Application of Genetics in Clinical Practice

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Abstract

Since the completion of the human genome project our understanding of the genes, and their function in various pathophysiological conditions, has advanced and revolutionized the medical field. From a simple chromosome study to complex DNA sequencing, array CHG, and Next-Generation sequencing technology has made it possible to diagnose many rare diseases which were thought of as nonexistence or difficult to diagnose. And many of these disorders can be treated if diagnosed early. The burden of genetic disorders seems to be high in India likely to be due to consanguinity practiced in many communities. The present article is a basic understanding of the field of genetics and when to suspect and, how to diagnose and manage in day-to-day clinical practice.

Keywords: Genes, DNA, NGS, DNA sequencing, chromosome

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Introduction

In recent years, due to increasing awareness of genetic disorders and the possibility of personalized medicine and treatment for many genetic disorders, there has been a growing interest in the field of genetics. With technological advancements and the introduction of high throughput techniques like next-generation sequencing (NGS), CGH microarray, fluorescent *in situ* hybridization (FISH) etc. in the last few decades,[1,2,3] a number of genetic disorders have been identified which makes it possible to provide a confirmed diagnosis to the family and clinician for better management and counseling. As more studies are aimed at deciphering the link between the genome and human health, there is certainly a significant role of genetics in medical practice. Here, we give a brief overview of the basic concepts in genetics and describe the common genetic disorders. We emphasize the importance of awareness among family physicians to address the challenges associated with these conditions.

Basic Concepts in Genetics

Deoxyribose nucleic acid (DNA) is the hereditary material in humans that encodes all the information necessary for the cell. It is composed of four nucleotide bases: adenine (A), guanine (G), cytosine (C) and thymine (T). Inside the cell, the DNA is arranged in structures called chromosomes. There are 46 chromosomes (23 pairs) in the human genome, 22 pairs of autosomes (numbered 1-22) and one pair of sex chromosomes (X and Y). The genetic information is transmitted from parents to offspring through chromosomes, as each parent contributes one member of each pair of chromosomes. The study of these chromosomes is

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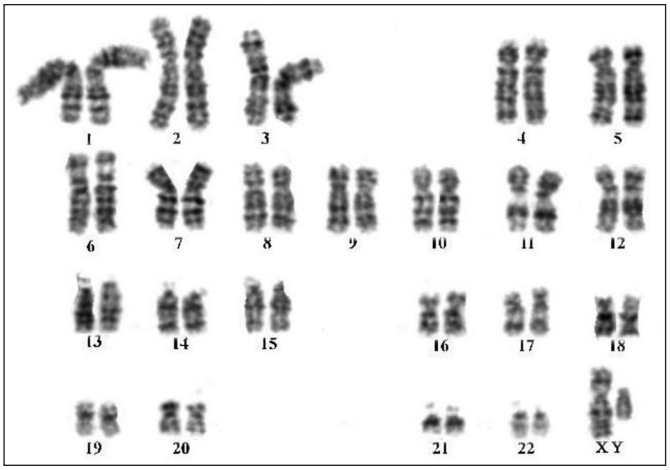

Figure 1: Chromosomal complement showing normal male karyotype-46, XY

called a karyotype which is the primary genetic investigation for common syndromes like Down syndrome, Klinefelter, Turner, Edward, Patau syndrome and any other genetic disorders that can be identified by chromosomal rearrangement (Figure 1).

A total of 6 billion base pairs comprises the human genome and approximately 21,000 genes are distributed across 23 pairs of chromosomes with an average composition of 500 to 1000 genes in each. Gene is the basic functional unit in human genetics. The simple definition of a gene is that it comprises a DNA sequence made up of four letters called nucleotides; A, T, G and C and can be divided into two regions: exons (protein-coding part) and introns (non-coding part) (Figure 2). This region is transcribed into messenger RNA (mRNA) and in turn, translated into a functional protein by the cellular machinery.

