral Domain Optical Coherence Tomography
WHO	World Health Organization

Abstract

Hereditary disorders are commonly observed in the Pakistani population, yet their inheritance pattern, etiology, prevalence, phenotypic variation, and risk factors in various subpopulations remain largely unexplored. The current descriptive clinical and epidemiological genetic study was carried out through a door-to-door survey to investigate the mode of inheritance, prevalence-pattern, and phenotypic manifestations of hereditary disorders in the general population of district Layyah (Punjab). Families and subjects with hereditary disorders were ascertained and pedigrees were drawn according to the family history of the disorder. A total of 500 independent families/subjects with various types of congenital and genetic abnormalities were ascertained. Among the major disease categories, neuromuscular disorders were most common (n=134) followed by neurological disorders (n=124), sensorineural defects (n=114), limb disorders (n=57) and visual impairments (n=37). Among the ascertained cases, males had higher presentation (68%, n=339) than females (32%, n=161). Sporadic (56%, n=282) and isolated cases (56%, n=282) representation was higher than familial (44%, n=218) and syndromic cases (44%, n=218), respectively. A high representation (45%) of subjects falls within the range of 9 to 19 years of age, and the majority belonged to low socio-economic category (80%). The highest incidence was observed with birth order of 1st parity (n=151). In 82% of cases, the malformation segregated in one generation. In this sample, major factor responsible for genetic disorders was consanguineous marriages found to be in 81% of cases (n=404). This study provides useful information about the prevalence of genetic disorders in the study area and can be potentially helpful for further analysis. Awareness programs about disorders, counseling and prenatal diagnosis can minimize the disease risks.

Chapter 01

Introduction

1.1 Genetic Disorders

Genetics is the study of inheritance and variation that are transferred from one generation to the next generation. The inherited traits may be physical, mental, and metabolic (Mirkin, 2006). The genetic disorder occurs due to the change in the sequence of nucleotides in DNA; change in DNA arises because of mutations. Based on the pattern of inheritance, genetic disorders may be familial or sporadic. The term 'familial' depicts the genetic disorders that run in a family and occur more frequently in each family and can predict the chance of inheritance in the next generation while the term 'sporadic' represent those disorders that do not run in the family and occur in an irregular pattern and cannot predict the chance and inheritance (Hemonta *et al.,* 2010). Based on the involvement of different organs of the body, the genetic disorders may be isolated or syndromic. In isolated genetic disorders, only a single organ of the body is affected while in syndromic, number of organs of the body are affected. Depending on causation, genetic disorders have been divided into four main types: single-gene disorders, multifactorial disorders, chromosomal abnormalities, and mitochondrial disorders (Copp *et al.,* 2020). Single gene disorders are further classified as; a) autosomal dominant; b) autosomal recessive; c) x-linked dominant; d) x-linked recessive (Dobyns *et al.,* 2004). Types of genetic disorders are listed below.

1.1.1 Signal Gene Disorder

This monogenic group of conditions occurs from a single gene mutation. e.g., cystic fibrosis, deafness (Martins *et al.,* 2010), Duchenne muscular dystrophy (Duan *et al.,* 2021), familial hypercholesterolemia is a type of high-cholesterol disease (Pan *et al.,* 2020), and neurofibromatosis type 1 (NF1) (Ferner *et al.,* 2013).

1.1.2 Chromosomal Disorder

In this category, patients have extra or missing chromosomal material, e.g., Fragile X syndrome (Hagerman *et al.,* 2017), Klinefelter syndrome (Wikstrom *et al.,* 2011), and Down syndrome (Bull *et al.,* 2020).

1.1.3 Complex Disorder

These multifactorial diseases are caused by numerous different things, including gene alterations. Chemical exposure of various compounds, nutrition intake of vitamins and hormones, usage of certain drugs, and cigarette used are a few among them e.g., autism spectrum disorder (Hossain *et al.,* 2017), cancer, metabolic disorders, diabetes, and spina bifida (Bowman *et al.,* 2001).

1.1.4 Mitochondrial Disorder

Mitochondrial diseases are acquired or inherited and caused by mutations within mitochondrial DNA (mtDNA). They may also be the result of acquired mitochondrial dysfunction due to contrary effects of drugs and other environmental influences. Mitochondrial disorders are divided roughly into ragged-red fiber disorders and non-ragged-red fiber ones based on subsarcolemmal accumulations, and their intense red appearance with histologic staining e.g., ragged-red fiber disorders include Mitochondrial Encephalopathy with Lactic Acidosis and Stroke-like episodes (Sproule *et al.,* 2008), Myoclonic epilepsy (Camfield *et al.,* 2013) and Progressive external ophthalmoplegia (McClelland *et al.,* 2016). Non-ragged-red fiber disorders include Leigh encephalopathy (Baertling *et al.,* 2014), ataxia (Ashizawa *et al.,* 2016), and retinitis pigmentosa (Hartong *et al.,* 2006).

1.2 Congenital Anomalies (CA)

CA are structural, functional, or metabolic anomalies that originate during intrauterine life and can interfere with body function (Francine *et al.,* 2014). They result from defective embryogenesis in the development process. Various systems have been used to categorize congenital abnormalities: These can be divided into significant and minor anomalies based on their severity (Shamim *et al.,* 2010).

International Classification of Diseases, (ICD), has classified CA according to the affected body parts. The rate of CA affecting the brain is reported to be 10/1000 live births, compared to the incidence of congenital anomalies affecting the heart (8/1000), kidneys (4/1000), limbs (1/1000) and combined (6/1000 live births). CA can also be categorized into genetic, environmental, and multifactorial although the exact cause of CA is still unknown in about 40-60% of cases. Both genetic and environmental causes have been identified in about 25 % of cases, and approximately 15% of CA are caused solely by genetic factors (Mohammed *et al.,* 2011).

Malformation caused by known environmental exposure (e.g., teratogens including maternal infections) is treated separately (Moorthie *et al.,* 2018). Teratogens may cause various alterations during the process of embryogenesis, including chromosomal breakage, gene mutation or enzyme inhibition. (Rizk *et al.,* 2014). These changes are affected by various factors, like the dose of the teratogen, frequency of exposure or the stage of embryo development. Malformations associated with chromosomal disorders are treated as part of the chromosomal syndrome, and malformations associated with single-gene disorders, are treated as inherited disorders. These steps leave a large group of CA with multifactorial or unknown reasons (Dutta *et al.,* 2015). Factors that may increase the risk of occurrence of CA include genetic

disorders, socioeconomic and demographic factors like parental consanguinity, maternal infections during pregnancy, drug abuse, ionizing radiation, and chemical and air pollution. Pregnancy-associated conditions such as insulin-dependent diabetes, and hypertension during pregnancy were also found to be associated with a higher incidence of congenital malformations in the baby. Ethnic and environmental influences affect the birth rate of specific groups of non-syndromic malformations, including neural tube defects and orofacial clefts. Many of these unexplained anomalies are caused by random accidents throughout the complex process of embryonic development, given their diversity and relatively consistent birth prevalence (Feldkamp *et al.,* 2017). Pregnancy-associated conditions such as insulin-dependent diabetes, hypertension during pregnancy such as antepartum hemorrhage, twin pregnancy, oligohydramnios, and polyhydramnios were also found to be associated with more CA (Rasmussen *et al.,* 2009; Blackburn *et al.,* 2017). Oligohydramnios interferes with fetal movement resulting in a cascade of developmental events leading to a fetal anomaly. Some CA can be prevented by the removal of risk factors or the establishment of protective factors. Important interventions during the antenatal period include ensuring adequate intake of vitamins, especially folic acid, and avoiding harmful substances such as tobacco (Blackburn *et al.,* 2017).

The sign and symptoms may range from mild, moderate, and severe to lethal. The congenital malformations may cause disfiguring of an individual or organ (Verma *et al.,* 2021). Structural defects usually arise in the first trimester of embryonic development (DeSilva *et al.,* 2016). In structural CA, the shape of the body is deformed like cleft palate, limb deformities, and neural tube defects while functional CA include those disorders in which the function of a certain body part or organ is interrupted (Wenger *et al.,* 2014). Birth defects can be isolated abnormalities or part of a syndrome that can cause infant mortality and morbidity (Wenger *et al.,* 2014). CA are the main cause of disability

and mortality in children in developing and developed countries. Hospitalization and treatment procedures for these children impose a financial burden on their families (Vatankhah *et al.,* 2017). The first three months of pregnancy are important for the proper development of an embryo, during these months the embryo is highly susceptible to external and internal factors which may cause CA (Goodway *et al.,* 2019). CA can or cannot be observed at birth but are often detected later in life as either structural or functional defect (Cassandrini *et al.,* 2017). CA are categorized as major and minor anomalies. Minor anomalies affect non-vital organs and cause little abnormalities while major anomalies which cause severe functional impairment. They require immediate correction for the normal development of the newborn (Wenger *et al.,* 2014). Major congenital malformations are drastic deviations from normal development that often results in perinatal deaths, require surgical treatment, and compromise an individual's ability to function normally in society (Bale, 2003). Major CA affects nearly 2% of human births (Dolk *et al.,* 2010).

1.3 Types of Congenital Anomalies

Several different CA have been found and categorized. CA can be hereditary or sporadic, singular or widespread, obvious or concealed, large or little, severe or mild (Wenger *et al.,* 2014). A conventional distinction used for CA in literature are major and minor anomalies. Anomalies that are minor or mild have little to no medical or investigative significance. While major or severe defects disrupt the normal function of the body, gross alterations, sometimes associated with death (prenatal and postnatal) have drastic effects and need extensive medical care or surgical corrections. They could be fatal or quite drastic (Verma *et al.,* 2021).

Minor congenital abnormalities can have an aesthetic impact but have little to no clinical or medical impact, e.g., prenatally derived preauricular pit, a developmental divergence from the expected structure had cosmetic effects but was unimportant structurally and functionally (DeSilva *et al.*, 2016).

There are several congenital anomalies present in human communities, ranging from neuromuscular disorders to neurological and limb deformities.

1.4 Neuromuscular disorders (NMD)

NMD is a collective term used to describe diseases that affect any part of the nervous system and muscles. Although many different forms vary in onset, severity, and prognosis, NMD can have an important direct and indirect impact on an individual leading to a loss of functional capacity (Dany *et al.*, 2017).

Different classification systems are available for neuromuscular disorders based on the involvement of the body part, the etiology, or presenting indication. Based on the anatomic involvement, NMD can be characterized into: 1) focal neuropathy, confined to a single limb; 2) peripheral neuropathy, involving nerves in the extremity; 3) motor neuron disease, involving motor nerve cells; 4) myopathy and junction disorders, involving muscles and the synapses between nerve and muscle; and 5) spinal cord injuries.

In neuromuscular disorders, Cerebral Palsy (CP) is one of the most common disorders (Dany *et al.*, 2017). Based on the presenting symptoms, NMD can be classified into disorders with sensory impairment, motor impairment, or both. Neuromuscular disorders can also be categorized roughly into hereditary or acquired. (McDonald *et al.*, 2012). The breakdown of classifications based on the type of impairment at presentation includes the

following categories: cerebral palsy, muscular dystrophy, hearing impairment, visual impairment, and musculoskeletal disorders.

1.4.1 Cerebral Palsy (CP)

There are many ways that cerebral palsy (CP) can present itself, including mental and physical dysfunction, isolated gait, cognitive, growth, and sensation issues. CP is a static neurological disorder caused by brain damage that occurs before the completion of cerebral development. CP can be caused by brain damage that occurs during the prenatal, perinatal, or postnatal periods because brain growth continues during the first two years of life (Krigger, 2006). Approximately 70-80% of cerebral palsy cases occur before birth, and the causes are mostly unknown. About 6% of individuals with congenital CP are affected by birth problems, such as hypoxia. Birth before 32 weeks of pregnancy, birth weight of less than 2,500 gm, intrauterine growth retardation, intracranial hemorrhage, and trauma are all neonatal risk factors. CP affects 10-20% of children after birth, primarily because of brain injury caused by bacterial meningitis, viral encephalitis, hyperbilirubinemia, and accidents (Taylor, 2001).

(i) Clinical features

Approximately 70-80% of CP individuals exhibit spastic clinical manifestations. Increased deep tendon reflexes, tremors, muscle hypertonicity, weakness, and a scissors gait with toe-walking may be seen in affected limbs. The dyskinetic kind of CP, which affects 10-20% of individuals, is marked by excessively sluggish, writhing motions of the hands, feet, arms, or legs, which are increased during stressful times and absent during sleep. Ataxic CP, the rarest kind, affects 5%-10% of people and primarily affects balance and coordination. About two-thirds of patients with CP have intellectual impairment. Seizures affect half of all pediatric patients. Neurological disorders such as decreased

vision or hearing, as well as abnormal touch and pain perceptions, are prevalent (Taylor, 2001).

(ii) Diagnosis

Early signs of CP diagnosis include abnormal posture, poor muscle tone, and slow motor development. It's necessary to examine persistent infantile reflexes. Children without CP rarely show the Moro reflex after the age of six months, and hand preference rarely appears before the age of twelve months. If spastic hemiplegia is present, hand preference might develop before the age of 12 months (Taylor, 2001). The methodology of the test is based on the clinical picture, disease pattern, family history, and other elements that affect the likelihood of a given diagnosis. The physical diagnostic technique includes targeted laboratory testing and cerebral imaging using computed tomography, magnetic resonance imaging, and ultrasound. The clinical evaluation and diagnosis might be aided by keeping an eye out for associated impairments like hearing and vision loss, seizures, problems with how one will perceive touch or pain, and cognitive dysfunction (Taylor, 2001).

1.4.2 Muscular Dystrophy (MD)

Muscular dystrophy is a group of diseases that causes progressive weakness and loss of muscle mass (Mercuri *et al.*, 2013). There are many kinds of muscular dystrophy. Symptoms of the most common variety begins in childhood. Other types don't surface until adulthood. There is no cure for muscular dystrophy. But medications and therapy can help manage symptoms and slow the course of the disease (Mercuri *et al.*, 2013). The two most common types of MD are discussed below.

1.4.2.1 Duchenne Muscular Dystrophy (DMD)

Duchenne muscular dystrophy (DMD) is a lethal X-linked recessive neuromuscular disorders caused by mutations in the dystrophin gene that result in absent or insufficient functional dystrophin, a cyto-musculoskeletal disorder protein that enables the strength, stability, and functionality of myofibers. Prevalence of DMD has been reported as 15.9 cases per 100,000 live male births in Pakistan and 19.5 cases per 100,000 live male births globally (Emery *et al.,* 2002). Progressive muscular damage and degeneration occur in people with DMD, resulting in muscular weakness, associated motor delays, loss of ambulation, respiratory impairment, and cardiomyopathy. Although the clinical course of muscle and cardiac involvement can be variable, death usually occurs because of cardiac or respiratory compromise (Mah *et al.,* 2011; Artasma *et al.,* 2006; Birnkrant *et al.,* 2018).

1.4.2.2 Becker Muscular Dystrophy (BMD)

Becker muscular dystrophy (BMD) is an X-linked recessive disorder due to a mutation in the dystrophin gene that results in progressive muscle degeneration and proximal muscle weakness (Thada *et al.,* 2021). This condition is less common and less severe than Duchenne muscular dystrophy (DMD). The onset of symptoms is late compared to Duchenne muscular dystrophy, although it varies widely between 5 to 60 years of age. In an investigation done in 67 patients exercising a standard protocol, the milder group had been found ambulant until their forties or beyond and the more severe group with the earlier loss of ambulation (Thada *et al.,* 2021)

Becker muscular dystrophy is a rare disease exclusively in males due to X-linked inheritance. The worldwide prevalence of Becker muscular dystrophy ranges from 0.1 to 1.8 per 10,000 male individuals. According to research conducted in Pakistan in 2010, the

prevalence of BMD for all age groups was 0.26 per 10,000 male individuals, and it was found more common among remote areas.

The study of Becker muscular dystrophy (per 10,000 males) suggests a prevalence of 0.01 in South Africa, 0.1 to 0.2 in Asia, and 0.1 to 0.7 in European countries. Isolated data shows BMD is three times less common then DMD (Salzberg *et al.,* 2018).

1.5 Neurological Disorders

Neurological disorders are medically defined as disorders that affect the brain as well as the nerves found throughout the human body and the spinal cord. Structural, biochemical or electrical abnormalities in the brain, spinal cord or other nerves can result in a range of symptoms. Examples of symptoms include paralysis, muscle weakness, poor coordination, loss of sensation, seizures, confusion, pain and altered levels of consciousness (Thakur *et al.,* 2016).

The specific causes of neurological problems vary but can include genetic disorders, congenital abnormalities or disorders, infections, lifestyle or environmental health problems including malnutrition, brain injury, spinal cord injury or nerve injury. There are many recognized neurological disorders, some relatively common, but many rare. Neurological disabilities include a wide range of disorders, such as epilepsy, learning disabilities, Intellectual disability (ID), Down Syndrome (DS) and Spina Bifida (Thakur *et al.,* 2016).

1.5.1 Intellectual disability (ID)

Intellectual disability (ID) is a mental health condition that mostly impairs cognitive function. It is defined by major lifelong developmental deficiencies in areas

such as learning, problem-solving, adaptive skills development, and independence, which usually begin before the age of 18 years (Levy, 2018; Vasudevan *et al.*, 2017). The functional impairment varies according to the severity of ID, which is measured by the intelligence quotient (IQ) score and ranges from mild to severe. Although genetic forms are becoming acknowledged as a significant etiological category, the etiology of ID is highly heterogeneous (Gilissen, 2014).

Pakistan has higher than estimated rates of ID, with a population of 160 million people, 45% of whom are under the age of 18 years. The prevalence estimates for major intellectual disability range from 19.1/1000 to 65/1000 for mild ID (Maulik *et al.*, 2011).

1.5.2 Down Syndrome (DS)

Trisomy 21 is the most common genetic cause of moderate intellectual disability. Different types of chromosome errors lead to Down syndrome. The associated factors that increase the risk of chromosome 21 malsegregation include advanced maternal age and recombination (Sherman *et al.*, 2007). Physical growth delays, mild to severe intellectual handicaps, and distinctive facial traits are frequently connected with it. A young adult with Down syndrome has an average IQ of 50, which is comparable to a child who is eight or nine years old but can vary greatly. Genetically, the affected person's parents are typically unaffected. There is no known behavioral activity or environmental element that alters the probability of the extra chromosome occurring; it is thought to happen randomly.

The incidence of Down syndrome in live births is approximately 1 in 733; the incidence at conception is more than twice that rate; the difference is accounted for by early pregnancy losses. Affected individuals are more prone to congenital heart defects (50%) (Mogra *et al.*, 2011).

(i) Diagnosis

All pregnant women, regardless of age, will be provided with the Down syndrome screening. Different tests with differing degrees of accuracy are utilized. To increase the rate of detection, they are frequently combined. None of these is conclusive, thus if a screening test is positive, a chorionic villus sample or an amniocentesis is needed to confirm the diagnosis. (Sherman *et al.*, 2007).

1.5.3 Spina Bifida

Spina bifida is a birth defect in which there is incomplete closing of the spine and the membranes around the spinal cord during early development in pregnancy (Castillo-Lancellotti *et al*., 2013).

Spina bifida occulta, meningocele, and myelomeningocele are the three primary kinds. Spina bifida cystica includes meningocele and myelomeningocele. Though it is rarely found in the middle back or neck, the lower back is where it most frequently occurs. (Castillo-Lancellotti *et al.*, 2013).

(i) Signs and symptoms

Physical signs of spina bifida may include:

- Leg weakness and paralysis.
- Orthopedic abnormalities (i.e., club foot, hip dislocation, scoliosis)
- Bladder and bowel control problems, including incontinence, urinary tract infections, poor kidney function and abnormal eye movement.
- Pressure sores and skin irritations (Mitchell *et al.*, 2004).

1.6 Sensorineural defects

When the ear's ability to transfer the vibratory mechanical energy of sound into the electrical energy of nerve impulses is impeded at birth, congenital hearing loss occurs. Hearing loss is classified based on the location of the lesion: hearing loss that affects the outer or middle ear is known as conductive hearing loss, whereas sensorineural hearing loss affects the inner ear, auditory nerve, or central auditory pathway. Both conductive and sensorineural are collectively referred to as mixed hearing loss (Boudewyns *et al.*, 2011). Sound waves cannot propagate through the ear in conductive hearing loss, which can be caused by maldevelopment of the middle ear, external ear, or both, and by transitory blockage of the middle ear caused by effusion. Deafness can be induced by both environmental and hereditary factors and is clinically and genetically heterogeneous. In Pakistan, the prevalence of profound bilateral hearing loss is estimated to be 1.6/100 individuals, and consanguineous families account for 70% of hearing loss (Williams *et al.*, 2019). In most developed nations, neonatal hearing-screening programs allow for early discovery; early intervention will prevent delays in speech and language development, as well as have long-term positive benefits on social and emotional development and quality of life. A search for an underlying etiology is frequently followed by a hearing loss diagnosis. Congenital hearing loss may be caused by environmental and prenatal factors that are more prevalent in low-income areas; congenital infections, especially cytomegalovirus, are also a significant cause of hearing loss (Grosse, 2008). In developed countries, genetic abnormalities are likely to account for most cases; mutations can disrupt any component of the hearing system, including inner ear homeostasis and mechano-electrical transmission. Hearing loss is caused by hereditary reasons in the majority of hearing-impaired children, most typically a single gene mutation. These defects can be inherited in a variety of ways and have varying

prevalence. Hearing loss is classified according to whether there is a co-inherited physical condition (syndromic hearing loss) or not (non-syndromic hearing loss) (Smith *et al.*, 2005). Syndromic hearing loss may account for up to 30% of prelingual deafness, which is usually of the conductive and mixed type. However, its relative contribution to all deafness is much smaller, reflecting the occurrence and diagnosis of post-lingual hearing loss. Genetic heterogeneity is represented by non-syndromic deafness. According to estimates, more than 70% of genetic hearing loss is non-syndromic (Petersen *et al.*, 2006).