During the process of DNA replication, errors (e.g., incorporating the wrong nucleotide) may occur, but most of them are repaired by the dedicated repair machinery in the cell. Yet, some errors that persist get transmitted from generation to generation. This process that ultimately results in a change in the genetic

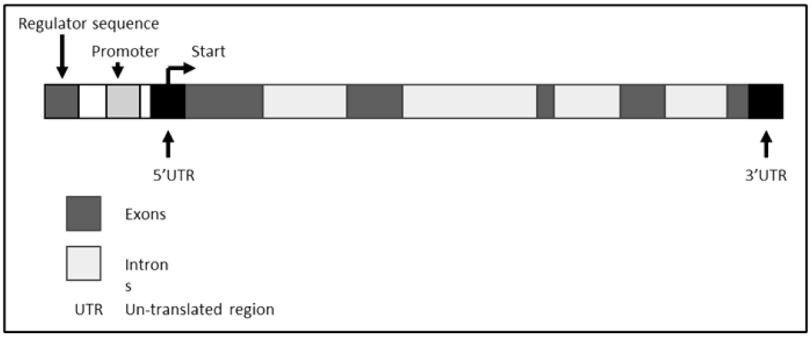

Figure 2: Basic structure of a gene

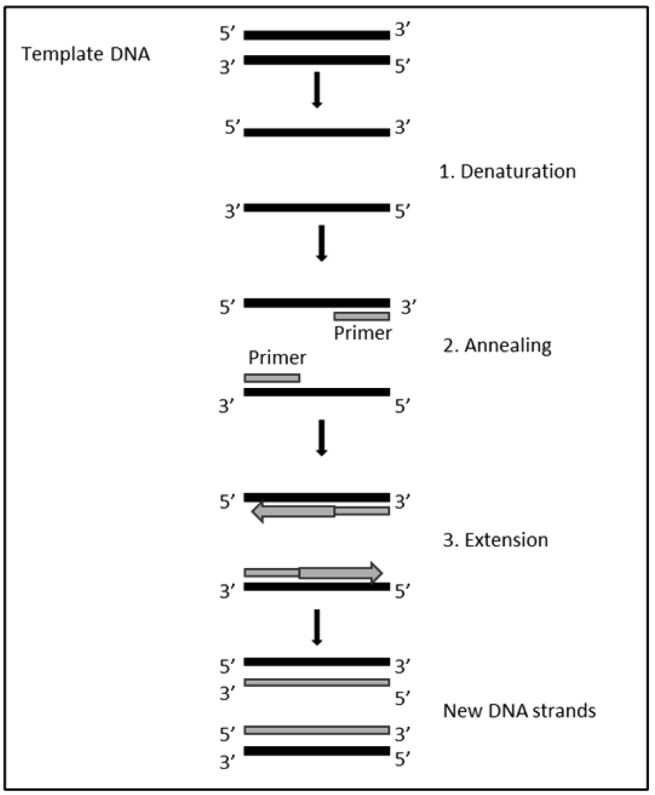

Figure 3: Polymerase chain reaction (PCR) and the steps involved.

material is defined as a mutation. A number of genetic disorders are a result of mutations in the gene that affects its normal functioning. It is now possible to identify these mutations with the help of various molecular techniques like PCR (Figure 3), Sanger sequencing, NGS etc. Likewise, molecular cytogenetic techniques like FISH (Figure 4) and microarray can aid in the detection of microdeletion or microduplication in the chromosomes.

PCR is a technique used to create multiple copies of target DNA. The key steps:

1. **Denaturation-** Template DNA is subjected to a high temperature to convert double-stranded DNA into a single-stranded form.
2. **Annealing-** In this step, short nucleotide sequences called primers bind to the complementary region on the template DNA.
3. **Extension-** with the help of the DNA polymerase enzyme, nucleotides complementary to the template strand are added, thus synthesizing a new DNA strand.

This 3-step process can be repeated for several cycles to produce multiple copies of

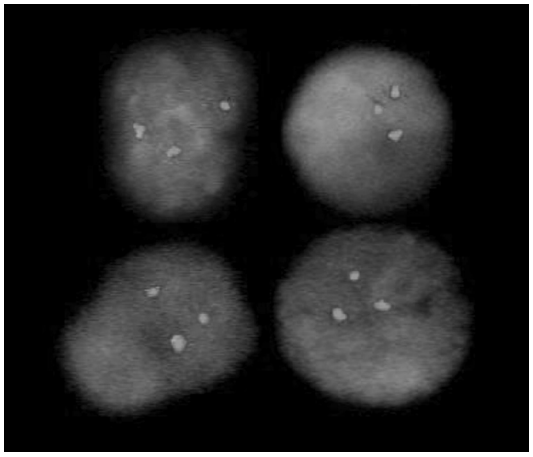

Figure 4: Interphase nuclei showing three orange signals for chromosome 21 in all cells indicating the presence of three copies of chromosome 21 (Down syndrome).

the original DNA.

Fluorescent *in situ* hybridization is a cytogenetic technique and is used for the detection of specific cytogenetic abnormalities such as chromosome number aberrations, chromosomal microdeletions etc. It involves the use of labeled DNA probes that attach to a high degree complementary parts of the chromosome and can emit colored signals.

No two individuals can share the same DNA sequence as there are different types of sequence variation that may occur anywhere in the human genome, within or outside of genes. Each human being has a unique DNA sequence due to polymorphic sites present in the DNA (about 1 in 1000 DNA bases is polymorphic). There are multiple types of DNA markers, the most common being single nucleotide polymorphisms (SNPs), which consist of single nucleotide change. Common SNPs are widely used in genetic association studies for common genetic disorders like thalassemia, sickle cell anemia, achondroplasia and other seven thousand genetic disorders. In addition to this, there are regions in the genome with repetitive sequences, called trinucleotide repeat sequence and are increasingly associated with human diseases like neurodevelopmental and neurodegenerative disorders, also called triple repeat expansion disorders.[4] The most common of these are Huntington, Fredrick ataxia, Fragile X, myotonic dystrophy and Spinocerebellar ataxia (SCA).

Human Genetic Disorders

Human genetic disorders are a result of a defect in the genetic material. These can be at the chromosomal level or at the DNA level. As a result of the defect, different processes inside the cell are impaired ultimately leading to a medical condition. These disorders can be broadly classified into three categories: chromosomal disorders, a single gene or mendelian disorders and metabolic disorders.

Chromosomal Disorders

These can occur either due to a change in the chromosome number or structure. Thus, they can be broadly classified into numerical abnormalities, structural abnormalities and abnormalities of the sex-chromosome. The most common disorder due to change in the chromosome number is Down syndrome (DS) (Figure 5A) with an incidence of ~1 in 800 live births. [3] In this case, there is an extra copy of chromosome number 21. The most common characteristic physical features are up-slanting palpebral fissures, single palmar crease, short stature, and hypotonia. In addition to this, neurological phenotypes such as intellectual disability, sleep apnea, seizures, behavioral problems and dementia are also visible in affected patients. In almost 95% of the DS cases, there is free trisomy i.e., an extra copy of chromosome

Figure 5A: Down syndrome patient. Key clinical indications are the flat round face, almond-shaped eyes, small nose, abnormally shaped ears.

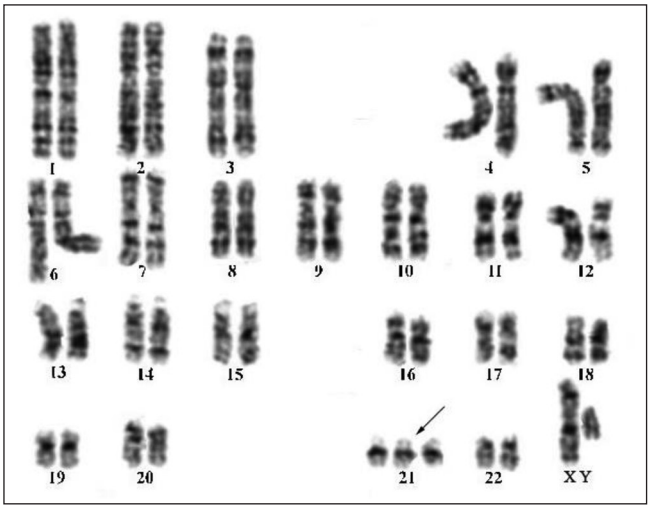

Figure 5B: Metaphase showing the presence of free trisomy 21 in a Down syndrome child, 47, XY, +21