1.7 Musculoskeletal disorders (MSD)

Musculoskeletal disorders (MSD) are a main group in the human musculoskeletal disorders system, including the joints, ligaments, muscles, nerves, tendons, and structures that support limbs, neck and back (Kumaraveloo *et al.*, 2018; Gatchel *et al.*, 2011). Musculoskeletal disorders do not include injuries in the musculoskeletal disorders system brought on by sudden trauma, such as a vehicle accident or fall. (Barbe *et al.*, 2013). MSD can affect many different parts of the body including the upper and lower back, neck, shoulders, and extremities (arms, legs, feet, and hands) (Mishra *et al.*, 2021). Examples of MSD include carpal tunnel syndrome, epicondylitis, tendinitis, back pain, tension neck syndrome, and hand-arm vibration syndrome (Gatchel *et al.*, 2011).

(i) Diagnosis

MSD are evaluated based on patient self-reports of symptoms. (Barbe *et al.*, 2013). To determine the cause of the pain, patient's medical history, recreational and occupational risks, the degree of the pain, a physical examination, and perhaps lab tests, X-rays, or an MRI are performed (Worasak *et al.*, 2018). Based on the location, nature,

and severity of pain as well as the sort of restricted or painful mobility a patient is experiencing, doctors look for particular criteria to diagnose each distinct musculoskeletal disorders illness (Barbe *et al.,* 2013). The Nordic Questionnaire, a widely used tool for assessing MSD, asks respondents to mark the body parts on which they have had discomfort and those where it has interfered with their daily activities (Cote *et al.,* 2013). Gait patterns generated by 3D motion capture devices can be used to detect musculoskeletal disorders by recent machine learning methods (Worasak *et al.,* 2018).

1.8 Limb disorders

Amputations and defects of the congenital limbs are missing or incomplete limbs at birth. Many of them are caused by intrauterine growth inhibition or disruptions caused by intrauterine destruction of normal embryonic tissues. The upper extremities are usually affected parts of the body. Congenital limb deficits can be caused by a variety of factors and are frequently a part of multiple congenital syndromes. Hypoplastic/absent limbs are known to be caused by teratogenic substances (e.g., thalidomide, vitamin A). Soft-tissue or vascular disruption disorders, such as amniotic band-related limb deficit, in which loose strands of amnion entangle or fuse with fetal tissue, are the most common cause of congenital limb amputations. The total prevalence of limb disorders is 7.9/1000 live birth (Boyd, 2021).

Limbs defects can be longitudinal and transverse. Longitudinal defects are characterized by particular malformations (e.g., complete or partial absence of the radius, fibula, or tibia). The most frequent upper-limb deficiency is radial ray deficiency, and the most common lower-limb deficiency is a hypoplasia of the fibula. All parts beyond a certain level are missing in transverse defects, and the limb resembles an amputation

stump. The most common cause is amniotic bands; the degree of deficiency varies depending on where the band is located, and there are usually no other malformations or anomalies (e.g., polydactyly and syndactyly).

1.9 Visual impairments

Visual impairment can be defined as a functional limitation of the eye. Visual impairment is classified by the (WHO) based on two factors: visual acuity fields, which is the area from which an individual can perceive visual information. The inability to look at light is known as photophobia. Low visual acuity, according to the (CDC) and the (WHO), is defined as eyesight between 20/70 and 20/400 with the best available correction, or a visual field of 20 degrees or less. Visual acuity of less than 20/400 with the best available correction, or a visual field of fewer than 10 degrees, is considered blind. Pakistan is rated third among South Asian countries, behind India and Bangladesh, with a total prevalence of blindness and vision impairment of 21.78 million people of all ages (Mandal, 2021).

1.10 Aim and Objectives

This comprehensive epidemiological research of congenital and inherited abnormalities was carried out:

- To estimate the prevalence of hereditary disorders in district Layyah and to observe their phenotypic pattern.
- To estimate the association of hereditary disorders with the rate of parental consanguinity.
- Association of congenital and hereditary anomalies (CHA) with maternal and genetic factors.
- To establish various clinical and phenotypic variants of CHA.
- To investigate how people with genetic abnormalities are distributed about different socio-economic demographic factors.

Chapter 02

Subjects and methods

2.1 Study area

The research study was carried out in the district Layyah located in the southern area of Punjab, Pakistan. Layyah was founded as a town in 1550 by Kamal Khan, a decedent of Ghazi Khan and founder of Dera Ghazi Khan (GOP, 2022). Layyah is surrounded on east by Jhang City, on western side River Indus flows along Dera Ghazi Khan, on north by Bhakkar and on the south by Muzaffargarhdistrict. It is comprised of three tehsils i.e., Layyah, Karor Lal Esan and Chaubara. The population of Layyah district is 1,823,995 (Census, 2017). Layyah is main administrative city of the district. Tehsil Layyah and Karor Lal Esan are well-developed agriculturally with large tracks of sand dunes and uncultivated land. The Chaubara tehsil is barren and consists of forest and sand dunes.

Geographically, district Layyah lies between 30–45° to 31–24° north latitudes and 70–44° to 71–50° east longitudes. The area consists of a semi-rectangular block of sandy land/ desert of Thal between two Rivers Indus and the Chenab in Sindh Sagar Doab. District Layyah has a covered area of 6289 km^2 with a width from east to west of 88 km and a length from north to south of 72 km.

District Layyah has an extremely hot climate. During summer highest temperature goes to 53°C. The average annual temperature in Layyah is 25.2°C. In winter it touches 02°C to 0°C due to area's nearness to Koh e Suleman range of Mountains.

Table 2.1 Demographic Variables of Layyah District

Variables	Estimates
Area	6289 km^2
Population	1.824 million (1,823,995)
❖ Rural	1,502,821
❖ Urban	321,174
❖ Male	924,837
❖ Female	899,016
❖ Transgender	142
Annual Growth Rate (1998-2017)	2.59%
Major Occupations	Civil, Services, Farming
Literacy Rate	56%
Industries	78
Hospitals	107
Infant mortality rate in Pakistan	66/1,000 live births
Maternal mortality ratio in Pakistan	178/100,000

(Census 2017)

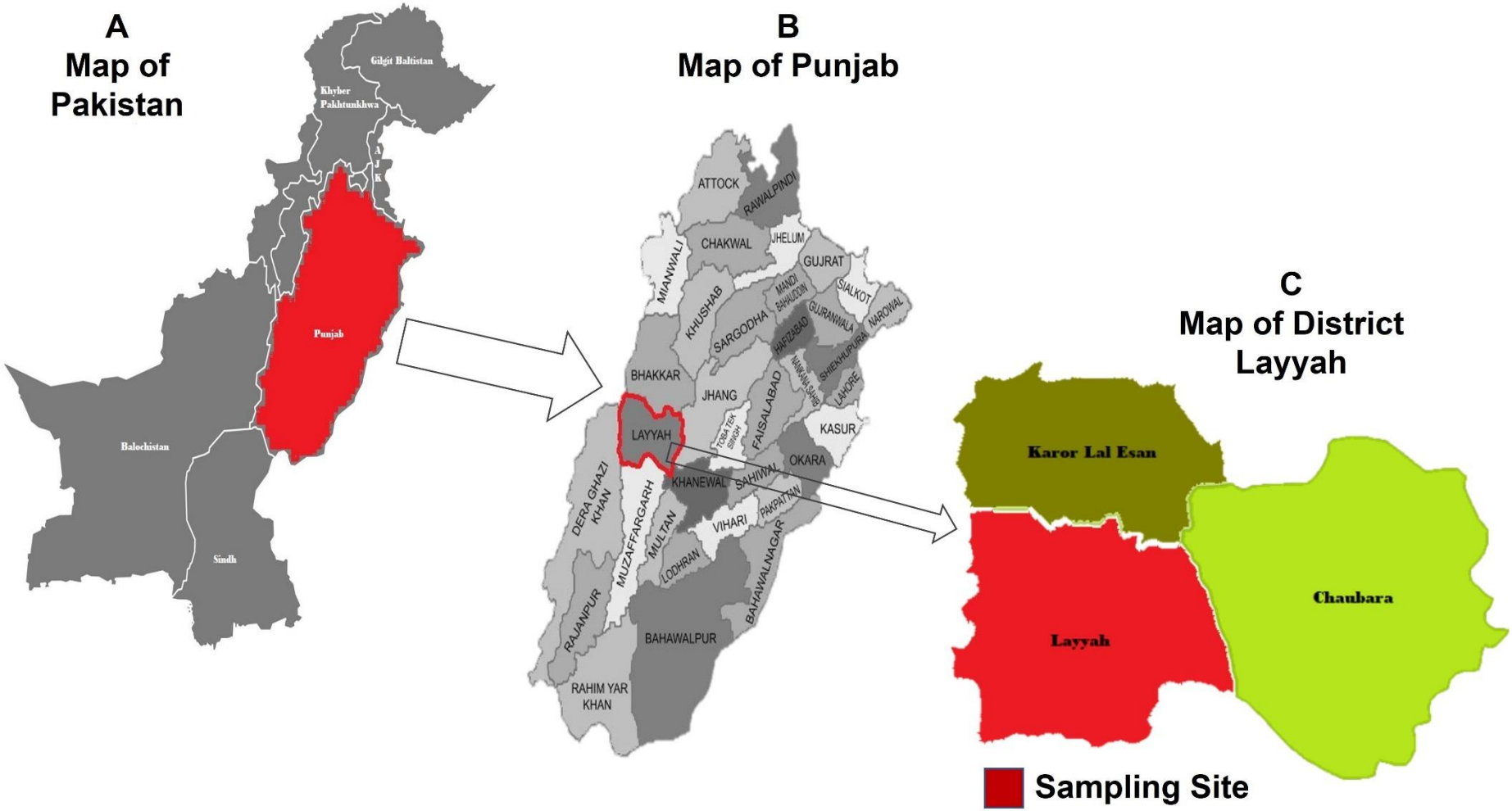


Fig.2.1 (A) Map of Pakistan, superimposed with (B) Map of Punjab and (C) Map of District Layyah.

2.2 Selection of Layyah as Study Area

Tehsil Layyah was selected as a study site due to the advantage of being my hometown ensured as the maximum cooperation zone for research. In this genetic study of congenital and hereditary anomalies, patients with physical appearance as affected were considered as subjects and some of those with a clinical history of disorder were diagnosed by any expert medical authority.

This clinical and genetic study was conducted in this Tehsil Layyah because of the existence of an enormous number of congenital anomalies. These anomalies were led by a major factor of consanguinity in rural as well as urban areas with lack of awareness about genetic disorders and their pattern of transmission. In days of increasing literacy rate, genetic studies are rare in that area and not a single program exists to provide awareness to people.

The basic purpose of genetic study in Layyah was to understand and explore the general/rare nature of congenital anomalies and their prevalence, prevailing for long in general population of that area and secondly, it was necessary to provide awareness and strategies for healthy descendant generations to the maximum population as per our access.

2.3 Study Duration

This study was conducted between March to June 2022 in a door-to-door survey manner to get the actual prevalence of congenital and hereditary anomalies in a specified area of Layyah as during these months weather conditions were suitable comparatively to travel and collect information to conduct this study.

2.4 Study Design

In the start of this research work, an organized study design was prepared to implement on the specified study area of District Layyah. Prior to start of epidemiological data collection, area visit and meetings with local inhabitants were conducted to understand the nature of the population and the prevalence of genetic anomalies prevailed. This was very helpful to get the proper cooperation of that specified population to pursue research work in a suitable way and estimated time span. This area was under-developed with most of the rural background and lack of proper facilitation and awareness about genetic disorders with consequences.

After understanding the situation, a moto of awareness was built to play a positive role in the betterment of that society with the collaboration of local educational and health institutes. Due to lack of awareness, there was no proper record of congenital anomalies in local government hospitals, to achieve the objective of exploring the spectrum of congenital and hereditary anomalies prevalent in the population of District Layyah, it was compulsory to conduct research in a door-to-door survey manner. To obtain an actual spectrum of anomalies in Layyah, it was ensured to perform complete surveys in specified population on priority bases. A total of 500 families with congenital and hereditary anomalies were ascertained in door-to-door survey with the proper consent of recruited families.

2.5 Proforma Designing

Through proper literature study and understanding the requirements to accomplish the aims and objectives of study, a questionnaire type of proforma was designed to keep

details of the affected subject recruited and family with physical, clinical, and developmental history of anomaly.

This proforma comprised three major parts as first one comprising of demographic variables of major subject like age, gender, language, caste, education, occupation, marital status, family type, residence, and socioeconomic status. The second part of the proforma comprised of family details of subject like family history of anomaly, parental age at birth of subject, parental consanguinity, pregnancy events of subject, parity/ birth order, number of normal siblings, married/ single status, and pedigree information. Third part of the proforma consists of clinical/ physical features of the affected subject such as weight, height, arm span, head circumference, neck circumference, chest circumference included with length of arm, leg and feet.

2.6 Ethical Approval

To study the human population on genetic basis, moral and ethical limitations prevail due to which consent from IRB and subject was ensured or from the guardian of the recruited family. Ethical review committee of IRB, Quaid-i-Azam University approved this research after analysis of all required parameters of the research.

2.7 Data Collection

These data were properly obtained from a specified area to ensure the actual prevalence of specific congenital anomalies in zone of research. To have a proper spectrum of anomalies, door-to-door survey was conducted with the consent of the ascertained subjects and their family. Only volunteer participants were considered for recruitment as subjects to get actual information in ethical way. Some people from the

research area were considered as a resource person for locating the affected subjects from their local area. After getting proper information, proforma were filled accordingly.

2.8 Pedigree Construction

A pedigree was drawn for every subject based on information provided by senior members of the family to get accurate information of family relations and history of anomaly through all generations. Pedigree is a presentation of a recruited family of subjects that contain information related to the inheritance pattern of a specific genetic anomaly/ disease. Through pedigree information related to affected and normal members of the family throughout generations, parental union type like consanguineous and non-consanguineous marriages, and pattern of inherited disorder like dominant or recessive nature was concluded.

In pedigree, standard symbols were used for representing males, females, twins, family relatives, affected and normal subjects in the family tree. Circles were used as symbols of normal females and filled circles as affected ones. Squares were symbols of normal males and filled squares showed affected males. For dead individuals, square and circles were divided by a slash as a symbol. Rhombus was used as symbol for an individual with unknown gender. A single horizontal line between parental square and circle shapes was drawn for non-consanguineous union and double line for consanguineous marriage. Siblings were shown by connected to same parents as branches in a horizontal row manner. The number of generations were mentioned on the left side of the pedigree in front of the concerned generation shapes, in form of Roman Numerals while the parity order of subjects in sibship was mentioned in Arabic Numerals. The mode of inheritance of anomaly was carefully observed for further studies.

2.9 Data Analysis and Storage

The whole data were put in an excel sheet and further analysis was performed. These data were stored with details of various socio-demographic variables like name, gender, age, residency, rural/urban, parental details like paternal and maternal age at birth of subject, sporadic/familial, isolated/syndromic, normal, and affected siblings, affected and dead subjects in family, onset, and nature of disease like progressive/ non-progressive.

2.10 Classification of congenital and hereditary anomalies

The classification on initial stages was performed by the help of physical symptoms, clinical reports, and family history of the affected subject with the proper consultancy of resident doctors and medical specialists. For further classification, a proper study of various genetics-based databases and a literature review was ensured to compare the initial classification to categorize anomalies into major and minor types. Online Mendelian Inheritance in Man (OMIM) and International Classification of Diseases (ICD-10; Version 2019) were major databases used for the classification of anomalies by comparing pictures, physical features/symptoms, clinical reports, developmental history, and family history types of information obtained through data collection in form of proformas.

Chapter 03

Results

In this epidemiological study, a total of 500 families suffering from various sorts of congenital and hereditary anomalies were ascertained in a door-to-door survey-based research field work. All the data were recruited from the Layyah district of Southern Punjab. The family recruitment was to assess the actual prevalence of congenital anomalies in that specified area. After accomplishing this survey, data were analyzed and categorized into major and minor categories based on different parameters like type of anomaly, gender, sporadic/familial, isolated/syndromic and age groups.

Major categories were neuromuscular disorders 27% (n=134), neurological disorders 25% (n=124), sensorineural defects 23% (n=114), limb disorders 11% (n=57), visual impairments 7% (n=37), musculoskeletal disorders 2% (n=12), Blood disorders 2% (n=10) and others 2% (n=12) including various anomalies in this category. As it is concerned with followed parameters of prevalence, males contributed 68% (n=339) more as compared to females 32% (n=161) while sporadic 56% (n=282) lead the table as an alternative for familial cases comprising only 44% (n=218).

3.1 Distribution of Subjects on basis of Demographic Attributes

This study was categorized into various groups based on the prevalence and nature of demographic variables. Most of the subjects belonged to rural areas comprising 81% (n=405) and urban contributed only 19% (n=95) to the total data of 500 affected families. In the aggregate of 68% (n=339) males subjects, the rural population contributed 54% (n=271) while urban shared 14% (n=68) comparatively. In these data, females were 32% (n=161), out of which rural and urban populations contributed 27% (n=134) and 5% (n=27), respectively.

In this total data of 500 families, sporadic cases were higher as compared with familial ones. In sporadic 56% (n=282) cases, rural were 46% (n=231) and urban 10% (n=51). While in familial 44% (n=218) subjects, there was rural 35% (n=174) and urban 9% (n=44) cases. Above mentioned demographic attributes are enlisted in Table 3.1.

Table 3.1 Distribution of Subjects on basis of Demographic Attributes

Demographic Variables	Gender		Familial attributes			Percentage
	Male	**Female**	**Familial**	**Sporadic**	**Total**	**(%)**
Origin (n=500)						
Rural	271	134	174	231	405	81.0
Urban	68	27	44	51	95	19.0
Total	**339**	**161**	**218**	**282**	**500**	
	$\chi2 = 0.77$; df=1		$\chi2=0.35$; df=1			
	p=0.3811; NS		p=0.5531; NS			
Age range (n=500)						
Up to 9	103	35	72	66	138	27.6
>9-19	140	84	97	127	224	44.8
>19-29	43	24	27	40	67	13.4
>29-39	26	5	10	21	31	6.2
>39	27	13	12	28	40	8.0
	$\chi2=9.91$; df=4		$\chi2=9.06$; df=4			
	p=0.042; *		p=0.0596; NS			
Caste/Ethnicity						
Jatt	120	52	77	95	172	34.4
Baloch	52	33	34	51	85	17.0
Syed	11	7	8	10	18	3.6
Rajpoot	14	2	11	5	16	3.2
Baryal	13	3	8	8	16	3.2
Gurmani	8	8	9	7	16	3.2
Sohea	9	2	9	2	11	2.2
Dullo	5	5	2	8	10	2.0
Thaheem	6	4	3	7	10	2.0
Others	101	45	57	89	146	29.2
	$\chi2=11.72$; df=9		$\chi2=16.76$; df=9			
	p=0.2295; NS		p=0.0527; NS			

Occupation (age>16 yrs; n=213)						
unemployed	122	48	61	109	170	79.8
Others	19	3	14	8	22	10.3
Student	15	6	10	11	21	9.8
	$\chi2$=2.1; df=2		$\chi2$=6.8; df=2			
	p=0.3401; NS		p=0.0328; *			
Literacy level (age >5 yrs; n=463)						
Illiterate	232	107	136	203	339	73.2
Literate (All)	88	36	64	60	124	26.8
Primary schooling (1-5 yrs)	55	23	39	39	78	16.8
Middle schooling (6-8 yrs)	22	7	15	14	29	6.3
High schooling (9-12 yrs)	8	5	8	5	13	2.8
Graduation and higher	3	1	2	2	4	0.8
	$\chi2$=1.34; df=5		$\chi2$=8.25; df=5			
	p=0.9303; NS		p=0.1429; NS			
Economic Status (n=500)						
Low	262	136	167	231	398	79.6
Middle	68	20	45	43	88	17.6
High	9	5	6	8	14	2.8
	$\chi2$=4.40; df=2		$\chi2$=2.47; df=2			
	p=0.1106; NS		p=0.2907; NS			
Merital Status (age>16 yrs; n=213)						
Single	124	44	75	93	168	78.9
Married	28	17	15	30	45	21.1
	$\chi2$=2.33; df=1		$\chi2$=1.86; df=1			
	p=0.1268; NS		p=0.1726; NS			
Family type (n=500)						
Extended	57	20	35	42	77	15.4
Nuclear	282	141	183	240	423	84.6
	$\chi2$=1.62; df=1		$\chi2$=0.13; df=1			
	p=0.2036; NS		p=0.7212; NS			

* (Significant)

NS: (Non-significant)

3.1.1 Distribution of subjects based on various Age Groups

All the recruited subjects were categorized according to their present age which ranges from few days to 75 years. These subjects were divided into six groups based on their age and placed in that specific category accordingly. First group ranges from newborn babies up to nine years of age. The largest age group with the maximum prevalence pattern was 9-19 years with 45% (224) subjects including both males and females (Fig. 3.1). The second largest group following the first one was 0-9 years sharing 28% (138) subjects out of the total data of 500 subjects. The male and female distribution with age categories was not statistically significant.