21 (Figure 5B), whereas 3-4% of cases are because of translocations, in which there is a transfer of genetic material from one chromosome to another. Our study has shown a similar observation with 84.8% cases due to free trisomy and 8.9% due to translocations. [5] Karyotyping is the widely used test for the diagnosis of DS as well as other numerical aberrations. Other than trisomy 21, trisomy 18 (Edward syndrome) and trisomy 13 (Patau syndrome) are also regularly observed.

Structural chromosome rearrangement is another phenomenon that takes place during events of chromosome breakage and rejoining in different configurations. When such rearrangements affect the functioning of critical genes, there can be serious clinical effects. Translocation, inversion, deletion and duplication are the different types of chromosomal rearrangements that take place.

In case of abnormalities of sex chromosomes, there is either loss or gain of sex chromosome. For instance, in the Turner syndrome, there is the presence of a single X chromosome (45, X). They are phenotypically female but they show clinical features like short stature, webbed neck, and there is incomplete or no development of secondary sex characteristics, leading to infertility. In contrast, Klinefelter syndrome (KS) that occurs in males shows the presence of an extra X chromosome (47, XXY). In the majority of cases, these conditions are diagnosed later in adulthood as the clinical indications become pronounced and can be picked up by clinicians. Generally, males showing any one of the following indications are suspected for KS: genital abnormalities at birth, tall stature, incomplete puberty, learning difficulties in adolescence or adult males being evaluated for infertility (~3% infertile males have KS). Even then, almost two-thirds of KS cases may remain undiagnosed, the major reason being variable phenotype and many cases having subtle findings. Table 1 gives an overview of the common genetic disorders in India, their prevalence and the key clinical features observed.

Single Gene or Mendelian Disorders

They are considered to be rare compared to other genetic disorders with an incidence of 4-5 per 1000 live births. [6] As the name suggests these disorders are a result of a mutation in a single gene and the disease follows the Mendelian pattern of inheritance. Disease or trait which is determined by a gene on an autosome is referred to as an autosomal inherited condition. Whereas when it is due to a gene on a sex chromosome, it is known to show sex-linked inheritance. When a disease can manifest in the presence of one copy of a mutant gene, it is referred to as an autosomal dominant condition. However, autosomal recessive disorders occur when both the copies of a gene are mutated.

Some of the most frequent and well-known monogenic disorders include β-thalassemia, sickle cell anemia, Duchenne muscular dystrophy (DMD), cystic fibrosis, spinal muscular atrophy (SMA) and several metabolic syndromes (e.g., phenylketonuria, G6PD deficiency etc.). Notably, there is an increased burden of these disorders in the present context in India owing to the high proportion of consanguinity and endogamy seen in several Indian communities. A number of independent studies have identified the common mutation spectrum for these single-gene disorders in the Indian population. As most of these disorders present in early childhood, awareness and knowledge about these disorders among physicians, especially pediatricians are critical in order to pick up the early signs and provide an accurate diagnosis. [7,8,9]