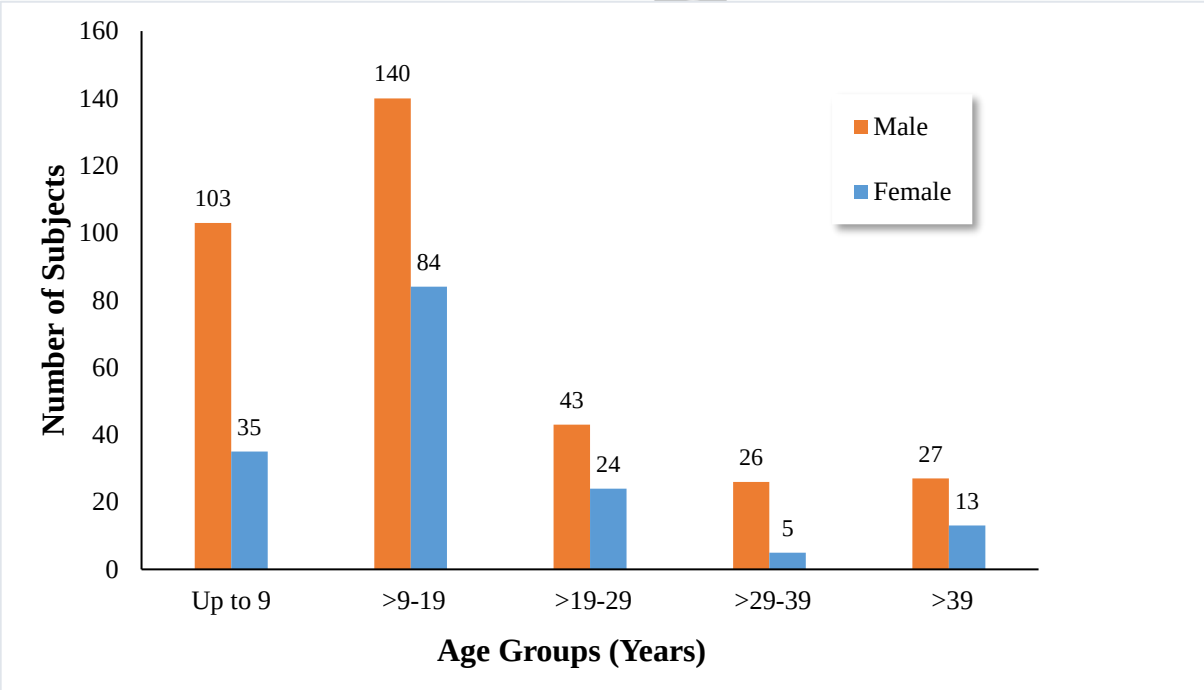


Fig. 3.1 Distribution of subjects based on various age groups

3.1.2 Distribution of subjects with respect to Ethnicity

Based on ethnic/caste system, data were categorized into 9 major groups with maximum number of cases and other castes sharing few cases were merged into a major group named as "others". The highest number of cases were ascertained from a major caste group of this area termed as Jatt, contributing 34% (n=172; Fig. 3.2).

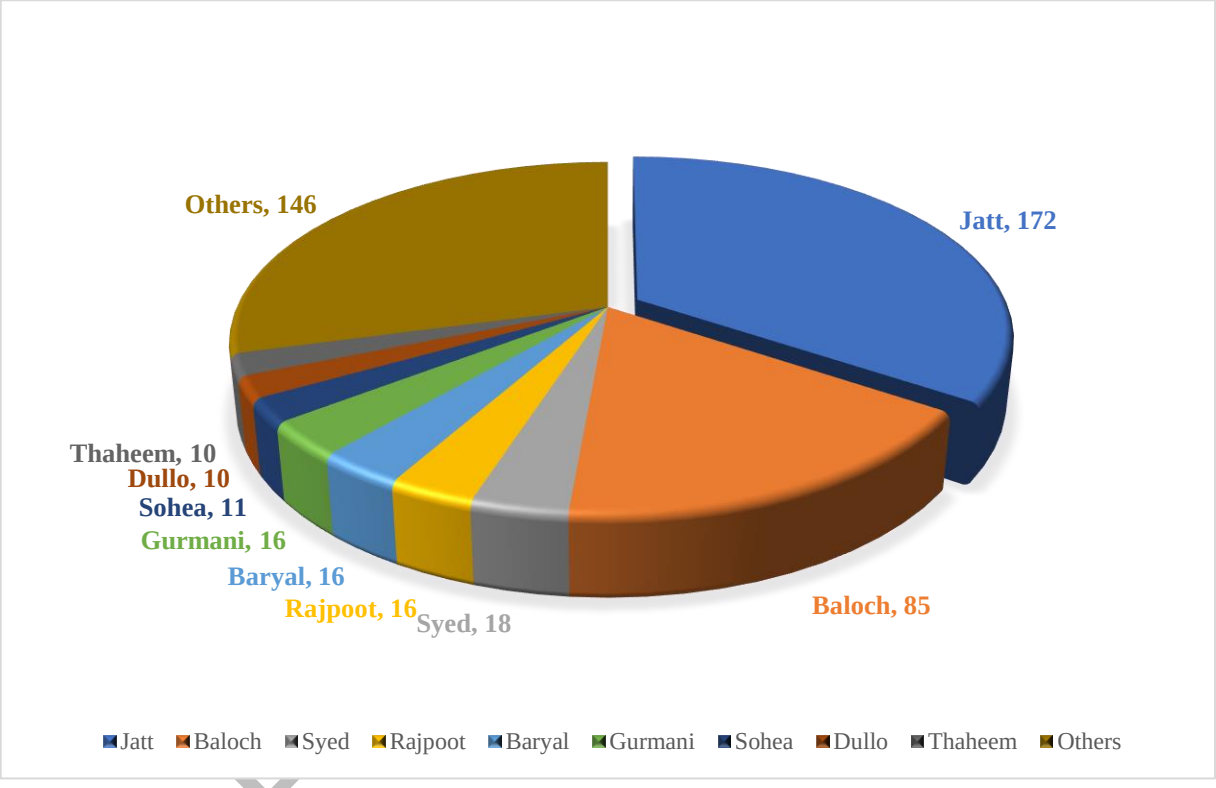


Fig. 3.2 Distribution of subjects with respect to Ethnicity

3.1.3 Distribution of subjects on basis of Literacy level

In 500 recruited cases, subjects (n=463) with age higher than 5 years were considered for further categorization based on their education level into two major groups termed illiterate with the highest number of 73% (n=339) subjects and literate with 27% (n=124) subjects (Fig. 3.3). The literate group was further sub-categorized on basis of different education levels like Primary, Middle, High schooling and Graduation (Fig. 3.4).

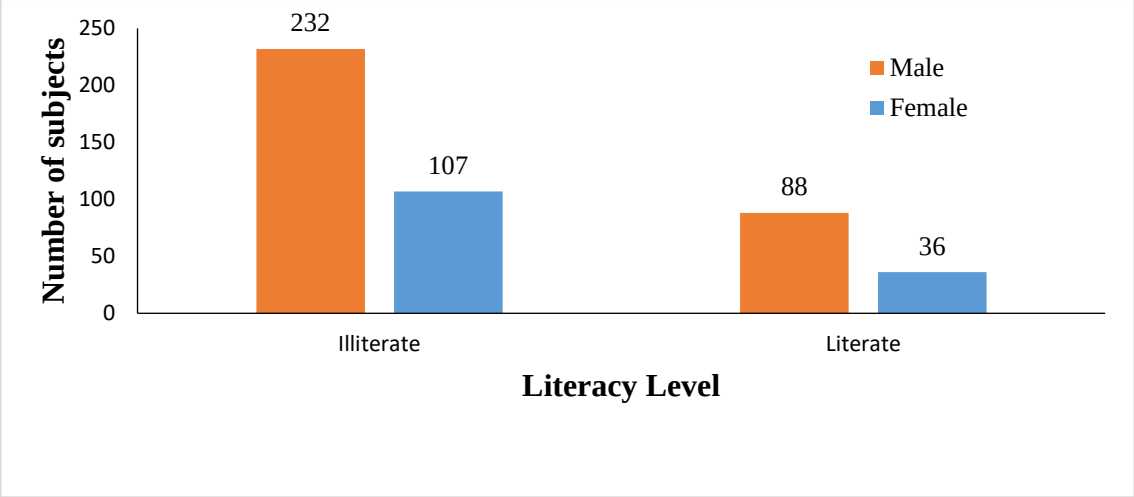


Fig. 3.3 Distribution of subjects on basis of Literacy level

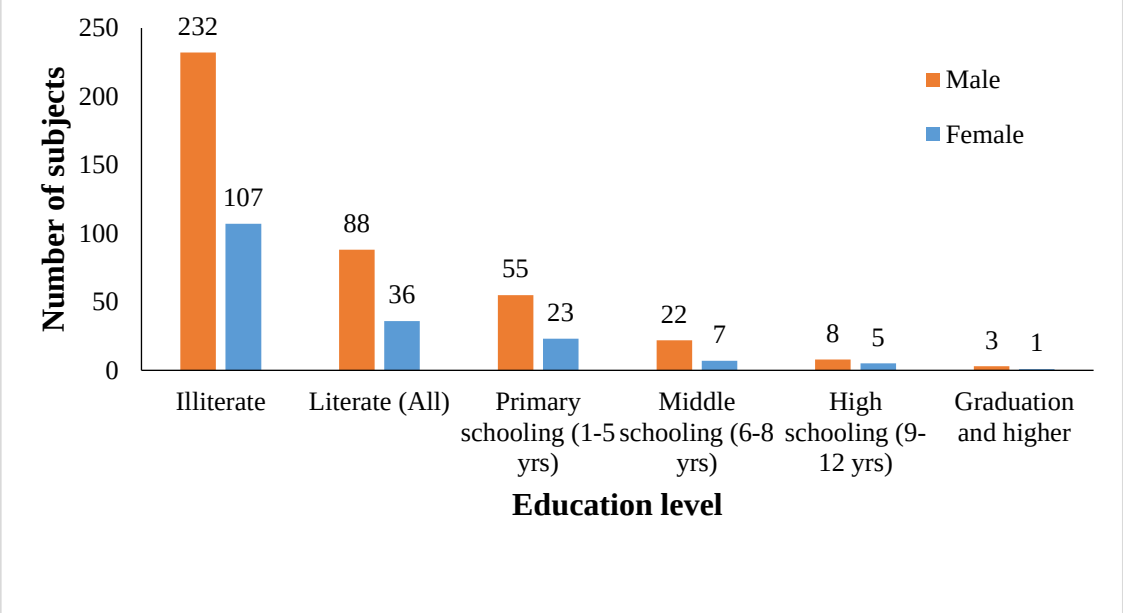


Fig. 3.4 Distribution of subjects on basis of Education level

3.1.4 Distribution of subjects based on their Occupation

To check the economic situation of the area under research, subjects with age higher than 16 years were analyzed on basis of their occupation and employment status. Out of 213 cases, 80% (n=170) of subjects were unemployed, two other categories include students and others having fewer subjects comparatively (Fig. 3.5).

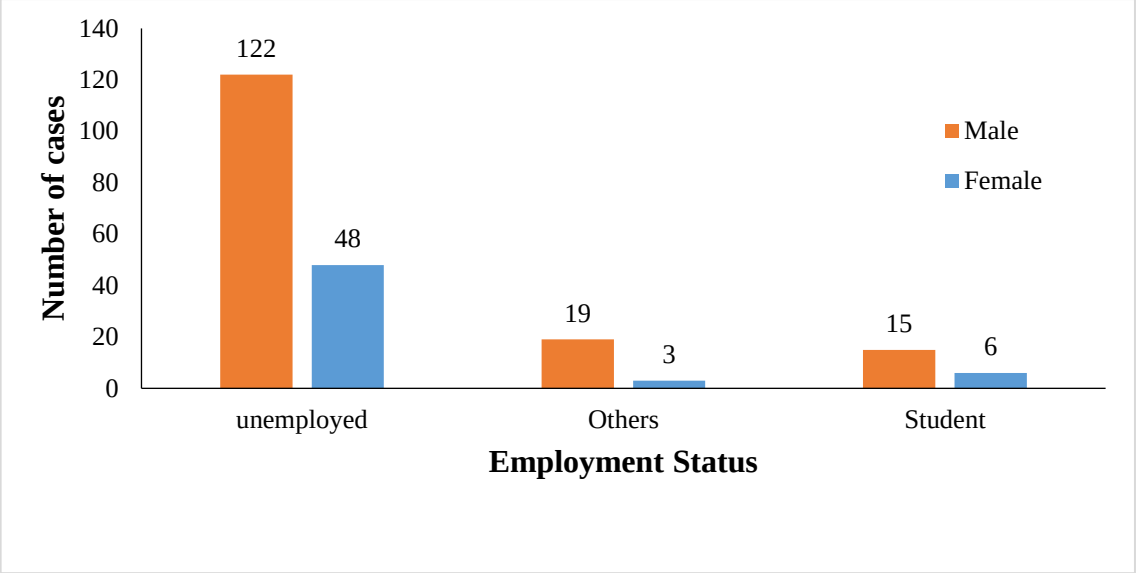


Fig. 3.5 Distribution of subjects based on their Occupation

3.1.5 Distribution of subjects based on Socio-economic Status

On basis of the socio-economic status of the population, ascertained subjects were classified into different categories like low, middle, and high after witnessing their living standards. In whole data, 80% (n=398) of subjects fall in the low category followed by 18% (n=88) in the middle and very few (n=14) in the high category (Fig. 3.6).

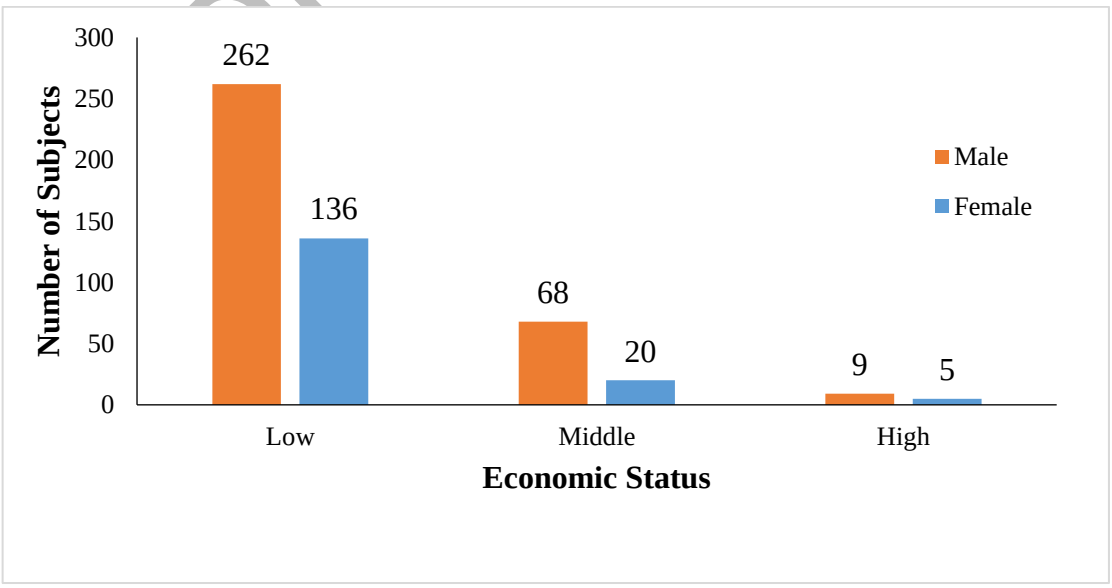


Fig. 3.6 Distribution of subjects based on Socio-economic Status

3.1.6 Distribution of subjects based on Marital Status

Subjects (n=213) with age higher than 16 years were considered for analysis and categorized based on their marital status leading to two major groups of single and married. A high number of subjects (79%; n=168) were categorized into a single group including males (58%; n=124) and females (21%; n=44). While married subjects contributed 21% (n=45) to the data (Fig. 3.7).

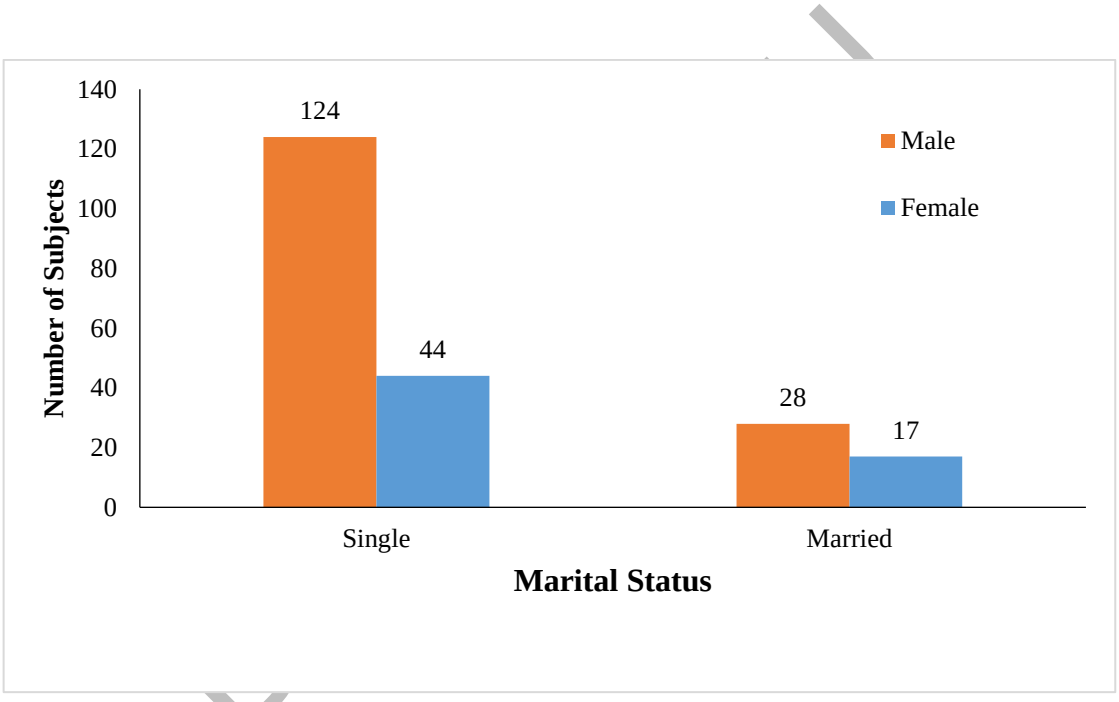


Fig. 3.7 Distribution of subjects based on Marital Status

3.1.7 Distribution of subjects on basis of family type

All the recruited subjects were categorized into two major divisions with respect to their family types, nuclear and extended. In these data, 85% (n=423) subjects fall in the category of nuclear division while only 15% (n=77) subjects were part of an extended family system (Fig. 3.8).

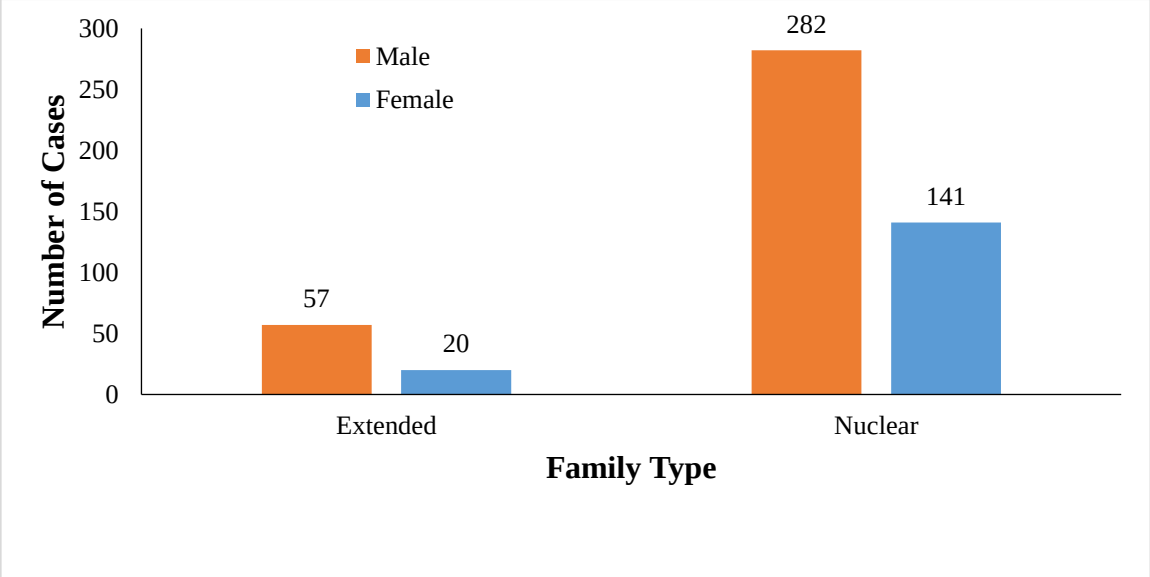


Fig. 3.8 Distribution of subjects on basis of family type

3.2 Genetic attributes of congenital and hereditary anomalies (CHA) under study

Congenital and hereditary anomalies were analyzed based on genetic attributes including familial/ sporadic nature, parity of subjects, marriage types, disease segregation among various generations, parental age at birth of subject, and isolated/syndromic nature. These anomalies were classified on basis of gene databases like OMIM and ICD-10 version 2019, and above-mentioned genetic attributes.

3.2.1 Distribution of congenital and hereditary anomalies (CHA) based on gender and familial attributes

In total ascertained data, subjects were analyzed further based on gender and familial attributes leading to eight major categories of congenital and hereditary anomalies. A concluding analysis of this categorization depicts that male subjects suffering from all sorts of anomalies were higher as compared to females. Neuromuscular

disorders share 27% (n=134) including males (73%; n=98) and 27% (n=36) females followed by neurological disorders contributing 25% (124) subjects with males (65%; n=80) relatively higher than 35% (n=44) females (Table 3.2A; Fig. 3.9). When classified on basis of familial and sporadic nature, neuromuscular disorders contribute 54% (n=72) sporadic and 46% (n=62) females, followed by neurological disorders with 65% (n=81) sporadic and 35% (n=43) familial cases (Table 3.2B). A smaller category of anomalies termed as "others" also exists with the diversity of anomalies and the same trend prevails that males and sporadic subjects were higher in number.