Beta-thalassemia is a common autosomal recessive disorder in India, with an estimate of ~10,000-20,000 affected children born every year.[10] It is caused due to reduced or no expression of beta-globin genes. There are 3 main types depending on the disease severity: major, intermediate and minor, major being the most severe. In thalassemia major children, there is a mutation on both the copies of beta-globin genes. They are normal at birth but develop severe anemia subsequently. To compensate for the falling hemoglobin and RBC levels, repeated blood transfusion is essential. A varying set of mutations in the *HBB* gene have been identified among different ethnic groups in India. For instance, in Gujarat, our study showed IVS-I-5, 619 bp deletion, codons 41/42, codons 8/9 and IVS-I-1 (G>T) to be the most common mutations for β-thalassemia.[11] Beta-thalassemia intermedia individuals may or may not show any symptoms. In the case of beta-thalassemia minor cases, the mutation is present only on one copy of the *HBB* gene, hence they are carriers and no treatment is required. Among other common monogenic disorders, cystic fibrosis is a lethal genetic disease that primarily affects the lungs and pancreas and less frequently other organs like the liver, kidney, intestine and male reproductive organs. It is a result of abnormal transport of chloride ions across the apical membranes of epithelial cells due to pathogenic mutation in the *CFTR* gene. The most common pathogenic variant reported in almost 70% of the affected Caucasian individuals is p. Phe508del, [12] however, in addition to this, several other *CFTR* gene mutations have been identi-

Table 1: Overview of the common genetic disorders in India, their prevalence and the key clinical characteristics

Name of disorder (chromosome/gene involved)	Estimated incidence (world)	Prevalence in India	Key clinical indications
Down syndrome (Trisomy 21)	1:800	1 in 750-1000	Mild to moderate intellectual disability, characteristic facial appearance, weak muscle tone, heart defects, digestive abnormalities, hypothyroidism, increased risk of hearing and vision problems, leukemia, Alzheimer's disease
Edwards syndrome (Trisomy 18)	1:5000	Unknown	Intrauterine growth retardation, low birth weight, heart defects and abnormalities of other organs, small, abnormally shaped head, small jaw and mouth, clenched fists, severe intellectual disability
Patau syndrome (Trisomy 13)	1:16000	Unknown	Severe intellectual disability, heart defects, brain or spinal cord abnormalities, small or poorly developed eyes, extra fingers or toes, cleft lip and palate, weak muscle tone
Turner syndrome (45, X)	1:2500	Unknown	Short stature, early loss of ovarian function, infertility, absence of puberty, webbing of the neck, skeletal abnormalities, kidney problems, heart defects
Klinefelter syndrome (47,XXY)	1:500-1:1000	Unknown	Small testes, low testosterone levels, delayed and incomplete puberty, breast enlargement, reduced facial and body hair, infertility, increased height, increased risk of breast cancer, learning disabilities, delayed speech and language development
Williams syndrome (7q11.3)	1:7500	Unknown	Distinctive elfin facies, including periorbital puffiness, full lips and cheeks, and a bulbous nasal tip; cardiovascular abnormalities, mainly supravalvular aortic stenosis (SVAS) and peripheral pulmonary stenosis (PPS), friendly social personality, endocrine abnormalities, mild to moderate mental retardation
Cri-du-chat syndrome (5p15.2)	1:15,000-1 in 50,000	Unknown	Microcephaly, hypertelorism, epicanthic folds, micrognathia, broad nasal bridge, low set ears, downturned corners of the mouth and severe psychomotor retardation. A high-pitched cat-like cry probably caused by an abnormal larynx or epiglottis, is one of the most characteristic features
Beta- thalassemia (*HBB*)	1:3,500	1 in 30	Severe anemia, failure to thrive, recurrent bouts of fever, diarrhea, splenomegaly
Cystic fibrosis (*CFTR*)	1:500-1:50,000	1 in 1,00,323	Failure to thrive, chronic bronchopulmonary infection, pancreatic insufficiency, high sweat sodium and chloride
Duchenne muscular dystrophy (*DMD*)	1:3500-5000 newborn males	30 in 100,000	Scoliosis, flexion contractures, calf muscle pseudohypertrophy, hypotonia, positive Gowers sign, waddling gait, high serum creatine kinase

contd...