Table 3.2A Distribution of major congenital and hereditary anomalies with respect to gender

Major Divisions	Gender		Total	Percentage (%)
	Male	Female		
Neuromuscular disorders	98	36	134	26.8
Neurological disorders	80	44	124	24.8
Sensorineural defects	80	34	114	22.8
Limbs disorders	35	22	57	11.4
Visual impairments	24	13	37	7.4
Musculoskeletal disorders	7	5	12	2.4
Blood disorders	7	3	10	2
Others	8	4	12	2.4
Total	**339**	**161**	**500**	
	$\chi 2$=4.39; df=7			
	p=0.7339; NS			

NS, Non-significant

Table 3.2B Distribution of major congenital and hereditary anomalies with respect to familial and sporadic attributes

Major Divisions	Familial/Sporadic		Total	Percentage (%)
	Familial	Sporadic		
Neuromuscular disorders	62	72	134	26.8
Neurological disorders	43	81	124	24.8
Sensorineural defects	55	59	114	22.8
Limbs disorders	23	34	57	11.4
Visual impairments	20	17	37	7.4
Musculoskeletal disorders	5	7	12	2.4
Blood disorders	6	4	10	2
Others	4	8	12	2.4
Total	**218**	**282**	**500**	
	$\chi 2=8.92$; df=7			
	p=0.2586; NS			

NS, Non-significant

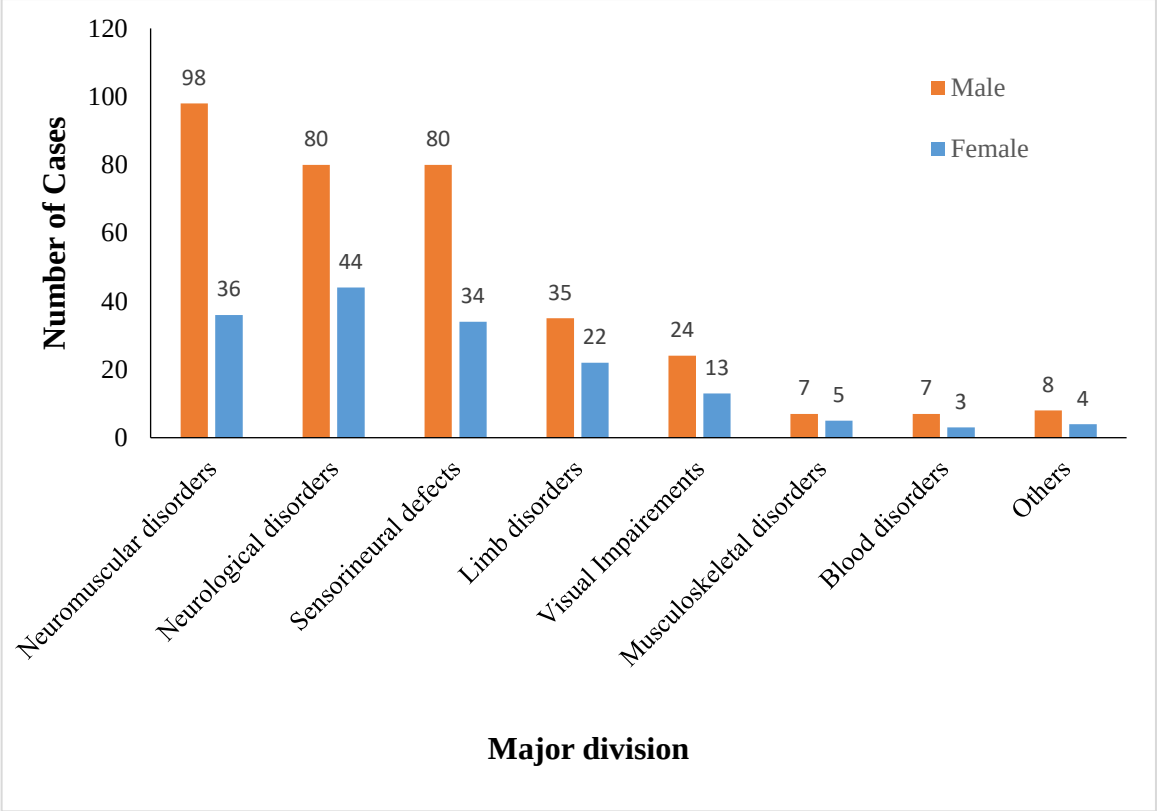


Fig. 3.9 Distribution of anomalies based on gender

3.2.2 Classification of congenital and hereditary anomalies (CHA)

In these data, a variety of genetic and congenital anomalies was observed in the general population of District Layyah. The major disease divisions were neuromuscular disorders, neurological disorders, sensorineural defects, limb disorders, visual impairments, musculoskeletal disorders, blood disorders and others. In these ascertained data of families with CHA, neuromuscular disorders (n=134), neurological disorders (n=124), sensorineural defects (n=114), and limb disorders (n=57) were more common among all anomalies. All the studied CHA are listed with their major division type and their sub-types (Table 3.3).

Table 3.3 Classification of Congenital and Hereditary anomalies

Major categories	Frequency	Proportion	95% CI	ICD-10 (2019)	OMIM
Neuromuscular disorders	134	0.268	0.229-0.307		
Cerebral Palsy	80	0.160	0.128-0.192	G80	
Ataxia	32	0.278	0.239-0.318	G80.4	
Ataxic diplegia	3	0.026	0.012-0.040	G80.1	
Athetoid pure	25	0.217	0.181-0.254	G80.3	
Athetoid dystonia	15	0.130	0.101-0.160	G80.3	
Spastic left hemiplegia	10	0.087	0.062-0.112	G80.2	
Spastic paraplegia	5	0.043	0.026-0.061	G82.1	
Spastic right hemiplegia	5	0.043	0.026-0.061	G80.2	
Spastic right leg monoplegia	4	0.035	0.019-0.051	G83.1	
spastic left leg monoplegia	1	0.009	0.001-0.017	G83.1	
spastic right arm monoplegia	1	0.009	0.001-0.017	G83.2	
Floppy left arm monoplegia	1	0.009	0.001-0.017	G83.2	
Spastic diplegia	3	0.026	0.012-0.040	G80.1	
spastic quadriplegia	9	0.078	0.055-0.102	G80.0	
Triplegic	1	0.009	0.001-0.017		
Ataxia	35	0.070	0.048-0.092	R27.0	160120
Muscular Dystrophy	17	0.034	0.018-0.050	G71.0	310200
Atrophy	1	0.002	-0.002-0.006		
Severe Muscle Hypotonia	1	0.002	-0.002-0.006	P94.2	300868
Neurological Disorders	124	0.248	0.210-0.286		
Intellectual disability	101	0.202	0.167-0.237	F79	
Down Syndrome	13	0.026	0.012-0.040	Q90	190685
Spina Bifida	5	0.010	0.001-0.019	Q05	182940
Occipital Encephalocele	2	0.004	-0.002-0.010		
Microcephaly	1	0.002	-0.002-0.006	Q02	251200

Hydrocephaly	1	0.002	-0.002-0.006	G91.9	236600
Chronic sensorineural polyneuropathy	1	0.002	-0.002-0.006	G60.9	162400
Sensorineural Defects	114	0.228	0.191-0.265		
Deaf and Mute	95	0.190	0.156-0.224	H91.3	304500
Deaf	8	0.016	0.005-0.027		
Mute	5	0.010	0.001-0.019		
Stuttering	6	0.012	0.002-0.022		
Limb disorders	57	0.114	0.086-0.142		
Talipes	31	0.062	0.041-0.083	Q66.0	119800
Polydactyly, postaxial	4	0.008	0.000-0.016	Q69	174200
Transverse Limb Amputation	4	0.008	0.000-0.016	Y83.5	
Limb discrepancy	3	0.006	-0.001-0.013	M21.7	
Constriction ring	3	0.006	-0.001-0.013		
Syndactyly	3	0.006	-0.001-0.013	Q70	609815
Synpolydactyly	2	0.004	-0.002-0.010	Q79.8	217100
Rickets	2	0.004	-0.002-0.010	E83.3	277440
Bifid thumb	1	0.002	-0.002-0.006		
Brachydactyly	1	0.002	-0.002-0.006	Q68.81	113000
Cleft Hand	1	0.002	-0.002-0.006		
Knocking knees	1	0.002	-0.002-0.006		
Femoral deficiency	1	0.002	-0.002-0.006		
Visual Impairments	37	0.074	0.051-0.097		
Blindness	28	0.056	0.036-0.076	H54	216900
Night Blindness	3	0.006	-0.001-0.013	H53.60	310500
Retinitis pigmentosa	1	0.002	-0.002-0.006		
Cataract	1	0.002	-0.002-0.006		
Cornea Elvis opacity	1	0.002	-0.002-0.006		
High myopia	1	0.002	-0.002-0.006	H52.10	

Horner syndrome	1	0.002	-0.002-0.006		
Nystagmus	1	0.002	-0.002-0.006		
Musculoskeletal Disorders	12	0.024	0.011-0.037		
Skeletal dysplasia	5	0.010	0.001-0.019		
Dwarfism	2	0.004	-0.002-0.010	E34.3	100800
Scoliosis	2	0.004	-0.002-0.010	M41	181800
Kyphoscoliosis	1	0.002	-0.002-0.006	M40	610170
Webbed neck	1	0.002	-0.002-0.006		
Others	1	0.002	-0.002-0.006		
Blood disorders	10	0.020	0.008-0.032		
Thalassemia	9	0.018	0.006-0.030	D56	613985
Hemophilia	1	0.002	-0.002-0.006	D66	306700
Others	12	0.024	0.011-0.037		
Cleft Lip	4	0.008	0.000-0.016	Q37	119530
Atrial Septal Defect	1	0.002	-0.002-0.006	Q21.1	108800
Facial dysmorphism	1	0.002	-0.002-0.006	G24.4	
Vitiligo	1	0.002	-0.002-0.006		
Ichthyosis	1	0.002	-0.002-0.006	L85.0	242300
Premature aging	1	0.002	-0.002-0.006		
Alopecia	1	0.002	-0.002-0.006	L63	104000
Obesity	1	0.002	-0.002-0.006		
Skin	1	0.002	-0.002-0.006		

3.2.3 Prevalence of major anomalies based on number of affected subjects

In major divisions of anomalies, the total number of 951 affected people included 593 (62%) males and 358 (38%) females. Neuromuscular disorders were at top of the list with the highest number of affected patients in ratios of 66% (n=174) males and 34% (n=88) females respectively. Neurological disorders show a major contribution with 115 males and 80 females affected with the anomaly (Table 3.4; Fig. 3.10). In all sorts of major anomalies, male number of patients were higher in number than females except for musculoskeletal disorders.

Table 3.4 Number of total Affected Persons (n=951)

Major divisions	Number of Affected		Total	Percentage (%)
	Male	Female		
Neuromuscular disorders	174	88	262	27.5
Neurological disorders	115	80	195	20.5
Sensorineural defects	148	100	248	26.1
Limb disorders	67	33	100	10.5
Visual Impairements	55	30	85	8.9
Musculoskeletal disorders	10	11	21	2.2
Blood disorders	15	8	23	2.4
Others	9	8	17	1.8
Total	593	358	951	
	$\chi 2$=7.33; df=7			
	p=0.3955; NS			

NS, Non-significant

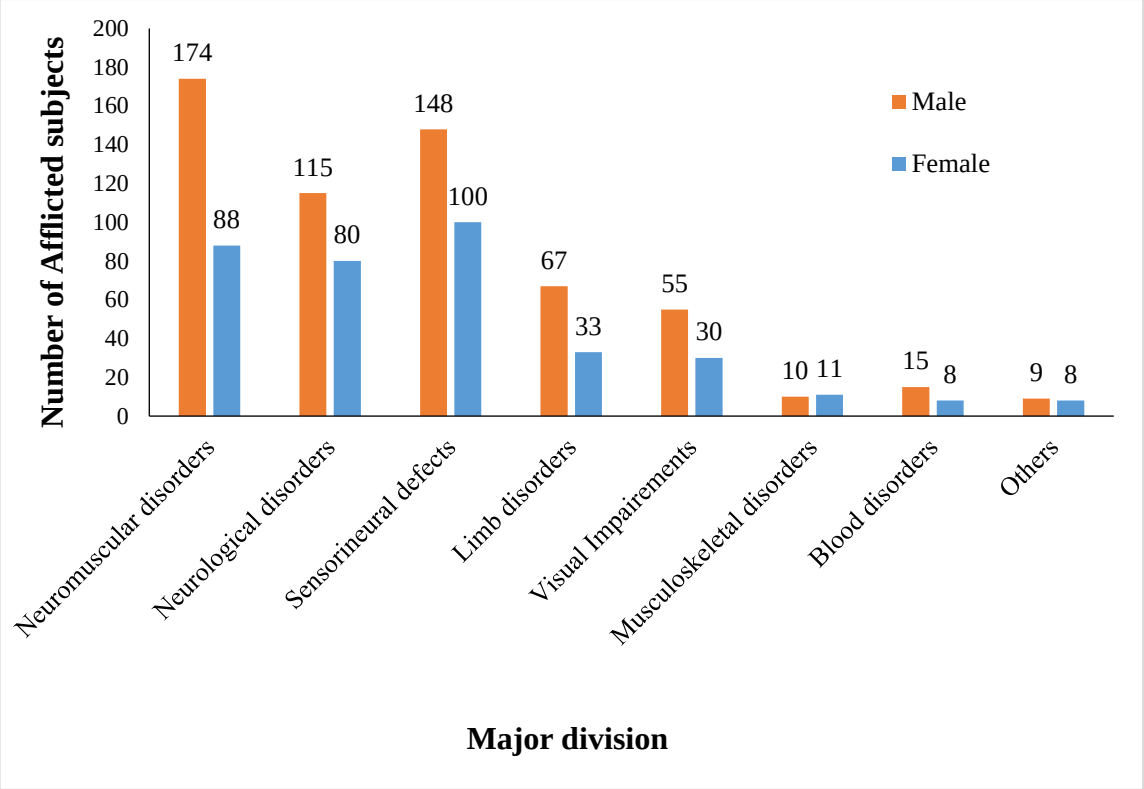


Fig. 3.10 Prevalence of major anomalies based on number of affected subjects

3.2.4 Distribution of subjects based on parity order

All major categories of hereditary anomalies were analyzed on basis of the parity order of subjects to find out any association of parity order with anomaly prevalence. The analysis showed that the majority of subjects 30% (n=151) out of the total 500 cases fall in category of first parity order followed by 2nd parity subjects with 25% (n=100) subjects in specific anomaly category. Afterward, 3rd and 4th parity contribute subjects with 13% (n=63) and 12% (n=62), respectively (Table 3.5).

Table 3.5 Parity of subject in major categories of congenital and hereditary anomalies

Major divisions	Parity of index subjects							Total	Percentage (%)
	1st	2nd	3rd	4th	5th	6th	>7th		
Neuromuscular disorders	45	30	14	16	11	5	13	134	26.8
Neurological disorders	40	18	13	18	11	4	20	124	24.8
Sensorineural defects	37	22	19	11	11	10	4	114	22.8
Limb disorders	12	9	11	9	5	4	7	57	11.4
Visual impairments	11	10	2	2	1	3	8	37	7.4
Musculoskeletal disorders	3	2	3	0	1	2	1	12	2.4
Blood disorders	2	2	0	3	0	2	1	10	2
Others	1	7	1	3	0	0	0	12	2.4
Total	**151**	**100**	**63**	**62**	**40**	**30**	**54**	**500**	

3.2.5 Distribution of subjects and affected persons with respect to disease segregation

In this research study, total 500 families were recruited with various numbers of congenital and hereditary anomalies studied among different major and minor categories. When these data got analyzed on basis of disease segregation among different generations, 82% of the cases have their subjects in 1st generation including both sporadic and familial cases, followed by 17% of subjects with two generations and only 1% of subjects with affected subjects in more than two generations.

These data were analyzed with respect to number of affected persons throughout few generations, the number of affected subjects in 1st generation were 65% (n=617) out of total 951 subjects, majorly contributed 175 (28%) affected subjects of neuromuscular disorders followed by 152 (25%) affected subjects of sensorineural defects and neurological disorders with 136 (22%) affected subjects. In familial cases, the number of affected persons with two generation were 296 (31%) with highest contribution by 87 (29%) sensorineural defects and 77 (26%) neuromuscular disorders. Affected persons with segregation in third (3rd) and fourth (4th) generations were 2.4% (n=23) and 1.6% (n=15) respectively (Table 3.6).

Table 3.6 Distribution of familial/sporadic cases in major disease divisions based on number of cases in different generations

Major Divisions (n=500)	Generation with disease				Total	Percentage (%)
	1st	2nd	3rd	4th		
Neuromuscular disorders	111	22	0	1	134	26.8
Neurological disorders	105	18	1	0	124	24.8
Sensorineural defects	92	20	1	1	114	22.8
Limb disorders	42	14	1	0	57	11.4
Visual Impairments	29	7	1	0	37	7.4
Musculoskeletal disorders	12	0	0	0	12	2.4
Blood disorders	9	1	0	0	10	2
Others	11	1	0	0	12	2.4
Total	**411**	**83**	**4**	**2**	**500**	
	Total affected subjects in different Generations					
Neuromuscular disorders	175	77	0	10	262	27.5
Neurological disorders	136	55	4	0	195	20.5
Sensorineural defects	152	87	4	5	248	26.1
Limb disorders	52	41	7	0	100	10.5
Visual Impairments	47	30	8	0	85	8.9
Musculoskeletal disorders	21	0	0	0	21	2.2
Blood disorders	19	4	0	0	23	2.4
Others	15	2	0	0	17	1.8
Total	**617**	**296**	**23**	**15**	**951**	

3.2.6 Distribution of Anomalies with respect to consanguinity and familial attributes

In recruited subjects (n=500), parents of 81% (n=404) subjects have consanguineous marriages resulting in 46% (n=186) familial and 54% (n=218) sporadic cases. On the other hand, parental marriages of only 96 (19%) subjects were non-consanguineous comprised of 33% (n=32) and 67% (n=64) familial and sporadic anomalies respectively.

Table 3.7 Gender and familial attributes wise distribution of subjects with reference to parental marriage types

Parental marriage types	Gender		Familial Attributes		Total
	Male	Female	Familial	Sporadic	
Consanguineous	267	137	186	218	404
Non-consanguineous	72	24	32	64	96
Total	**339**	**161**	**218**	**282**	**500**
	$\chi 2$=2.82; df=1		$\chi 2$=5.09; df=1		
	p=0.0930; NS		p=0.0240; *		

*, Significant

NS, Non-Significant

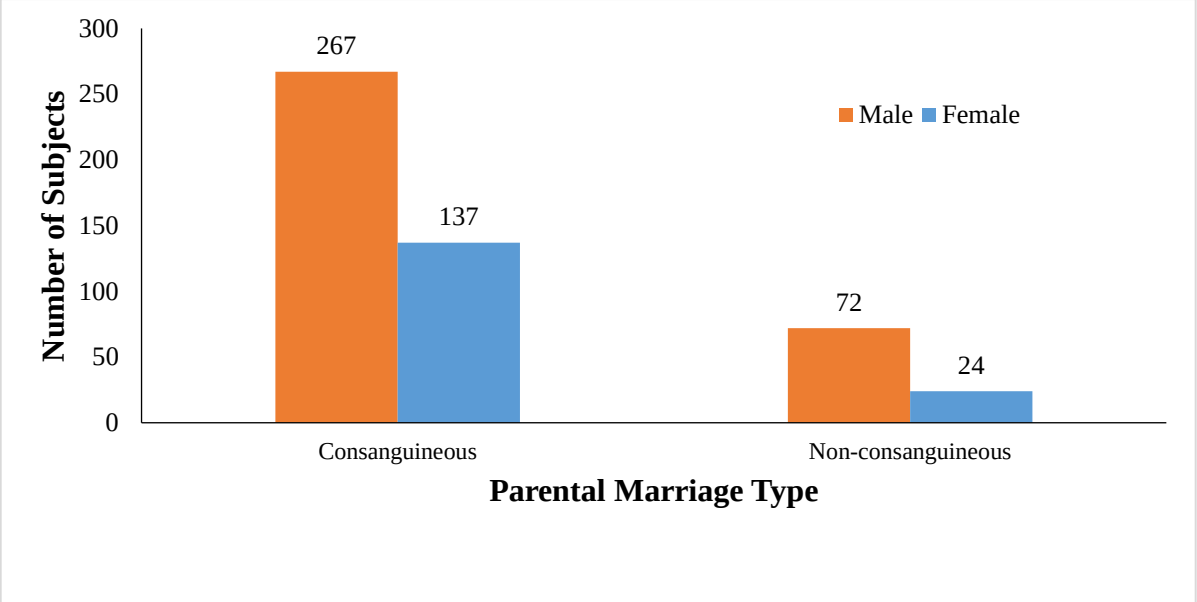


Fig. 3.11 Distribution of anomalies with respect to consanguinity

3.2.7 Distribution of major anomalies based on parental age at birth and isolated/syndromic attributes

These data shows the association of parental age at birth of subjects with the prevalence of various congenital and hereditary anomalies. These data were analyzed and comparative study performed for means of paternal ages at birth of major subjects (Table 3.8A). When observed on basis of isolated and syndromic attributes, 56% (n=282) subjects were contributed by isolated and 44% (n=218) subjects by sporadic nature of anomalies. In major anomalies, neuromuscular disorders and neurological disorders show a higher ratio of syndromic attributes as compared to isolated ones. In contrast, sensorineural defects and limb disorders show a higher ratio of isolated attributes comparatively. Neuromuscular disorders contribute 89 syndromic and 45 isolated cases, neurological disorders with 103 syndromic and 21 isolated cases and sensorineural defects have 108 isolated and 6 syndromic cases. In this detailed study, visual impairments and blood disorders show only isolated cases (Table 3.8B).

Table 3.8A Distribution of anomalies with respect to parental age at birth

Major divisions	Parental age	
	Paternal age	Maternal age
Neuromuscular disorders	31.5±7.4	29.1±6.9
Neurological disorders	32.5±8.1	29.9±7.6
Sensorineural defects	29.8±6.7	27.5±6.6
Limb disorders	32.5±8.3	29.6±7.6
Visual impairments	31.5±8.5	29.2±8.1
Musculoskeletal disorders	31.2±10.1	28.2±8.0
Blood disorders	29.5±7.2	29±7.4
Others	29.3±4.9	27.1±4.4
Total	**t=7.132; df=6; p=0.0004**	

P<0.05, "Significant"

Table 3.8B Distribution of anomalies with respect to isolated/syndromic attributes

Major divisions	Isolated/Syndromic		Total
	Isolated	Syndromic	
Neuromuscular disorders	45	89	134
Neurological disorders	21	103	124
Sensorineural defects	108	6	114
Limb disorders	46	11	57
Visual impairments	37	0	37
Musculoskeletal disorders	5	7	12
Blood disorders	10	0	10
Others	10	2	12
Total	**282**	**218**	**500**
	$\chi 2$=229.7; df=7		
	p<0.0001; ***		

***, Highly Significant

3.3 Spectrum of congenital and hereditary anomalies (CHA)

A detailed spectrum of congenital and hereditary anomalies in studied population is shown for some major categories of anomalies which includes classification and sub-categorization of more prevalent disorders in data.

3.3.1 Neuromuscular disorders

Neuromuscular disorders were a major category with the highest prevalence in the specified population of site under study contributing 27% (n=134) to total of 500 recruited cases. This major category was further sub-divided into cerebral palsy, muscular dystrophy, atrophy and severe muscle hypotonia. Cerebral palsy as a major category of neuromuscular disorders that shared 86% (n=115) of subjects followed by muscular dystrophy 12% (n=17) and 2% (n=2) others (Fig. 3.13).

3.3.1.1 Distribution of Neuromuscular disorders with respect to gender, familial/sporadic and isolated/syndromic attributes

In this study, neuromuscular disorders show that cerebral palsy counts 115 cases (83 males, 32 females) including 59% (n=68) sporadic, 41% (n=47) familial cases, 28% (n=32) isolated and 72% (n=83) syndromic cases. Muscular dystrophy has 17 (13 males, 4 females) subjects with 13 sporadic and 4 familial subjects having a similar proportion of isolated to syndromic subjects (Table 3.9; Fig. 3.12).

Table 3.9 Distribution of neuromuscular disorders based on gender, familial/sporadic and isolated/syndromic attributes

Anomaly	Gender		Familial Attributes		Isolated/syndromic		Total
	Male	Female	Sporadic	Familial	Isolated	Syndromic	
Cerebral Palsy	83	32	68	47	32	83	115
Muscular Dystrophy	13	4	13	4	13	4	17
Atrophy	1	0	0	1	0	1	1
Severe Muscle Hypotonia	1	0	0	1	0	1	1
Total	**98**	**36**	**81**	**53**	**45**	**89**	**134**
	$\chi2$=0.89; df=3		$\chi2$=4.97; df=3		$\chi2$=16.74; df=3		
	p=0.8290; ns		p=0.1743; ns		p=0.0008; ***		

***, Highly Significant

NS, Non-significant

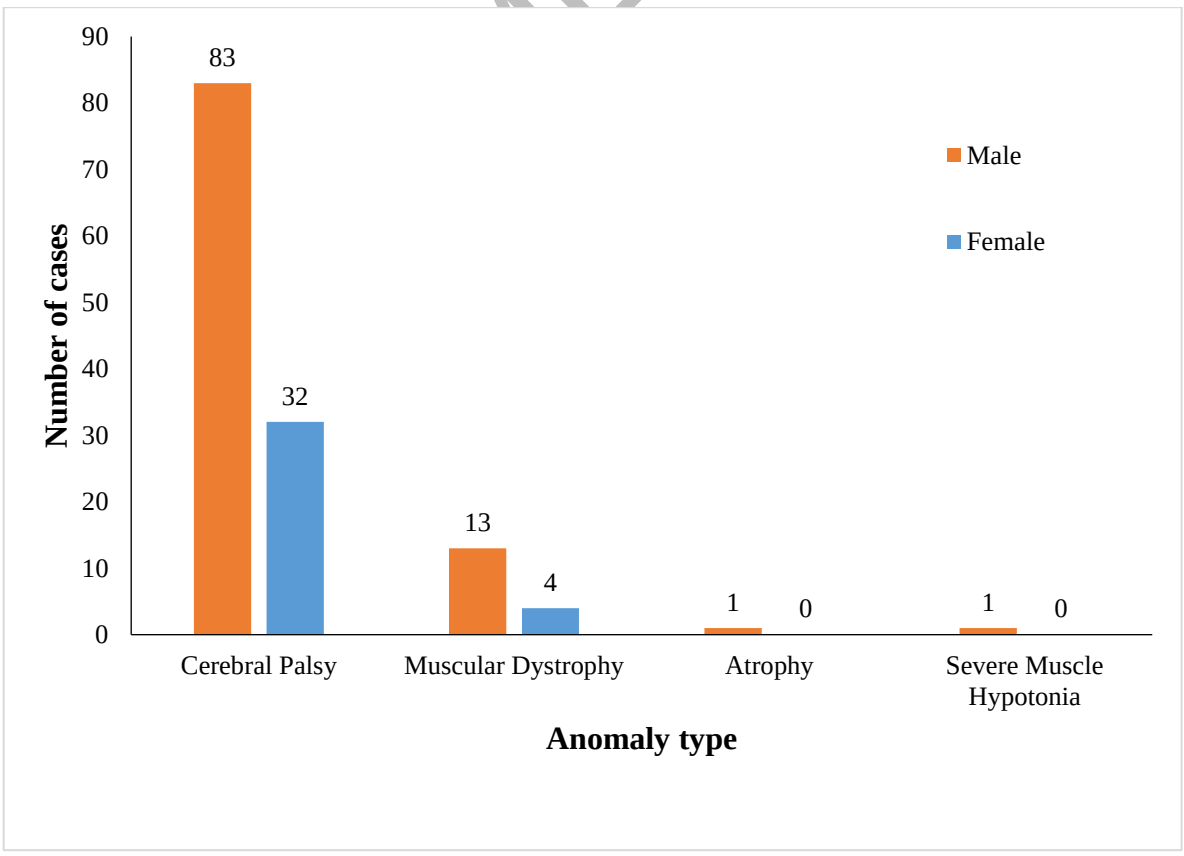


Fig. 3.12 Distribution of neuromuscular disorders based on gender

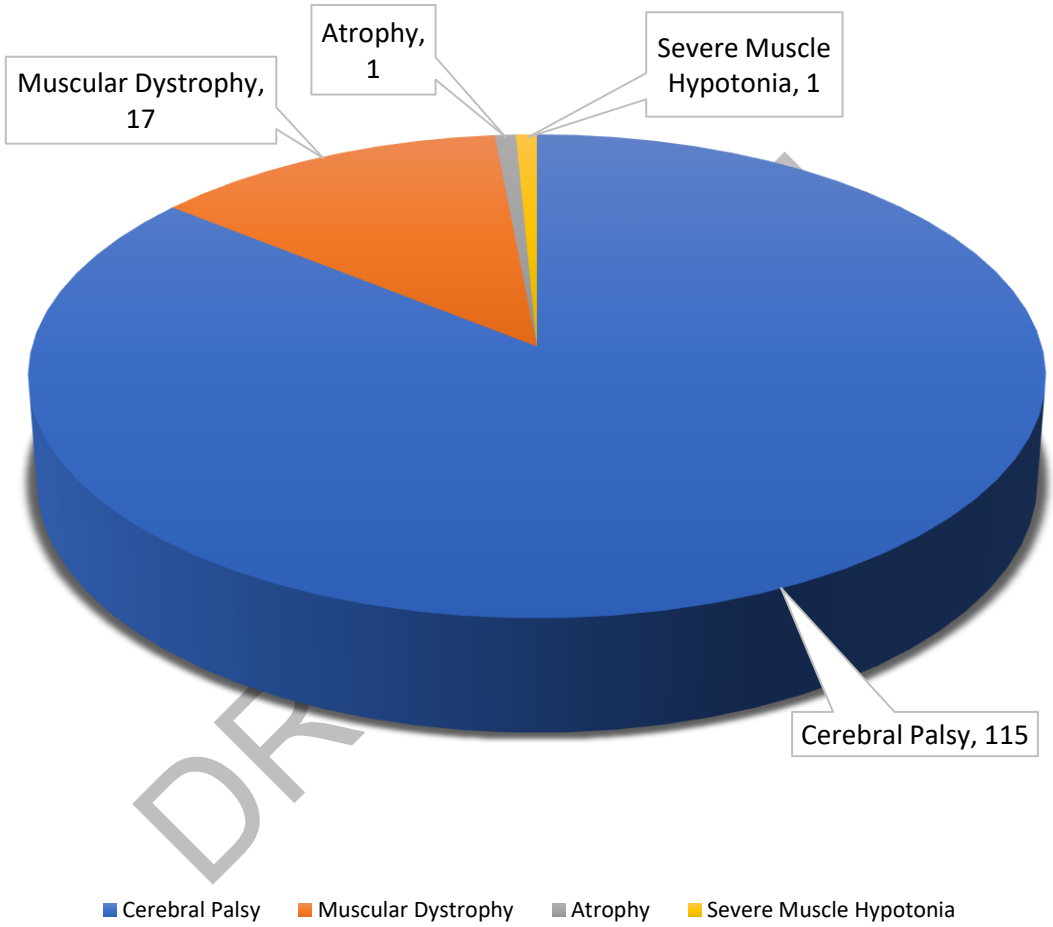


Fig. 3.13 Distribution of Neuromuscular disorders

3.3.1.2 Categorization of Cerebral Palsy

Subjects were further analyzed using different physical and clinical diagnostic parameters to categorize cerebral palsy into its types evaluating the prevalence of different categories of CP. Various types of CP observed in these data were athetoid, ataxia, spastic hemiplegia (right and left), diplegic, paraplegic, monoplegia, quadriplegic, and triplegic.

Table 3.10 Classification of Cerebral Palsy

Anomaly Type	Gender		Sporadic/Familial		Isolated/Syndromic		Total
	Male	Female	Sporadic	Familial	Isolated	Syndromic	
Athetoid	25	15	23	17	1	39	**40**
Ataxia	27	8	20	15	12	23	**35**
Monoplegia	6	1	6	1	4	3	**7**
Diplegic	2	1	2	1	1	2	**3**
Paraplegic	4	1	5	0	3	2	**5**
Hemiplegia	9	6	9	6	7	8	**15**
quadriplegic	9	0	7	2	3	6	**9**
Triplegic	1	0	1	0	1	0	**1**
Total	**83**	**32**	**73**	**42**	**32**	**83**	**115**

Table 3.11 Classification of Cerebral Palsy into sub-categories on basis of disease onset and staging

Cerebral Palsy (n=115)	ICD-10 Version 2019	Onset		Disease Staging		Total	Percentage (%)
		Congenital	Late	Progressive	Non- Progressive		
Ataxia	G80.4	30	2	6	26	32	27.8
Ataxic diplegia	G80.1	3	0	0	3	3	2.6
Athetoid pure	G80.3	24	1	3	22	25	21.7
Athetoid dystonia	G80.3	15	0	0	15	15	13.0
Spastic left hemiplegia	G80.2	10	0	0	10	10	8.7
Spastic paraplegia	G82.1	5	0	0	5	5	4.3
Spastic right hemiplegia	G80.2	5	0	1	4	5	4.3
Spastic right leg monoplegia	G83.1	4	0	0	4	4	3.5
spastic left leg monoplegia	G83.1	1	0	0	1	1	0.9
spastic right arm monoplegia	G83.2	1	0	0	1	1	0.9
Floppy left arm monoplegia	G83.2	1	0	0	1	1	0.9
Spastic diplegia	G80.1	3	0	0	3	3	2.6
spastic quadriplegia	G80.0	9	0	0	9	9	7.8
Triplegic		0	1	1	0	1	0.9
Total		**111**	**4**	**11**	**104**	**115**	

3.3.2 Neurological disorders

In the total of 500 ascertained subjects, neurological disorders were the second highly prevalent disorders in the studied population. These disorders were further categorized into Intellectual disability (79.83%, n=99), followed by down syndrome (10.48%, n=13), spina bifida (4.0%, n=5), microcephaly (2.41%, n=3), hydrocephaly (n=1), chronic sensorineural neuropathy (n=1) and occipital encephalocele (1.61%, n=2). These data include a higher ratio of males (64.51%, n=80) than females (35.48%, n=44). Similarly, sporadic (64.32%, n=81) and syndromic (83.06%, n=103) cases were more prevalent than familial (35.67%, n=43) and isolated (16.93%, n=21) cases comparatively.

Table 3.12 Distribution of Neurological disorders with respect to gender, familial/sporadic and isolated/syndromic perspectives

Anomaly type	Gender		Familial/Sporadic		Isolated/Syndromic		Total
	Male	Female	Familial	Sporadic	Isolated	Syndromic	
Intellectual disability	66	33	37	62	13	86	99
Down Syndrome	9	4	4	9	0	13	13
Spina Bifida	3	2	0	5	4	1	5
Microcephaly	2	1	1	2	0	3	3
Hydrocephaly	0	1	0	1	1	0	1
Chronic sensorineural polyneuropathy	0	1	1	0	1	0	1
Occipital Encephalocele	0	2	0	2	2	0	2
Total	**80**	**44**	**43**	**81**	**21**	**103**	**124**
	$\chi2$=7.65, df=6 p=0.2649; NS		$\chi2$=6.54; df=6 p=0.3656; NS		$\chi2$=38.04, df=6 p<0.0001; ***		

***, Highly Significant

NS, Non-Significant

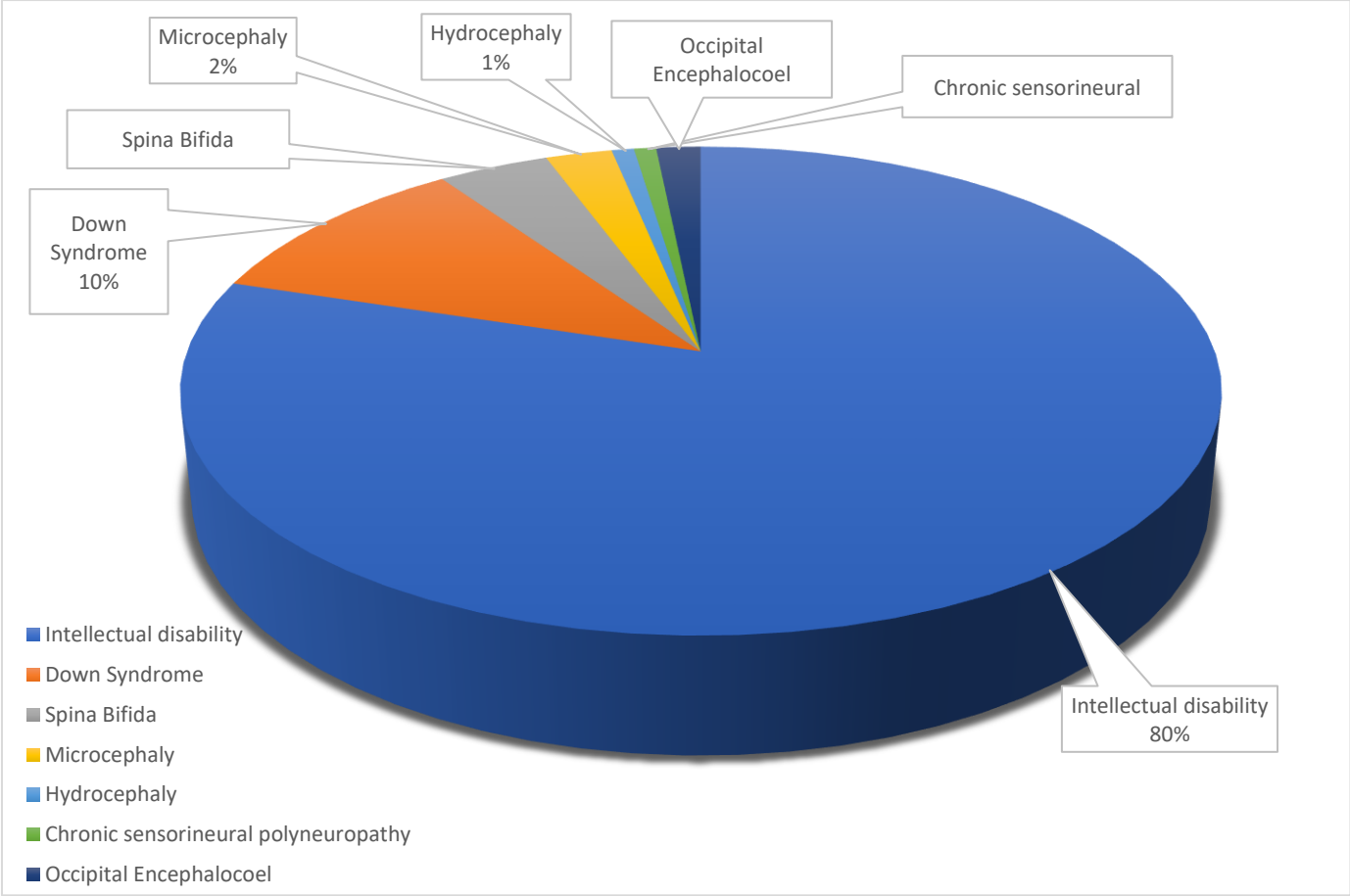


Fig. 3.14 Distribution of Neurological disorder

3.3.2.1 Classification of Intellectual disability

Intellectual disability is more prevalent as a neurological disorder. Based on the severity of disease and IQ level of subjects, Intellectual disability is categorized into four categories named mild, moderate, severe, and profound. Most subjects fall in severe (34.5%, n=35) and moderate (31.68%, n=32) followed by profound (28.71%, n=28.71) and mild (4.95%, n=5) category (Fig. 3.15).

Among 101 total cases of Intellectual disability, most of cases (n=100) have congenital onset and only 1 case of late onset. In disease staging based study, non-progressive ID cases were 87.12% (n=88) and progressive with 12.87% (n=13) cases (Table 3.13).

Table 3.13 Classification of Intellectual disability based on severity

Intellectual disability (n=100)	ICD-10; Version 2019	Onset		Disease staging		Total
		Congenital	Late	Progressive	Non-progressive	
Mild	F70	5	0	1	4	5
Moderate	F71	32	0	3	29	32
Severe	F72	34	1	7	28	35
Profound	F73	29	0	2	27	29
Total		**100**	**1**	**13**	**88**	**101**

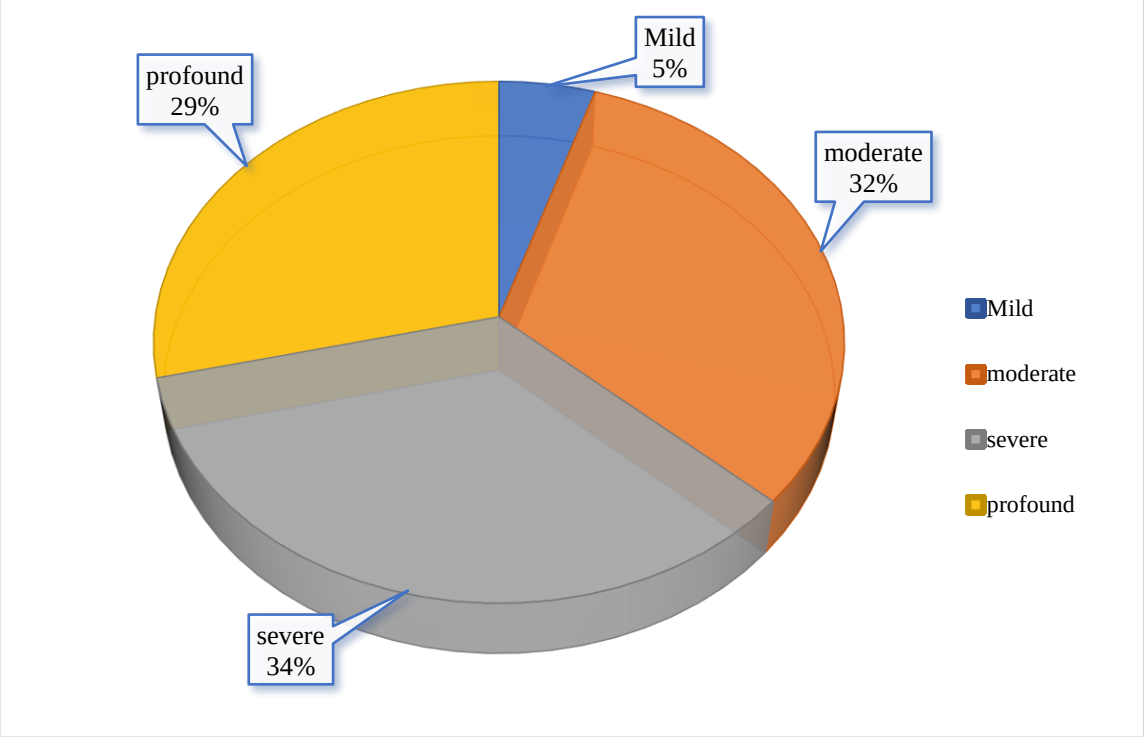


Fig. 3.15 Classification of Intellectual disability based on severity

3.3.3 Sensorineural Defects

Out of the total ascertained subjects (n=500), sensorineural defects were third most contributing anomaly in this population under study. Data results depict 22.8% (n=114) of patients with sensorineural defects including 80 males and 34 females. Further categorization leads to 83.33% (n=95) deaf and mute, followed by 7.017% (n=8) deaf, 4.3% (n=5) mute and 5.26% (n=6) cases of stuttering. There were 48.24% (n=55) familial and 51.75% (n=59) sporadic cases in the data. Similarly, isolated cases (n=108) were more frequent than sporadic cases (n=6).

Table 3.14 Distribution of Sensorineural defects with respect to gender, familial/sporadic and isolated/syndromic attributes

Sensorineural defects	Gender		Familial/sporadic		Isolated/syndromic		Total
	Male	Female	Familial	Sporadic	Isolated	Syndromic	
Mute and Deaf	65	30	45	50	90	5	95
Deaf	7	1	7	1	7	1	8
Mute	5	0	1	4	5	0	5
Stuttering	3	3	2	4	6	0	6
Total	**80**	**34**	**55**	**59**	**108**	**6**	**114**
	$\chi 2$=4.58; df=3 p=0.2054; NS		$\chi 2$=7.09; df=3 p=0.0688; NS		$\chi 2$=1.45; df=3 p=0.6935; NS		

NS, Non-significant

3.3.3.1 Classification of Sensorineural defects

Sensorineural defects were further analyzed and categorized into different types including mute and deaf, deaf, mute, and stuttering. In these data contribution of these sub types out of total subjects is mentioned in the Fig. 3.16.