Spinal muscular atrophy (*SMN1*)	1-2:100,000	Unknown	Respiratory failure, muscle weakness and atrophy, EMG shows neurogenic abnormalities
Phenylketonuria (*PAH*)	1:18,300	1 in 68	Microcephaly, cataract, dry skin
Gaucher disease (*GBA*)	1:50,000	Unknown	Pulmonary hypertension, hepatosplenomegaly, interstitial lung disease, bone pain, anemia, high plasma chitotriosidase
Tay-Sachs disease (*HEXA*)	1:100,000	Unknown	Increased startle response, hypotonia, cherry red spot of the macula, seizures
MPS II (*IDS*)	1:100,000	Unknown	Macrocephaly, coarse facies, short neck, hepatosplenomegaly, pes cavus, seizures, hypertrichosis, flexion contractures

fied in the Indian population with p. Phe508del seen in about 30% of the affected cases,[13] (Dr. Jayesh Sheth & group, personal communication 2021). The clinical manifestations in CF mimic other disorders like bronchitis, asthma, tuberculosis etc., hence, diagnosis can be easily missed due to a low index of suspicion. A large proportion of males, approximately 1 in 3500 live births are affected with Duchenne muscular dystrophy (DMD), a progressive disorder causing muscular weakness and early death. The mutation is present in the dystrophin gene located on the X chromosome. Due to the X-linked recessive mode of inheritance, females are generally carriers and males are affected (50% probability of carrier mother to pass the defective gene to son). The most common mutation in the dystrophin gene is the deletion of exons in the hot-spot region (exon 44-55) [14] followed by point mutations and duplications. At present, there is no definitive therapy for DMD except a few drugs to treat the symptoms and improve the quality of life of DMD patients. Though exon skipping therapy seems to be a promising one to treat these children with exon 51 deletion.[15] SMA is another muscle weakness disorder due to mutation or deletion in the *SMN1* gene. It is characterized by poor balance, weight-related issues, pain, deformities such as dislocated joints, scoliosis, knee contractures. Recently a drug has been approved for the treatment of SMA.[16] It is based on an antisense oligonucleotide strategy that helps in restoring the function of the *SMN2* gene, a homolog of *SMN1* in its absence. A major hurdle, however, is the exorbitant costs of this therapy and hence patients have to depend on charity access programs.[17]

Thus, continuous efforts are directed towards developing therapeutic approaches for several monogenic disorders as they are associated with high morbidity and mortality. Providing an early and accurate genetic diagnosis is the most important step in the management of these diseases. There are a number of genetic tests available for the diagnosis that apply the principles of molecular techniques like polymerase chain reaction (PCR), multiple ligation probe analysis (MLPA), Sanger sequencing, q-PCR etc.

Metabolic Disorders

A number of metabolic pathways within the cell consist of a series of reactions catalyzed by different enzymes. Due to mutation in genes encoding these enzymes, there can be a substantial decrease in the enzyme activity causing impaired metabolism with subsequent clinical manifestations. All the different conditions characterized by defects in metabolism belong to the group of metabolic disorders. These are individually rare but have a combined incidence of >1 in 5000 birth,[18] making them a significant health issue to be addressed.

One of the most common metabolic disorders is phenylketonuria (PKU), first described by a Norwegian physician, Asbjørn Følling.[19] This condition is due to deficiency of phenylalanine hydroxylase enzyme, encoded by the *PAH* gene which can be diagnosed by a simple urine screening of the common inborn error of metabolic disorders (IEMs).

In addition to this, there are about 250 metabolic disorders which can be identified by the newborn screening using tandem mass screening (TMS) from the blood on a filter paper or from the urine sample by gas chromatography-mass spectrometry technique known as