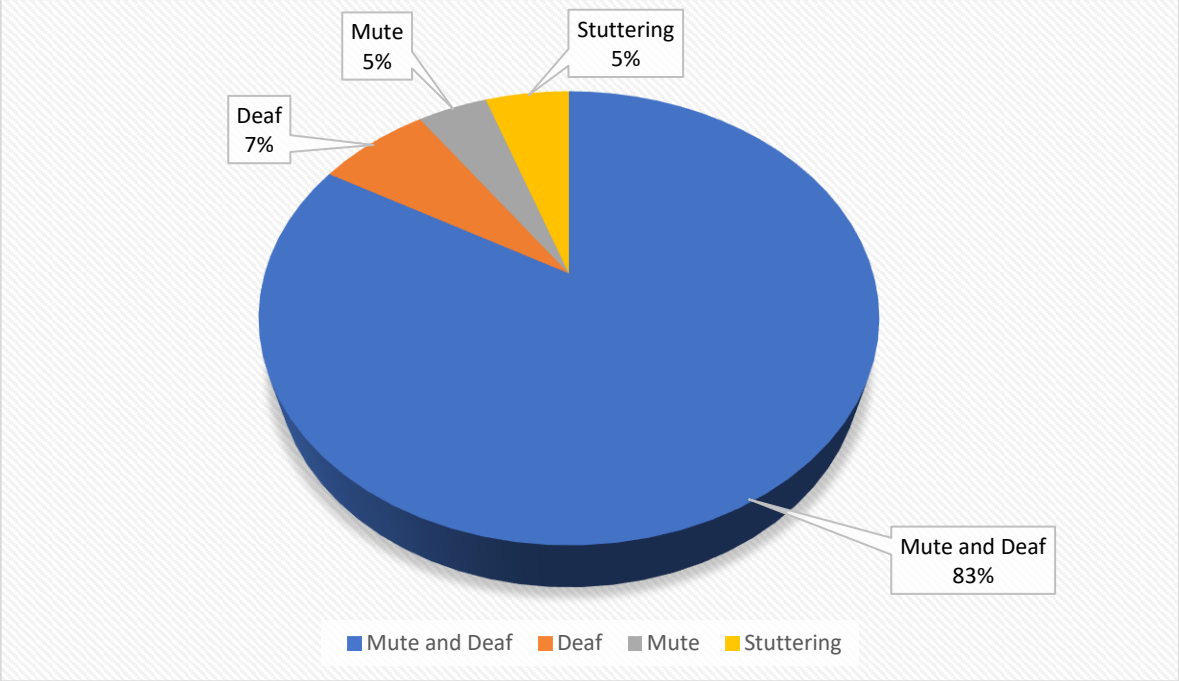


Fig. 3.16 Classification of Sensorineural defects

3.3.4 Limb disorders

Limb disorders show 11.4% prevalence in total ascertained data (n=500). Out of 57 patients, limb disorders were further categorized into talipes contributing majorly 54% (n=31), followed by polydactyly and transverse limb amputations (7% each), limb discrepancy (5%), constriction ring and syndactyly contributing 5% each. Synpolydactyly and rickets contribute only 4% each. The category named as “others” is a collection of three types of limb anomalies with low prevalence in the studied population (Fig. 3.17).

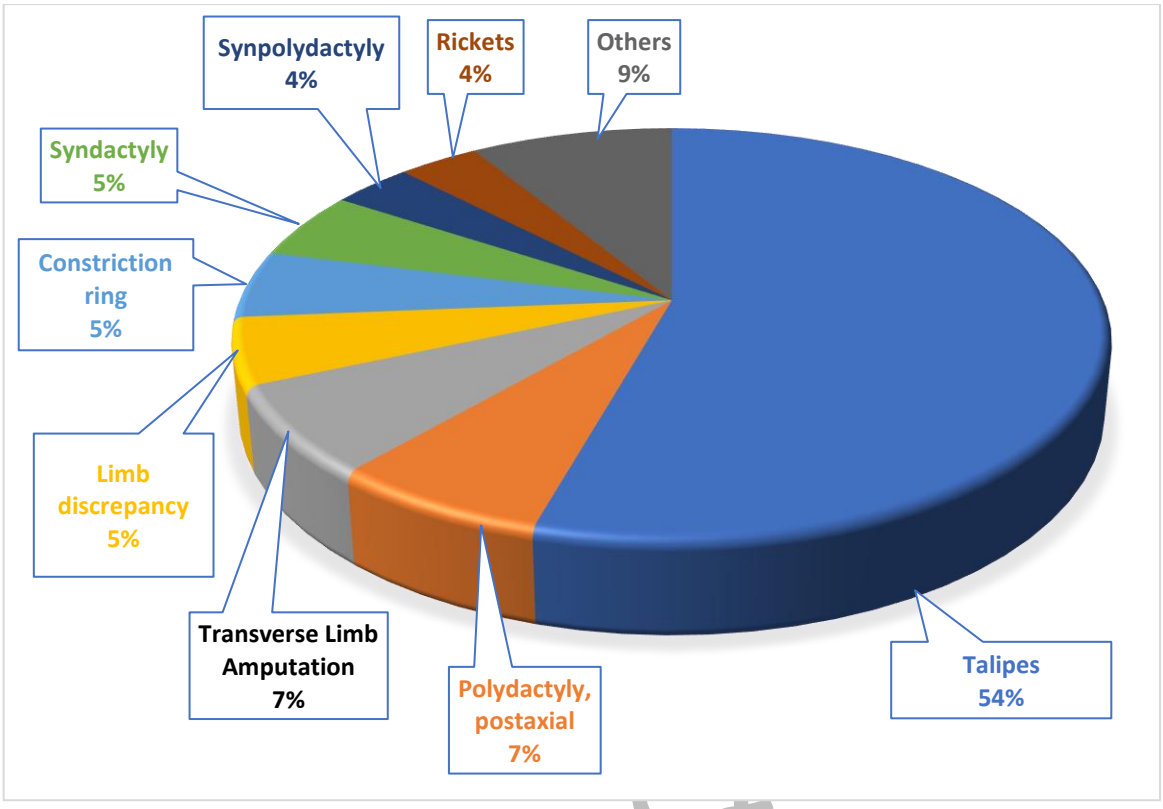


Fig. 3.17 Categorization of Limb disorders

3.3.4.1 Distribution of Limb disorders based on gender, familial/sporadic and isolated/syndromic attributes

The ascertained subjects with limb disorders were analyzed on basis of gender, familial/sporadic and isolated/syndromic attributes. In the case of gender, males were 61.40% (n=35) relatively high as compared to females 38.59% (n=22). Similarly, sporadic (59.64%, n=34) and isolated (80.7%, n=46) were more frequent than familial (40.35%, n=23) and syndromic (19.29%, n=11) cases respectively (Table 3.15).

Table 3.15 Distribution of Limb disorders based on gender, familial/sporadic and isolated/syndromic attributes

Limb disorders	Gender		Familial/sporadic		Isolated/Syndromic		Total
	Male	Female	Familial	Sporadic	Isolated	Syndromic	
Talipes	17	14	11	20	26	5	31
Polydactyly, postaxial	2	2	3	1	2	2	4
Transverse Limb Amputation	4	0	2	2	3	1	4
Limb length discrepancy	1	2	0	3	1	2	3
Constriction ring	2	1	0	3	3	0	3
Syndactyly	2	1	2	1	3	0	3
Synpolydactyly	2	0	2	0	1	1	2
Rickets	2	0	0	2	2	0	2
Others	3	2	3	2	5	0	5
Total	**35**	**22**	**23**	**34**	**46**	**11**	**57**
	$\chi2$=6.88; df=8 p=0.5493; NS		$\chi2$=12.49; df=8 p=0.1307; NS		$\chi2$=11.35; df=8 p=0.1829; NS		

NS, Non-significant

3.4 Pedigree construction to show inheritance patterns of congenital and hereditary disorders

To investigate the inheritance pattern of hereditary disorders diagnosed from ascertained families. Different familial disease segregation throughout generations, living and dead affected members, and paternal marriage types (consanguineous/ non-consanguineous) were displayed by a family pedigree.

3.4.1 Pedigree I: A Family with Skeletal dysplasia

The presentation of this family with skeletal dysplasia showing that the subject was associated with knocking knees, bowed spine, and camptodactyly. The pedigree shows autosomal recessive mode of inheritance. Only two male subjects were afflicted in this pedigree. There are no abnormal phenotypes in normal subjects (Fig. 3.18).

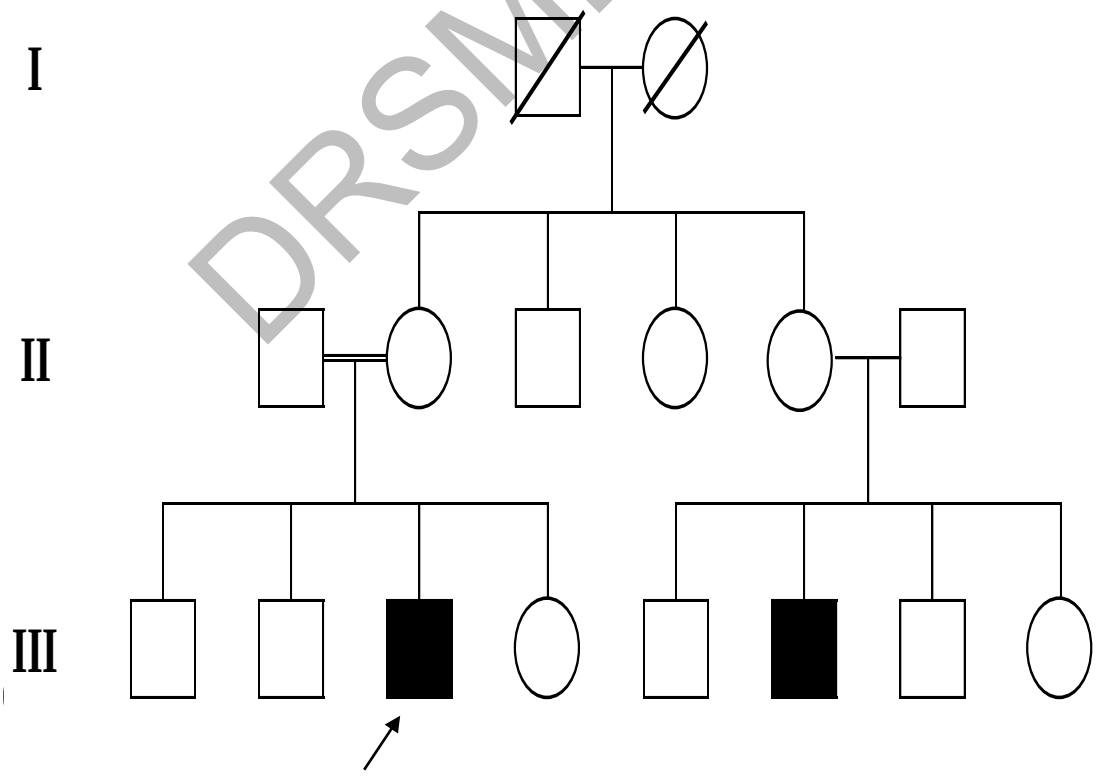


Fig. 3.18 Pedigree I

3.4.2 Pedigree II: A Family with Cerebral Palsy

The pedigree II was recruited from the urban area of tehsil Layyah. In this pedigree, the effected subjects (7 males and 2 females) showed the phenotype of cerebral palsy with talipes in two consecutive generations (IV and V). In this family, autosomal recessive pattern with consanguinity is prominent and could be the possible risk factor in transmission of this disorder among the subjects (Fig. 3.19).

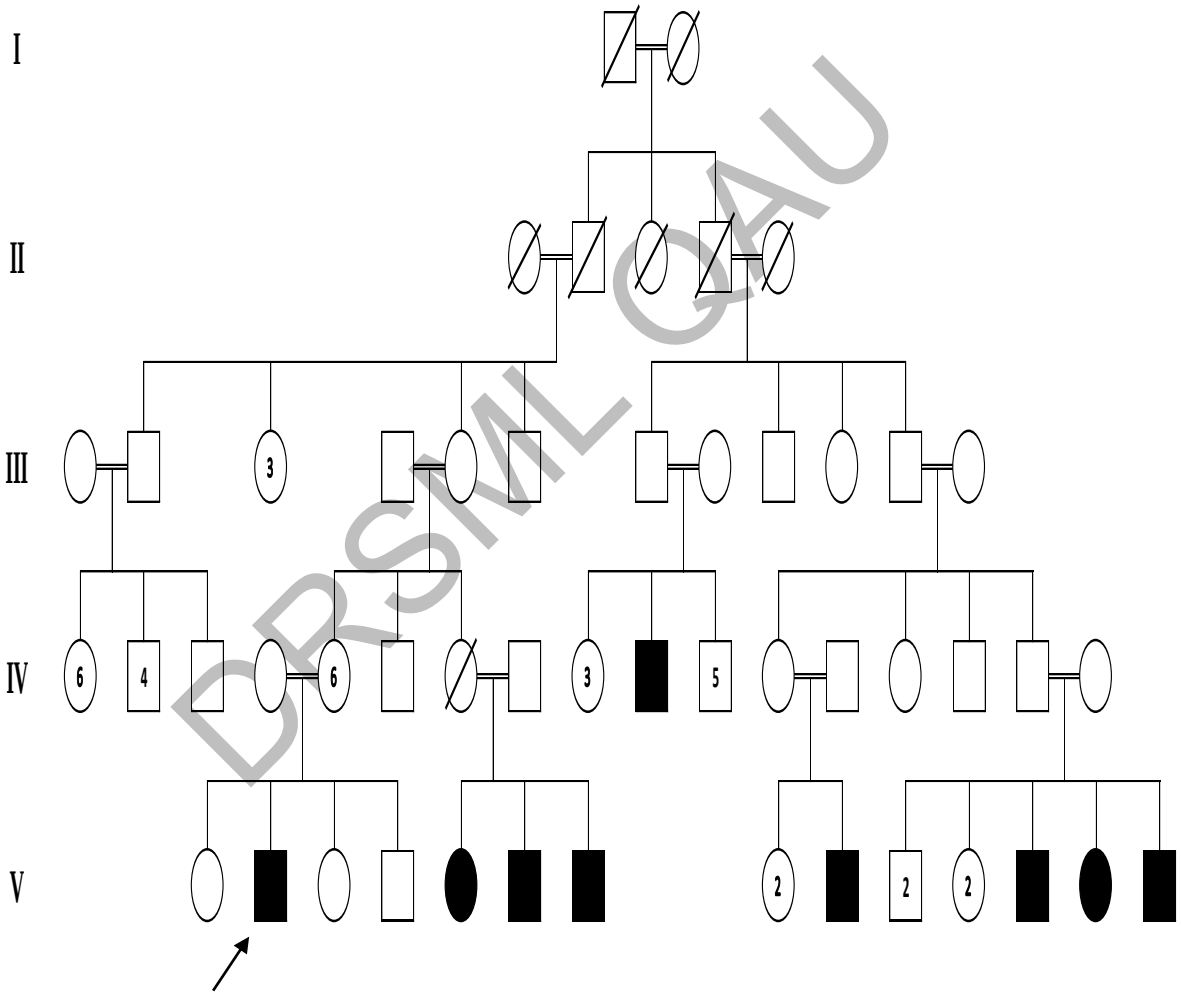


Fig. 3.19 Pedigree II

3.4.3 Pedigree III: A Family with Intellectual disability (ID)

The pedigree III was ascertained from remote area of tehsil Layyah. In this family the prominent feature of severe intellectual disability was observed. Four males and two females were affected. The clinical analysis of all phenotypes in normal subjects shows that close marriage indicates the autosomal recessive pattern in this family (Fig. 3.20).

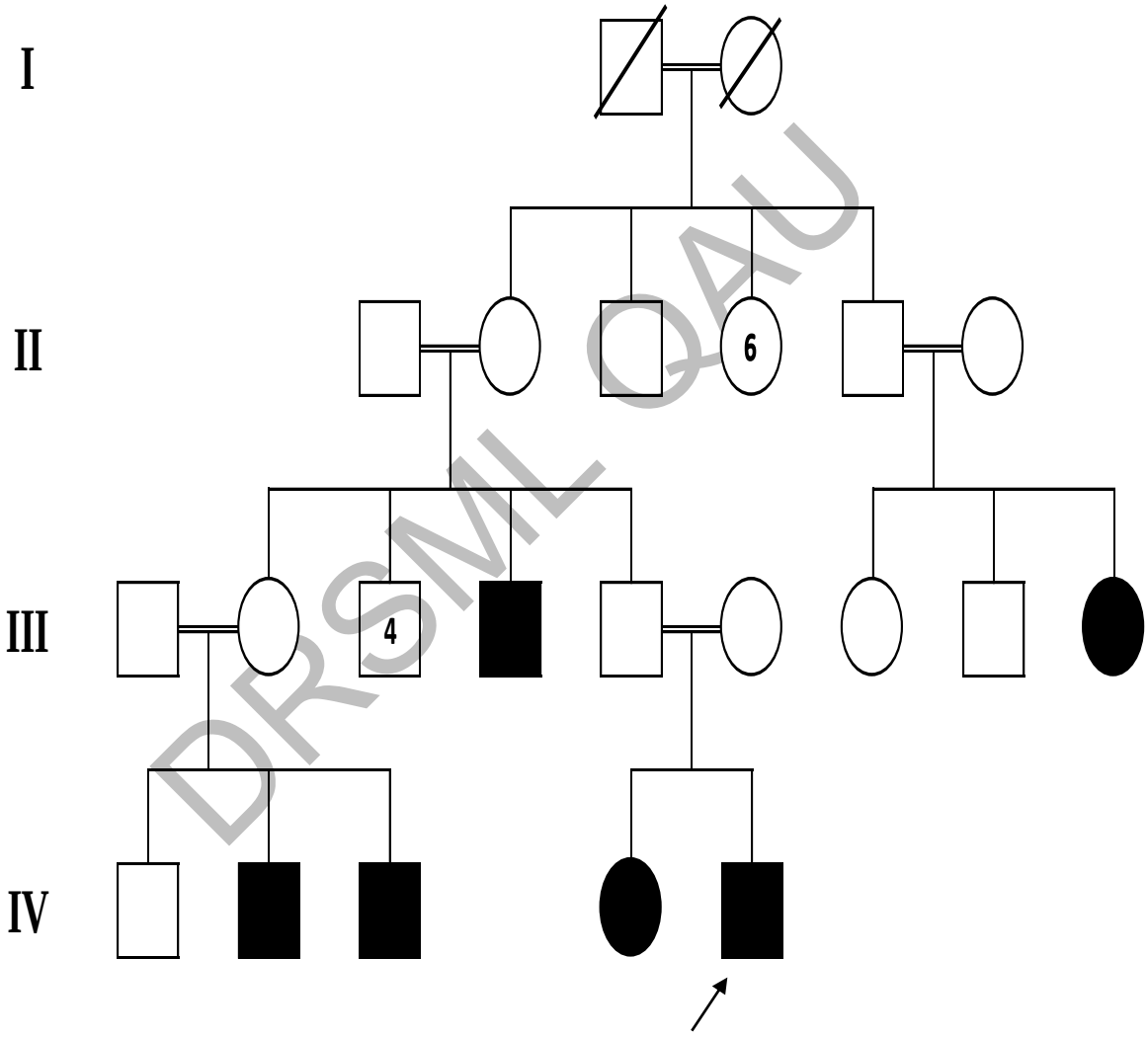


Fig. 3.20 Pedigree III

3.4.4 Pedigree IV: A Family with Becker's Muscular Dystrophy (BMD)

The pedigree IV with autosomal dominant mode of inheritance, indicates the disease progression of Becker's Muscular Dystrophy. In this family the head of the family with (3 males and 4 females) were deceased. While the subject was 40 years old male with prominent features of walking difficulty, blindness, and weakness of muscles (Fig. 3.21).