GCMS. The clinical phenotype of affected patients includes growth failure, vomiting, diarrhoea microcephaly, seizures and intellectual impairment caused by the accumulation of toxic by-products. Alkaptonuria is another classical example of metabolic disorder due to impaired tyrosine metabolism and accumulation of homogentisic acid. The affected patients are normal at birth but develop progressive arthropathy as the affected tissues become weak and brittle with time. In addition to these, there is another group of storage disorders particularly lysosomal storage disorder (LSDs), that comprise ~70 different conditions due to deficiency of various lysosomal enzymes. Impaired lysosome function due to mutation in genes encoding any one of the following: lysosomal enzymes (hydrolases), integral lysosomal membrane proteins (ion transporters), or cytosolic proteins (enzyme modifiers or activators) is the underlying cause of LSDs. Generally, affected children present with the initial symptoms within the age of 6 months to 10 years and the most common features are coarse facial features, hepatosplenomegaly, skeletal dysplasia, respiratory issues, and neurodegeneration.[18] Our study has previously shown a large burden of LSDs in India with Gaucher disease being the most common.[8] Notably, the majority of metabolic disorders show the autosomal recessive mode of inheritance.

The introduction of newborn screening has enabled primary screening of infants for some of the common metabolic disorders like organic acidemia, PKU, maple syrup urine disease (MSUD), congenital hypothyroidism (CH), cystic fibrosis (CF), congenital adrenal hypoplasia (CAH). This has made it possible to save a number of children with a lifelong permanent disability. Though unfortunately, to date, India does not have a universal national newborn screening program even for the most common treatable disorders.

Early diagnosis of metabolic disorders aids in the improvement of outcomes associated with these conditions as treatment interventions is available for some of them. In the case of PKU, a low phenylalanine diet can help in preventing the development of neurological and psychological changes by normalizing the phenylalanine concentration in the body. A key development in treatment options for metabolic disorders is mainly dietary supplementation and enzyme replacement therapy. It is now an approved therapy for a number of LSDs namely Gaucher disease, Fabry disease, Pompe disease, MPS I, MPS II, MPS IV A and MPS VI.[20]

Prenatal Diagnosis

For genetic disorders, prevention is better than cure as nearly 95% of these diseases have no treatment. In such conditions, prenatal diagnosis by testing the unborn fetus for a specific genetic condition is the only tool available to prevent the burden of these disorders. For this, a fetal sample is collected, generally by two common methods namely: chorionic villi sampling (done between 10 and 13 weeks of gestation) and amniocentesis (done between 14 and 20 weeks of gestation). The introduction of prenatal diagnosis has increased the number of referrals to genetic clinics as it gives parents the option to terminate the pregnancy when a genetic abnormality is detected. Chromosomal abnormalities like Down syndrome, metabolic disorders like Gaucher disease and single-gene disorders like thalassemia, SMA etc. can be detected early with the help of prenatal diagnosis. A prenatal diagnosis study carried out by Sheth et al in western India showed 7.2% of the total cases to have chromosomal abnormalities, most common being Down syndrome (2.7%).[21] In addition to this, the use of chorionic villi and cultured amniotic fluid cells for prenatal diagnosis of LSDs by enzyme and molecular methods was also demonstrated in a study by Sheth *et al*.[22] Thus, a number of studies have shown a successful outcome of prenatal diagnosis in cases where the index case is diagnosed with a genetic condition. Hence, prenatal diagnosis is an important strategy for the prevention of genetic conditions. However, providing genetic counselling to the couple explaining to them the risks, benefits and limitations of the procedure is important.

Conclusion

Overall, there are a considerable number of genetic disorders that are prevalent in India and contribute significantly to infant morbidity and mortality. The accurate incidence of many of these disorders in the country is still not completely known. There is an alarming need to create awareness among family physicians, specialists of the different medical fraternity and people about these disorders by a specific genetic investigation to identify such cases and provide them early and correct diagnosis for early interventional therapy and management. These disorders being genetic in origin are not completely curable. Yet, with continuous technological advancements, in addition to therapeutic interventions, prevention by prenatal diagnosis is widely available for all genetic disorders, except complex diseases like diabetes, cardiac disorders and many cancers. In short, a genetic diagnosis can improve

the presenting symptoms and the overall quality of life of the affected individuals. Thus, collective efforts of the clinicians, scientists and patients can aid in overcoming the challenges posed by this group of inherited and non-inherited diseases and to reduce their burden.

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