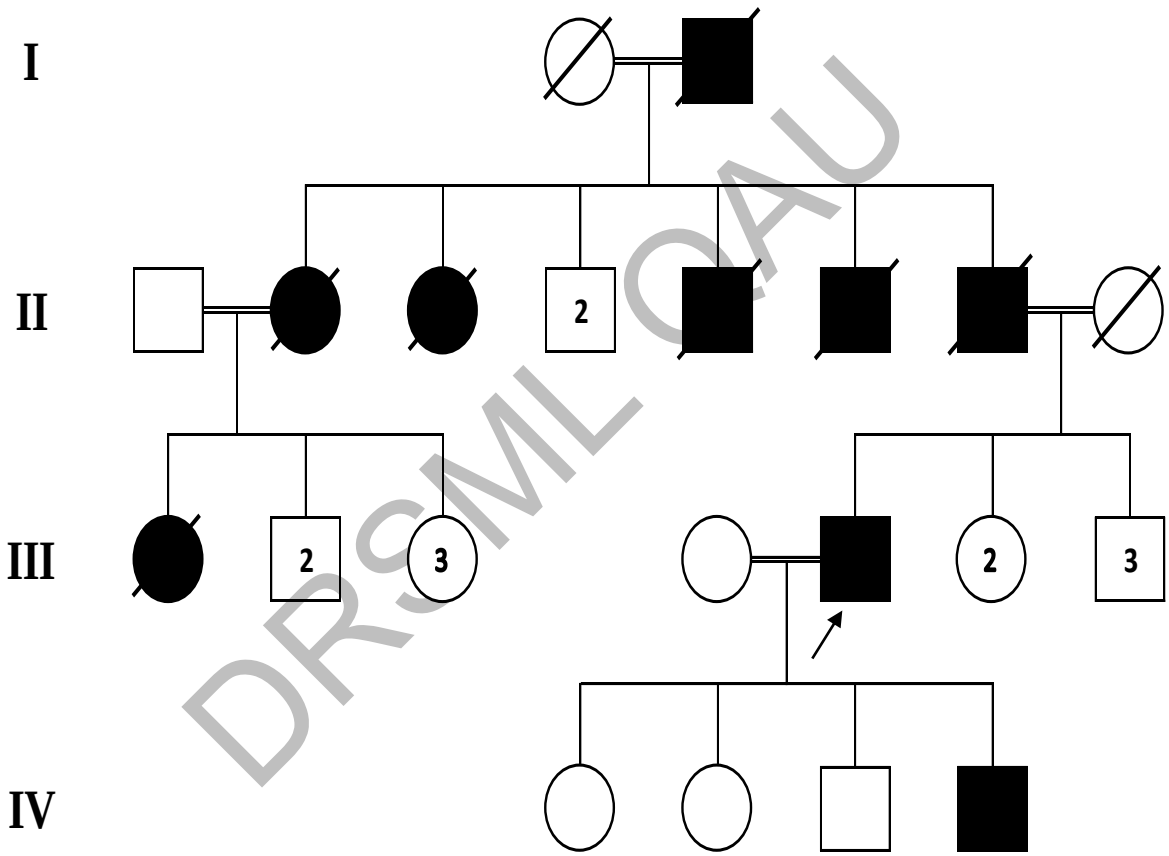
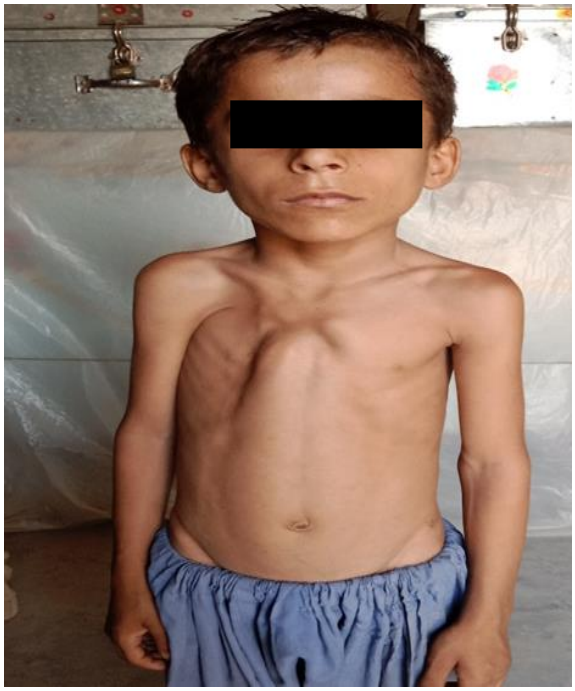
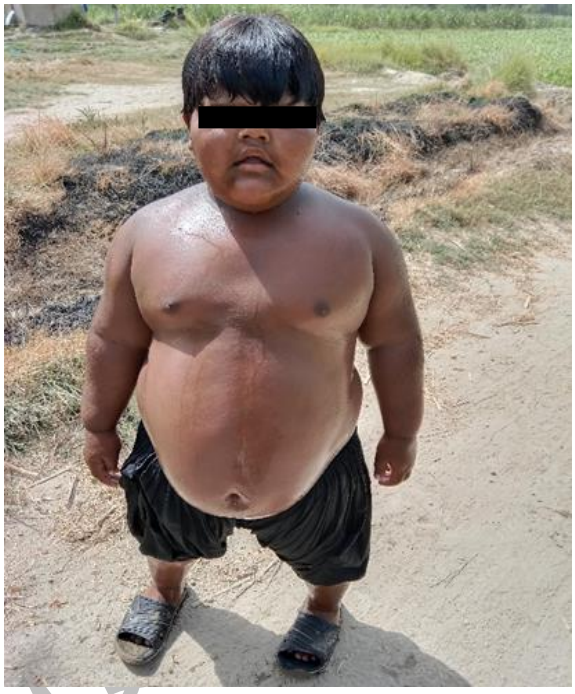


Fig. 3.21 Pedigree IV

3.5 Field survey; Pictures of Subjects with Hereditary disorders



Skeletal dysplasia

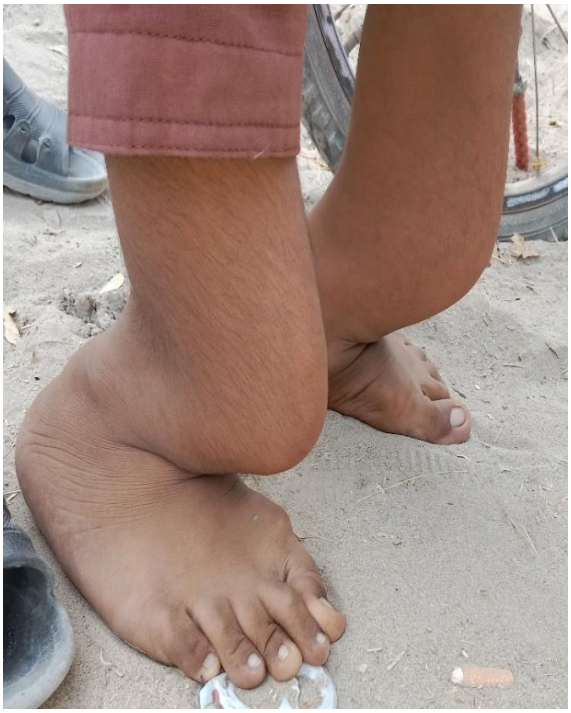


Obesity

Dwarfism

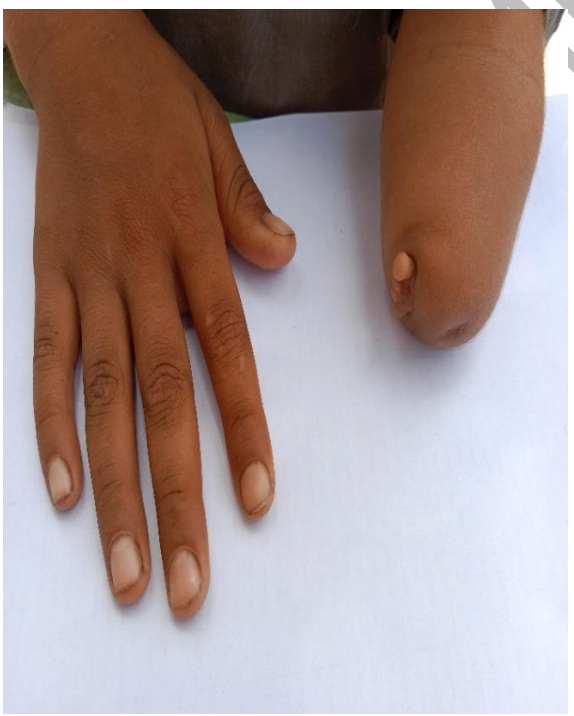
Ataxia

3.6 Field survey; Clinical manifestation of recruited subjects

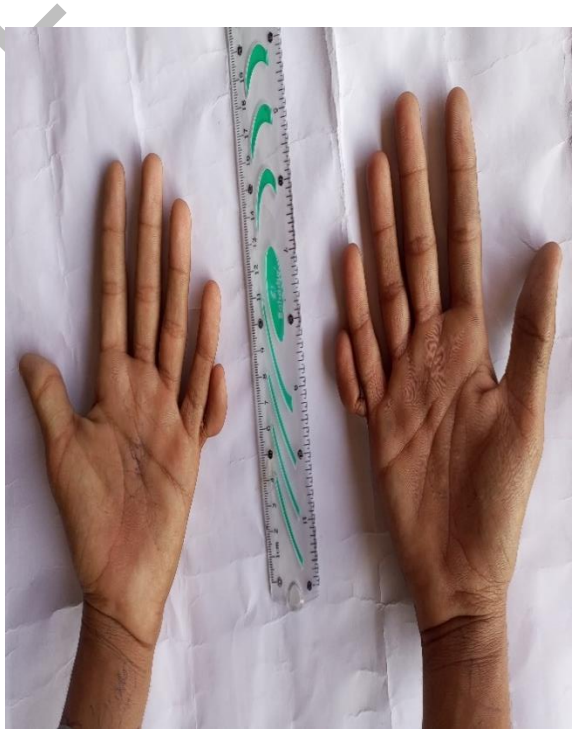


Rickets

Cleft Hands



Amputation



Polydactyly

Chapter 04

Discussion

This study was first time conducted in Layyah, based on prevalence and genetic epidemiological aspects of congenital and hereditary malformations. Tehsil Layyah witnesses high prevalence of hereditary and congenital anomalies due to lack of proper health care, maternal illness, and consanguineous marriages on a large scale. Factors like low socio-economic conditions, lack of health care, and lack of awareness can be the major causes of congenital anomalies prevailing in Layyah for long.

The health care system with flaws of management has no proper support for families with congenital anomalies and as a result, those families and society suffer at a large scale socially, economically, and psychologically. Proper documentation of congenital and hereditary malformations is not present due to lack of proper health infrastructure. Nearly 6% to 9% of prenatal deaths in Pakistan are mainly caused due to congenital anomalies (Korejo *et al.,* 2007).

The research work in this area was carried out to know the risk factors for certain congenital anomalies to ensure better health. Some prenatal medical tests during pregnancy like chorionic villus sampling, amniocentesis, and ultrasonography can be helpful in early diagnosis of congenital anomalies.

This was first research study of genetic disorders conducted in Layyah city of Pakistan and no such previous record exists. In these data, out of total 500 recruited cases, results showed that most common congenital anomalies were neuromuscular disorders (26.8%) followed by neurological disorders (24.8%), sensorineural defects (22.8%), limb disorders (11.4%), visual impairments (7.4%), musculoskeletal disorders (2.4%), blood disorders (2%) and others (2.4%). In this analysis, more contribution of male and sporadic cases was observed as compared to females and familial cases. Different etiological factors like environmental factors, dietary issues, lethality, and nascent mutation may be

accounted as the major reason of the higher prevalence of sporadic cases as compared to familial cases. Consanguinity is found to be a prominent factor even in sporadic cases relatively higher than familial ones and factors like lack of awareness, poor health care, improper record management, lack of penetrance and expressivity may be major reason that several familial cases were accounted as sporadic.

The higher prevalence of familial cases in the recruited area was mainly due to consanguineous marriages. In both sporadic and familial cases, consanguineous marriages were high in number as compared to non-consanguineous marriages. So, in population of district Layyah, consanguinity may be major contributing factor in the higher incidence of congenital and genetic disorders. Consanguinity was the major cause of birth defects among children leading to a significant result (p value ≤ 0.05) showing prevalence of congenital birth defects as 14% (Langah *et al.*, 2022).

A study of gross congenital malformations at birth was carried in Nishtar Hospital Multan, which witnessed that central nervous system anomalies (38.88%) were most common followed by cleft lip/palate (11.11%) indicating the highest prevalence of nervous disorders in southern areas of Punjab (Jahangir *et al.*, 2009). In comparison, our study similarly showed higher prevalence of neuromuscular disorders (26.8%) and neurological disorders (24.8%) followed by sensorineural (22.8%) and limbs defects (11.4%) depicting the higher prevalence of congenital nervous disorders in Layyah. In both studies, parental consanguinity was observed in 55.5% and 80.8% for malformed cases respectively. In this study, consanguinity is the highly influential factor causing anomalies.

In another similar study, conducted on neonates in Combined Military Hospital, Rawalpindi, the results showed higher prevalence of Central Nervous System (CNS)

disorders (40%), followed by musculoskeletal disorders (40%) and genitourinary defects (18%) indicating neuromuscular disorders as most common (Khan *et al.,* 2012), while in our study, neuromuscular disorders and neurological disorders hold the position of highly prevalent anomalies similarly.

A coherent study of congenital anomalies, conducted in northwestern population of Kurram Tribal Agency, indicated a higher prevalence of neurological disorders (n=83/246, CI=0.278, 0.397) followed by musculoskeletal disorders (n=56), limb disorders (n=52) and sensorineural defects (Zahra *et al.,* 2016). In contrast, similar results of this study, indicate that most common anomalies place is held by neuromuscular disorders and neurological disorders followed by sensorineural defects and limb disorders. Another coherent comparison between both studies was found between frequent occurrence of sporadic as compared to familial cases and isolated presentation was more common than syndromic appearance. Presence of more sporadic cases indicates the causation of these anomalies by environmental factors as well.

In Pakistan, a study of congenital and hereditary anomalies carried in Sialkot city, indicates the higher prevalence of limb disorders (p=0.469; CI= 0.406–0.532), followed by neurological disorders (p=0.315; CI= 0.257–0.374), musculoskeletal disorders and neuromuscular disorders (Bhatti *et al.,* 2019). Similarly, in these data, above mentioned anomalies were more common in prevalence. In both studies, males were more affected than females and sporadic show high prevalence than familial cases.

In both previous studies by (Zahra *et al.,* 2016) and (Bhatti *et al.,* 2019), cerebral palsy was added as sub-category of neurological disorders but in current study, CP is included in the major category of neuromuscular disorders with the maximum number of cases in this category of congenital anomalies. In an earlier study of congenital anomalies

conducted in Mosul City of Iraq, results indicate that disorders of central nervous system (39.62%) were highly prevalent, followed by Cleft lip and palate (3.71%) and Down syndrome which contributes comparatively less (2.78%) in the prevalence of hereditary anomalies (Taboo *et al.*, 2012) in that population showing similarities with our study with higher prevalence of neuromuscular disorders followed by neurological disorders.

A study conducted in Azad Jammu and Kashmir about the prevalence pattern of congenital anomalies indicates that most found anomalies were limb disorders (42.75/1000) and sensorineural defects (4/1000) which might be due to easily diagnosable physical features (Jabeen and Malik, 2014), whereas current study show results having sensorineural defects (114/500) and Limb disorders (57/500).

In these data, parental consanguineous marriages were higher than non-consanguineous, sporadic cases were higher than familial, and males were affected more than females. A study conducted on hereditary anomalies reported a higher ratio of male subjects (60%) as compared to females (40%) affected with anomalies (Ochoga *et al.*, 2018). Another research clinical study on disabled patients observed in Assam, India, resulted in an abundant ratio of affected males (58%) to affected females (42%) comparatively (Baruah *et al.*, 2019). In the congenital malformations study of prevalence in Egyptian children, results express a higher number of affected males than females (Shawky and Sadik, 2011). In epidemiological study of limbs and musculoskeletal disorders in Chitral, results in more affected males than females in a ratio of 2:1 (Ullah *et al.*, 2015). In previous studies, it is proved by evidence that males lead the anomalies than females which may be due to nature of the population, it is difficult to communicate with females due to certain ethical reasons.

A contrast of result is found in ratio of affected isolated to syndromic nature of cases in comparison with previously conducted studies on the prevalence of congenital anomalies. Most of the cases (n=282) out of 500 were syndromic and lesser number of affected subjects (n=218) were isolated in nature. In neuromuscular disorders and neurological disorders, syndromic cases were (n=103) and (n=89) as compared to (n=21) and (n=45) isolated cases respectively. In a study of congenital anomalies reported number of isolated cases was higher (n=37) as compared to only (n=4) syndromic cases (Najmabdi *et al.,* 2011). This sort of contrast could be mainly due to highest prevalence of neuromuscular disorders and neurological defects which have syndromic symptoms.

This study depicts, congenital anomalies were analyzed based on age groups of the subjects in which most common anomalies show a prevalence of 45% (n=224) including both males and females in age group of 9-19 years, followed by second highest prevalence of 28% (n=138) by age category of 0-9 years and lowest by age category 29-39 years with 6.2% (n=31). This study shows similarity in results, with previous studies on the prevalence of congenital and hereditary anomalies. A study previously conducted, shows results with most of subjects having age group of up to 17 years (Taye *et al.,* 2019). Similarly, another study in Sialkot, indicates that the majority of subjects fall in the category of age group of 9-19 years (Bhatti *et al.,* 2019). Another study reported that the majority of subjects have age category of 10-19 years (Zahra *et al.,* 2016).

These data when analyzed on basis of socio-economic status of families with congenital anomalies, resulted in low category contributing 80% (n=398) of total 500 cases, followed by mid category with 18% (n=88) cases and few number of cases (n=14) in high category. A study results show majority of families in mid category with 49% cases and low-income families with 43% anomalies (Taye *et al.,* 2019). Both these studies show a contrast due to occupational variety of studied populations.

In current study, analysis was performed on parity basis, resulting in cases with first parity order (30%, n=151) followed by second parity order (25%, n=100) and third (13%, n=63). Another study of congenital anomalies showed similar results having the highest first parity order (31%) followed by second parity order with contribution (18%) in total data (Mahela, 2016). A study conducted in Chitral about congenital limbs defects suggests that most of the subjects belonged to first parity (43%) (Ullah *et al.,* 2015). The results of these studies were consistent with each other.

Recruited subjects in this study, when analyzed on basis of disease segregation among different generations, results indicated that most of the cases segregate in one generation (n=411) including both familial (25.8%, n=129) and sporadic cases (56.4%, n=282), followed by familial cases with two generations (16.6%, n=83) and higher generations (1.2%, n=6). A previous study also indicates that most of the cases segregate in one generation (Zahra *et al.,* 2016), indicating similarity in results of both studies.

Consanguinity is a major causative factor for congenital and hereditary anomalies prevailing in this population. In recruited 500 cases, parental consanguinity is estimated to be 81% (n=404) including 46% (n=186) familial and 54% (n=218) sporadic cases, while non-consanguineous marriages contribute 19% (n=96) cases in total data comprising of familial (67%, n=64) and sporadic (33%, n=32) cases. There is a statistically non-significant difference between both types of marriage unions (p=0.668) in all major categories of congenital and hereditary anomalies (Bhatti *et al.,* 2019). Findings of another previous study, show that difference between consanguineous and non-consanguineous marriages was statistically non-significant but consanguineous marriages were more common (Zahra *et al.,* 2016). A previous study in Oman showed that mortality rate of newborn with congenital anomalies was significantly associated with high parental consanguinity such as higher association of intellectual disability in

children (Rajab *et al.,* 2014). There is a major role of parental consanguinity in causing autosomal or sex-linked recessive disorders in offspring and consanguineous marriages are considered as common practice in Middle East (Hudgin *et al.,* 2006). In Pakistan, as estimated more than 80% of parents are first cousins, followed by blood relatives (7%), intra-caste marriages (6%) and a lower ratio (4%) of inter-caste marriages (Akram *et al.,* 2008; Ullah *et al.,* 2017). As parental consanguinity is not same in all studies of congenital and hereditary disorders which indicates the influence of environmental factors in the causation of genetic disorders in the studied population.

The prevalence of affected subjects in each category was divergent because of different populations and varying methods of study. Some of the studies have been conducted in Hospitals while this study was purely field-based (door-to-door survey) to get actual prevalence of congenital and hereditary anomalies throughout a specific population. Results of these studies cannot be compared with those conducted in developed countries with collaborative and high standard surveillance system. The fluctuation in figures may also be due to population specific risk factors like geographical distribution, ethnicity, socio-demographic status, maternal health, nutritional requirements, and consanguinity.

Certain anomalies were present with low representation in our study because such anomalies are less in nature and their diagnosis require complete medical examination and tests. This study was of prospective nature and all efforts were made to include all maximum anomalies prevailing in the studied population. In this study, some severe anomalies causing prenatal and postnatal deaths might be less in ratio as compared to data ascertained from hospitals with proper record management. This study might reflect bias in ascertainment because this survey was conducted in Tehsil Layyah and tried to

ascertain all villages in a consecutive way to get actual estimate of the prevalence of congenital and hereditary anomalies throughout the District Layyah.

This study is a pioneer study because conducted for the first time in this population of District Layyah in which anomalies subject belong to every age group and gender without any discrimination of ascertainment. Most of the demographic parameters were studied in detail to understand their relevance with the genetic basis of anomaly. Clinical parameters, test reports, and family history of the anomaly were explained with help of pedigree construction including an indication of consanguinity in this study.

Some myths and superstitious beliefs also prevail in the population due to a lack of awareness and education in some areas of the population. Families were found to believe that whatever disability is, it is only by Allah and there is no need to understand and go for medical examinations because it was due to some of their acts in the past. They believed that Allah is the supreme power and there is no existence of genetic patterns of disorders like congenital anomalies. Such beliefs were like hurdles to have their consent and perform research survey in those areas with ethical manners.

4.1 Future Direction

Differential diagnosis of genetic disorders is one of the most difficult aspects of clinical practice because clinical symptoms often overlap with each other. For instance, afflicted subjects with neuropsychiatric disorders (Autism, Parkinson's, and Schizophrenia), have a variety of clinical phenotypes linked to a gene, environment, hormonal and metabolic defects. On the other hand, muscular and neurological deformities, often overlap due to similar features.

There is no single definitive diagnosis for these disorders, a variety of collective modalities, to assist and reduce the possibilities, examinations including nerve

conduction, electrophysiological, brain imaging, psychological, and neurophysiologic exams, as well as genetic testing, might be used.

The clinical presentations of muscular dystrophies are also varied, with early muscle weakness, involvement of calf hypertrophy, myalgia, and cognitive dysfunction. Visual impairments are often clinically heterogeneous, making these dystrophic sub-types. Diagnosis is generally dependent on the patient's previous medical record. Because they are clinically undifferentiated in the early stages, it is crucial to distinguish between stationary and progressive kinds of retinal dystrophy when making a diagnosis. Therefore, the progression of the disease is measured by regular check-ups by a consultant physician. In addition, modern advanced and state of the art techniques such as MRI, CT-scan, ERG and SD-OCT, which are not routinely performed in most clinics to provide insight into the nature of the disease, are needed. To make an accurate diagnosis at the molecular level in the Pakistani environment, suitable clinical facilities must be built.

4.2 Genetic Testing in Pakistan

Many people suffer from recessive form of the disease, especially in rural areas of populated developing South Asian countries such as Pakistan, India, Bangladesh, and Afghanistan. Inbreeding is primarily caused by close marriages, which are more common in Muslim populations since people live in small clusters and are divided into tribes (Gilani *et al.,* 2007; Riaz *et al.,* 2019). Consanguinity is one of the major contributing factors to the high prevalence of recessive disorders in these populations in general (Riaz *et al.,* 2019).

In addition, a lack of funding and poor healthcare facilities (for differential diagnosis and possible clinical treatment options) lead to a rise in the incidence of

numerous neurological, muscular, and retinal dystrophies and other conditions that, while not curable, can be treated. Marriages between two individuals with related disease entities are another occurrence in such high-burden populations (e.g., with mental retardation, thalassemia and sensorineural defects) (Gilani *et al.*, 2007). When a recessive pattern of genetic defects occurs, all the progeny is affected, increasing the disease burden. Moreover, the lack of education among members of such families exacerbates the effects of economic conditions. The above details help to explain why developing countries like Pakistan have such a large number of patients. If we want to reduce the incidence of these diseases in our country, as seen in developing countries around the globe, we must implement a national genetic testing policy method.

Few academic researchers are focusing on various aspects of Pakistan's heterogeneous groups of genetic defects, with the majority of them focusing on identifying the genetic changes that cause these diseases. In comparison to other developing countries, it appears that none of the groups is interested in the systematic provision of genetic testing and prenatal diagnosis to patients, based on published research results. It is recommended that the organizations must use their resources to reduce the risk of disease in our patients.

4.3 Genetic counseling

Children and families of hereditary illnesses are enrolled for genetic testing to establish the genetic origin of diseases. After the causative mutations have been identified, the implications can be addressed with the affected relatives, who can be advised on how to avoid further diseased offspring in the family. In addition, subjects should also be forwarded to a consultant for appropriate treatment and therapy through clinical intervention. Accurate diagnosis and appropriate genetic testing are needed for

genetic therapy. Counselling may be very effective tool to established genotype-phenotype relationships. As a result, doctors and geneticists who work with such families should be professionally qualified as genetic counselors. It should be noted that genetic counseling in families with de novo mutations is a difficult job for genetic counselors due to the lack of family background and the assumption that the parents are stable and do not bear the disease-causing mutations.

Genetic counseling facilities are limited in Pakistan, and the prevalence of genetic disorders is not adequately addressed due to the cultural pattern of consanguineous marriages. As a result, the number of people affected is growing every day. Furthermore, there is no established prenatal diagnosis service for families with severely afflicted subjects in Pakistan, genetic counseling programs must be developed immediately to inform affected families about the benefits of such services.

A high incidence of genetic disorders places a significant economic burden on families as well as the country's economy. Since a high percentage (%) of the population lives in poverty, the majority of the suffering families are incapable to afford care and rehabilitation for their affected siblings, and full regaining is hardly possible. Individual with severe genetic illness are marginalized in society and have limited access to health services, education, and personal growth opportunities. For the sake of those families, it is recommended that the Ministry of Health Department must take urgent steps to avoid the recurrence of genetic disorders, such as.

- Launch public awareness campaigns through electronic media about the connection between close marriages and birth defects.

- A qualified medical practitioner should provide free prenatal testing and screening to people in state hospitals to prevent the recurrence of disease in coming generations.
- In the region, there are only a few research institutions that conduct relevant research. For planned research in this field, more institutions and funding should be provided.
- Simultaneously, international agreements should be established to conduct advanced research so that Next Generation Sequencing (NGS) technologies can be developed in the country soon.

4.4 Conclusion

The clinical and genetic epidemiological study of congenital and hereditary anomalies provides a descriptive status of anomalies (n=500) and their possible causation factors in the population of District Layyah for the first time. The neuromuscular disorders and neurological defects showed the highest prevalence in the population followed by sensorineural defects and limb disorders added with more sporadic nature as compared to familial cases and supported by the highest rate of parental consanguinity concluding that there is more influence of genetic-based factors as compared to environmental causes. Moreover, an improved health-care system and proper genetic counseling can reduce these anomalies to a large extent. Due to limitations of study period and resources, this study was confined to some specified areas of Layyah giving an initial overview of the congenital anomalies in the population and possible risk factors of major cause.

Further, large-scale studies with extensive time periods and resources should be conducted in the region to get high level of prevalence of congenital anomalies and develop strategies to minimize their effect on the population.

Chapter 5

References

Akram, D. S., Arif, F., & Fayyaz, J. F. (2008). How frequent are consanguineous marriages?. Journal of the Dow University of Health Sciences (JDUHS), 2(2), 76-79.

Ashizawa, T., & Xia, G. (2016). Ataxia. Continuum: Lifelong Learning in Neurology, 22(4 Movement Disorders), 1208.

Baertling, F., Rodenburg, R. J., Schaper, J., Smeitink, J. A., Koopman, W. J., Mayatepek, E., ... & Distelmaier, F. (2014). A guide to diagnosis and treatment of Leigh syndrome. Journal of Neurology, Neurosurgery & Psychiatry, 85(3), 257-265.

Bale, J. R., Stoll, B. J., & Lucas, A. O. (2003). Reducing birth defects: meeting the challenge in the developing world. Washington: National Academies Press.

Barbe, Mary F; Gallagher, Sean; Massicotte, Vicky S; Tytell, Michael; Popoff, Steven N; Barr-Gillespie, Ann E (2013). The interaction of force and repetition on musculoskeletal and neural tissue responses and sensorimotor behavior in a rat model of work-related musculoskeletal disorders. BMC Musculoskeletal Disorders. 14: 303. doi:10.1186/1471-2474-14-303.

Baruah, M., Das, R. K., Vishwakarma, D., & Malakar, A. J. (2019). A clinical study of patients attending disability clinic in a tertiary care hospital of Assam, India. International Journal of Research in Medical Sciences, 7(5), 1572.

Bhatti, N. A., Mumtaz, S., & Malik, S. (2019). Epidemiological study of congenital and hereditary anomalies in Sialkot District of Pakistan revealed a high incidence of limb and neurological disorders. Asian Biomedicine, 13(2), 49-60.

Birnkrant, D. J., Bushby, K., Bann, C. M., Apkon, S. D., Blackwell, A., Brumbaugh, D., ... & DMD Care Considerations Working Group. (2018). Diagnosis and management of Duchenne muscular dystrophy, part 1: diagnosis, and neuromuscular, rehabilitation, endocrine, and gastrointestinal and nutritional management. The Lancet Neurology, 17(3), 251-267.

Blackburn, S., Maternal, Fetal, & neonatal physiology-E-book: a clinical perspective. 2017: Elsevier Health Sciences.

Boudewyns, A., Declau, F., Van den Ende, J., Van Kerschaver, E., Dirckx, S., Hofkens-Van den Brandt, A., & Van de Heyning, P. (2011). Otitis media with effusion: an underestimated cause of hearing loss in infants. Otology & Neurotology, 32(5), 799-804.

Bowman, R. M., McLone, D. G., Grant, J. A., Tomita, T., & Ito, J. A. (2001). Spina bifida outcome: a 25-year prospective. Pediatric neurosurgery, 34(3), 114-120.

Boyd, A.S. (2021, December 3). Congenital Limb Abnormalities. MSD MANUAL Professional-version. https://www.msdmanuals.com/professional/pediatrics/congenital-craniofacial-and-musculoskeletal-abnormalities/congenital-limb-abnormalities.

Bull, M. J. (2020). Down syndrome. New England Journal of Medicine, 382(24), 2344-2352.

Camfield, C. S., Striano, P., & Camfield, P. R. (2013). Epidemiology of juvenile myoclonic epilepsy. Epilepsy & Behavior, 28, S15-S17.

Cassandrini, D., Trovato, R., Rubegni, A., Lenzi, S., Fiorillo, C., Baldacci, J., ... & Santorelli, F. M. (2017). Congenital myopathies: clinical phenotypes and new diagnostic tools. Italian Journal of Pediatrics, 43(1), 1-16.

Castillo-Lancellotti, C., Tur, J. A., & Uauy, R. (2013). Impact of folic acid fortification of flour on neural tube defects: a systematic review. Public Health Nutrition, 16(5), 901-911.

Census 2017, Pakistan.

Center for Disease Control and Prevention (CDC)

Copp, A. J., Stanier, P., & Greene, N. D. (2020). Genetic basis of neural tube defects. Textbook of Pediatric Neurosurgery, 2275-2294.

Cote, Julie N.; Ngomo, Suzy; Stock, Susan; Messing, Kisn; Vézina, Nicole; Antle, David; Delisle, Alain; Bellemare, Marie; Laberge, Marie; St-Vincent, Marie (2013). "Quebec Research on Work-related Musculoskeletal Disorders". Relations Industrielles. 68 (4): 643. doi:10.7202/1023009ar.

Dany, A., Rapin, A., Réveillère, C., Calmus, A., Tiffreau, V., Morrone, I., ... & Boyer, F. C. (2017). Exploring quality of life in people with slowly-progressive neuromuscular disease. Disability and rehabilitation, 39(13), 1262-1270.

DeSilva, M., Munoz, F. M., Mcmillan, M., Kawai, A. T., Marshall, H., Macartney, K. K., ... & Brighton Collaboration Congenital Anomalies Working Group. (2016). Congenital anomalies: Case definition and guidelines for data collection, analysis, and presentation of immunization safety data. Vaccine, 34(49), 6015.

Dobyns, W. B., Filauro, A., Tomson, B. N., Chan, A. S., Ho, A. W., Ting, N. T., ... & Ober, C. (2004). Inheritance of most X-linked traits is not dominant or recessive, just X-linked. American Journal of Medical Genetics Part A, 129(2), 136-143.

Dolk, H., Loane, M., & Garne, E. (2010). The prevalence of congenital anomalies in Europe. Rare Diseases Epidemiology, 349-364.

Duan, D., Goemans, N., Takeda, S. I., Mercuri, E., & Aartsma-Rus, A. (2021). Duchenne muscular dystrophy. Nature Reviews Disease Primers, 7(1), 1-19.

Dutta, S., Human teratogens and their effects: a critical evaluation. IJIRR, 2015. 2(3): p. 525-536.

Emery, A. E. (2002). The muscular dystrophies. The Lancet, 359(9307), 687-695.

Feldkamp, M. L., Carey, J. C., Byrne, J. L., Krikov, S., & Botto, L. D. (2017). Etiology and clinical presentation of birth defects: population based study. British Medical Journal, 357.

Ferner, R. E., & Gutmann, D. H. (2013). Neurofibromatosis type 1 (NF1): diagnosis and management. Handbook of Clinical Neurology, 115, 939-955.

Francine, R., S. Pascale, and H. Aline, Congenital anomalies: prevalence and risk factors. Mortality, 2014. 1: p. 2.

Gatchel, R. J., & Kishino, N. (2011). Pain, musculoskeletal injuries, and return to work. In J. C. Quick & L. E. Tetrick (Eds.), Handbook of Occupational Health Psychology (2nd ed.). Washington, DC: American Psychological Association.

Gilani, A. I., Jadoon, A. S., Qaiser, R., Nasim, S., Meraj, R., Nasir, N., ... & Ahmad, U. (2007). Attitudes towards genetic diagnosis in Pakistan: a survey of medical and

legal communities and parents of thalassemic children. Public Health Genomics, 10(3), 140-146.

Gilissen, C., Hehir-Kwa, J. Y., Thung, D. T., van de Vorst, M., van Bon, B. W., Willemsen, M. H., & Veltman, J. A. (2014). Genome sequencing identifies major causes of severe intellectual disability. Nature, 511(7509), 344-347.

Goodway, J. D., Ozmun, J. C., & Gallahue, D. L. (2019). Understanding motor development: Infants, children, adolescents, adults. Jones & Bartlett Learning.

Grosse, S. D., Ross, D. S., & Dollard, S. C. (2008). Congenital cytomegalovirus (CMV) infection as a cause of permanent bilateral hearing loss: a quantitative assessment. Journal of Clinical Virology, 41(2), 57-62.

Hagerman, R. J., Berry-Kravis, E., Hazlett, H. C., Bailey, D. B., Moine, H., Kooy, R. F., ... & Hagerman, P. J. (2017). Fragile X syndrome. Nature reviews Disease primers, 3(1), 1-19.

Hartong, D. T., Berson, E. L., & Dryja, T. P. (2006). Retinitis pigmentosa. The Lancet, 368(9549), 1795-1809.

Hemonta, D., & Giriraj, K. (2010). Congenital malformations in Assam. Journal of Indian Association of Pediatric Surgeons, 15(2), 53.

History of District Layyah from https://layyah.dc.lhc.gov.pk

Hossain, M. D., Ahmed, H. U., Jalal Uddin, M. M., Chowdhury, W. A., Iqbal, M. S., Kabir, R. I., ... & Sarker, M. (2017). Autism Spectrum disorders (ASD) in South Asia: a systematic review. BMC Psychiatry, 17(1), 1-7.

Hudgin L, Cassidy SB. Congenital anomalies. In: Marten RJ, Fanaroff AA, Walsh MC, editors. Neonatal – perinatal medicine. Philadelphia: Mosby – Elsevier; 2006. 561–86.

ICD. International Classification of Disease. (2021). Retrieved 10 October, 2021, from https://icd.who.int/browse10/2019/en#/G80.3

Jabeen, N., & Malik, S. (2014). Prevalence of congenital anomalies and non-communicable diseases in women of age 12-75 years in District Bhimber, Azad Jammu and Kashmir, Pakistan. Iranian Journal of Public Health, 43(1), 42.

Jehangir, W., Ali, F., Jahangir, T., & Masood, M. S. (2009). Prevalence of gross congenital malformations at birth in the neonates in a tertiary care hospital. Annals of Punjab Medical College (APMC), 3(1), 47-50.

Kancherla, V., Wagh, K., Johnson, Q., & Oakley Jr, G. P. (2018). A 2017 global update on folic acid-preventable spina bifida and anencephaly. Birth Defects Research, 110(14), 1139-1147.

Khan, A. A., Khattak, T. A., Shah, S. H. A., Roshan, E., & Haq, A. U. (2012). Pattern of congenital anomalies in the newborn. Journal of Rawalpindi Medical College, 16(2).

Korejo, R., Bhutta, S., Noorani, K. J., & Bhutta, Z. A. (2007). An audit and trends of perinatal mortality at the Jinnah Postgraduate Medical Centre, Karachi. Parity, 31(40), 40.

Krigger, K. W. (2006). Cerebral palsy: an overview. American Family Physician, 73(1), 91-100.

Kumaraveloo, K Sakthiaseelan; LunnerKolstrup, Christina (3 July 2018). "Agriculture and musculoskeletal disorders in low- and middle-income countries". Journal of Agromedicine. 23 (3): 227–248. doi:10.1080/1059924X.2018.1458671. PMID 30047854. S2CID 51719997.

Langah, A., Hussain, A., Baig, S., Riffat, S., Qureshi, J. A., & Afreen, U. (2022). Prevalence of Congenital Birth Defects among Pediatric Patients of Interior Punjab. Pakistan Journal of Medical & Health Sciences, 16(05), 273-273.

Levy, Y. (2018). 'Developmental delay' reconsidered: The critical role of age-dependent, co-variant development. Frontiers in Psychology, 9, 503.

Mah, J. K., Selby, K., Campbell, C., Nadeau, A., Tarnopolsky, M., McCormick, A., ... & Yoon, G. (2011). A population-based study of dystrophin mutations in Canada. Canadian Journal of Neurological Sciences, 38(3), 465-474.

Mahela, S., & Talukdar, B. (2016). Prevalence of congenital abnormalities on routine ultrasound scan of second and third trimester pregnancy. International Journal of Reproduction, Contraception, Obstetrics and Gynecology, 5(1), 182-186.

Mandal, A. (2021 December 7). What is visual impairment?. NEWS Medical Life Sciences. https://www.news-medical.net/health/What-is-visual-impairment.aspx.

Martins, L. M., Camargos, P. A., Becker, H. M., Becker, C. G., & Guimarães, R. E. (2010). Hearing loss in cystic fibrosis. International Journal of Pediatric Otorhinolaryngology, 74(5), 469-473.

Maulik, P. K., Mascarenhas, M. N., Mathers, C. D., Dua, T., & Saxena, S. (2011). Prevalence of intellectual disability: a meta-analysis of population-based studies. Research in developmental disabilities, 32(2), 419-436.

McClelland, C., Manousakis, G., & Lee, M. S. (2016). Progressive external ophthalmoplegia. Current neurology and neuroscience reports, 16(6), 1-10.

McDonald, C. M. (2012). Clinical approach to the diagnostic evaluation of hereditary and acquired neuromuscular diseases. Physical Medicine and Rehabilitation Clinics, 23(3), 495-563.

Mercuri, E., & Muntoni, F. (2013). Muscular dystrophy: new challenges and review of the current clinical trials. Current opinion in pediatrics, 25(6), 701-707.

Mirkin, S. M. (2006). DNA structures, repeat expansions and human hereditary disorders. Current opinion in structural biology, 16(3), 351-358.

Mishra SD, Sarkar K (January 2021). "Work-related musculoskeletal disorders and associated risk factors among urban metropolitan hairdressers in India". Journal of Occupational Health. 63 (1): e12200. doi:10.1002/1348-9585.12200. PMC 7883474. PMID 33586840.

Mitchell, L. E., Adzick, N. S., Melchionne, J., Pasquariello, P. S., Sutton, L. N., & Whitehead, A. S. (2004). Spina bifida. The Lancet, 364(9448), 1885-1895.

Mogra, R., V. Zidere, and L. Allan, prenatally detectable congenital heart defects in fetuses with Down syndrome. Ultrasound in obstetrics & gynecology, 2011. 38(3): p. 320-324.

Mohammed, Y. A., Shawky, R. M., Soliman, A. S., & Ahmed, M. M. (2011). Chromosomal study in newborn infants with congenital anomalies in Assiut University hospital: Cross-sectional study. Egyptian Journal of Medical Human Genetics, 12(1).

Moorthie, S., Blencowe, H., Darlison, M. W., Lawn, J., Morris, J. K., Modell, B., ... & Yunnis, K. A. (2018). Estimating the birth prevalence and pregnancy outcomes of congenital malformations worldwide. Journal of community genetics, 9(4), 387-396.

Najmabadi, H., Hu, H., Garshasbi, M., Zemojtel, T., Abedini, S. S., Chen, W., ... & Ropers, H. (2011). Deep sequencing reveals 50 novel genes for recessive cognitive disorders. Nature, 478(7367), 57-63.

Ochoga, M., Tolough, G., Michael, A., Ikuren, I., Shogo, A., & Abah, R. (2018). Congenital Anomalies at Benue State University Teaching Hospital, Makurdi, Benue State: A Three-year Review. J Adv Med Med Res, 25(11), 1-7.

OMIM. Online Mendelian Inheritance in Man. (2021). Retrieved 17 September,2021, from https://www.omim.org/

Petersen, M. B., & Willems, P. J. (2006). Non-syndromic, autosomal-recessive deafness. Clinical genetics, 69(5), 371-392.

Rajab, A., Al Salmi, Q., Jaffer, J., Mohammed, A. J., & Patton, M. A. (2014). Congenital and genetic disorders in the Sultanate of Oman. First attempt to assess healthcare needs. Journal of community genetics, 5(3), 283-289.

Rasmussen, S. A., Erickson, J. D., Reef, S. E., & Ross, D. S. (2009). Teratology: from science to birth defects prevention. Birth Defects Research Part A: Clinical and Molecular Teratology, 85(1), 82-92.

Riaz, M., Tiller, J., Ajmal, M., Azam, M., Qamar, R., & Lacaze, P. (2019). Implementation of public health genomics in Pakistan. European Journal of Human Genetics, 27(10), 1485-1492.

Rizk, F., P. Salameh, and A. Hamadé, Congenital anomalies: prevalence and risk factors. Universal Journal of Public Health, 2014. 2(2): p. 58-63.

Salzberg, D. C., Mann, J. R., & McDermott, S. (2018). Differences in race and ethnicity in muscular dystrophy mortality rates for males under 40 years of age, 2006–2015. Neuroepidemiology, 50(3-4), 201-206.

Shamim, S., N. Chohan, and Q. Sobia, Pattern of congenital malformations and their neonatal outcome. Journal of Surgery Pakistan, 2010. 15(1): p. 34-7.

Sharp, D. (2005). Genetic epidemiology: strengths, weaknesses, and opportunities. The Lancet, 366(9489), 880.

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).

Sherman, S. L., Allen, E. G., Bean, L. H., & Freeman, S. B. (2007). Epidemiology of Down syndrome. Mental retardation and developmental disabilities research reviews, 13(3), 221-227.

Smith, G. D., Ebrahim, S., Lewis, S., Hansell, A. L., Palmer, L. J., & Burton, P. R. (2005). Genetic epidemiology and public health: hope, hype, and future prospects. The Lancet, 366(9495), 1484-1498.

Sproule, D. M., & Kaufmann, P. (2008). Mitochondrial encephalopathy, lactic acidosis, and strokelike episodes: basic concepts, clinical phenotype, and therapeutic management of MELAS syndrome. Annals of the New York Academy of Sciences, 1142(1), 133-158.

Taboo, Z. A. (2012). Prevalence and risk factors for congenital anomalies in Mosul city. The Iraqi Postgraduate Medical Journal, 11(4), 458-70.

Taye, M., Afework, M., Fantaye, W., Diro, E., & Worku, A. (2019). Congenital anomalies prevalence in Addis Ababa and the Amhara region, Ethiopia: a descriptive cross-sectional study. BMC pediatrics, 19(1), 1-11.

Taylor, F. (2001). National Institute of Neurological Disorders and Stroke (USA); Office of Science and Health Reports. Cerebral palsy: hope through research. Bethesda: MD Institute.

Thada, P. K., Bhandari, J., & Umapathi, K. K. (2021). Becker muscular dystrophy. In StatPearls [Internet]. StatPearls Publishing.

Thakur, K. T., Albanese, E., Giannakopoulos, P., Jette, N., Linde, M., Prince, M. J., ... & Dua, T. (2016). Neurological disorders. Mental, Neurological, and Substance Use Disorders, 87.

Ullah, M. A., Husseni, A. M., & Mahmood, S. U. (2017). Consanguineous marriages and their detrimental outcomes in Pakistan: an urgent need for appropriate measures. Int J Community Med Public Health, 5(1), 1-3.

Ullah, S., Dasti, J. I., & Malik, S. (2015). Descriptive epidemiology of hereditary musculoskeletal and limb defects in the isolated population of Chitral, North-West Pakistan. Pakistan Journal of Medical Sciences, 31(5), 1047.

Vasudevan, P., & Suri, M. (2017). A clinical approach to developmental delay and intellectual disability. Clinical medicine, 17(6), 558.

Vatankhah, S., Jalilvand, M., Sarkhosh, S., Azarmi, M., & Mohseni, M. (2017). Prevalence of congenital anomalies in Iran: A review article. Iranian journal of Public Health, 46(6), 733.

Verma, R. P. (2021). Evaluation and risk assessment of congenital anomalies in neonates. Children, 8(10), 862.

Wenger, T. L., McDonald-McGinn, D. M., & Zackai, E. H. (2014). Genetics of common congenital syndromes of the head and neck. In Congenital Malformations of the Head and Neck (pp. 1-22). Springer, New York, NY.

Wikstrom, A. M., & Dunkel, L. (2011). Klinefelter syndrome. Best practice & research clinical endocrinology & metabolism, 25(2), 239-250.

Williams, R. E. (2019). Neuronal Ceroid Lipofuscinoses. The Causes of Epilepsy: Diagnosis and Investigation, 352.

Worasak, Rueangsirarak; Jingtian, Zhang; Nauman, Aslam; Hubert P. H., Shum (2018). "Automatic Musculoskeletal and Neurological Disorder Diagnosis with Relative Joint Displacement from Human Gait". IEEE Transactions on Neural Systems and Rehabilitation Engineering. 26(12): 2387–2396.

World Health Organization (WHO).

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