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GENETICS

PATTERNS OF GENETIC TRANSMISSION

Dhanya Lakshmi Narayanan

Abstract: *Genetic disorders are broadly classified into three major groups: chromosomal disorders, single gene disorders and multifactorial disorders. Single gene disorders, also known as Mendelian disorders are characterized by their patterns of transmission in families. The pattern of genetic transmission of single gene disorders depends on whether the phenotype is dominant or recessive and whether the gene is located on autosomes or sex chromosomes. Understanding the patterns of inheritance is essential in practice of clinical genetics and is the first step in genetic counseling. This is an overview about the Mendelian and Non Mendelian patterns of genetic transmission.*

Keywords: *Mendelian inheritance, Single gene disorders, Genetic counseling.*

The three major groups of genetic disorders are chromosomal disorders, single gene disorders and multifactorial disorders. Single gene disorders are also known as Mendelian disorders and have a characteristic pattern of genetic transmission. It was Gregor Mendel who first identified the patterns of genetic transmission based on his experiments on pea plants. Based on whether a phenotype is dominant or recessive and the presence of a gene on an autosome or sex chromosome, Mendelian disorders can be autosomal dominant, autosomal recessive, X linked or Y linked. Atypical patterns of inheritance (Non Mendelian) are genomic imprinting, uniparental disomy, mitochondrial inheritance, oligogenic inheritance, triplet repeat disorders, mosaicism and multifactorial inheritance. A basic understanding of the patterns of inheritance is essential for a clinician to provide genetic counseling to families. A pedigree is a pictorial representation of an individual's family history and aids in identification of the pattern of genetic transmission.

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Mendelian patterns of inheritance

The five different Mendelian patterns of inheritance are: a) Autosomal dominant, b) Autosomal recessive, c) X linked dominant, d) X linked recessive and e) Y linked.

a. Autosomal dominant inheritance

A condition is said to be dominant if the homozygotes and heterozygotes are indistinguishable from each other. In autosomal dominant conditions, a person having one mutant allele and one normal or wild type allele (the allele that encodes the phenotype most common in a particular natural population) will manifest clinical symptoms. The features of autosomal dominant inheritance (Fig.1) are :

- Both females and males can be affected in equal proportions.
- Multiple individuals in multiple generations are affected.
- Both males and females can transmit the disease to offspring.
- There is at least one instance of male-to-male transmission.

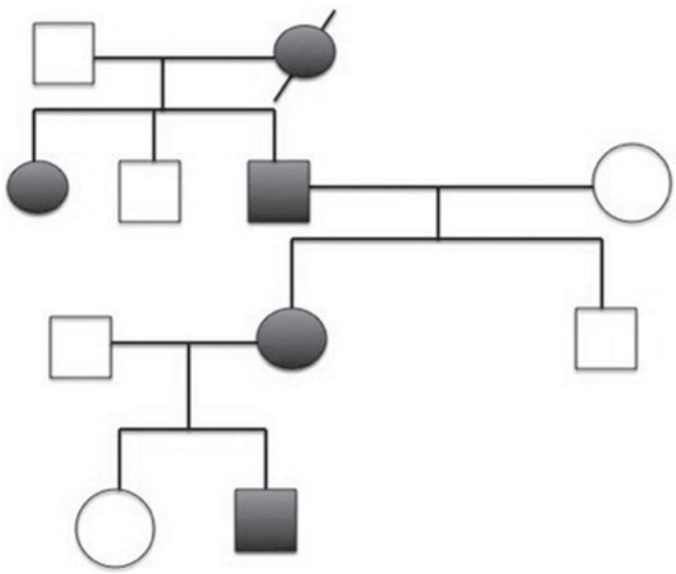

Fig.1. Autosomal dominant inheritance

Some examples for genetic diseases with autosomal dominant inheritance are tuberous sclerosis complex, neurofibromatosis, Marfan syndrome, achondroplasia, etc., Autosomal dominant disorders can exhibit pleiotropy, variable expressivity and varying degree of penetrance.

Pleiotropy: Autosomal dominant disorders can affect different systems of body in different ways and thus a single gene mutation can give rise to effects in different organs. Tuberous sclerosis is a condition, which occurs due to a heterozygous variation (mutation) in *TSC1* or *TSC2* and can cause multiple effects on different organ systems like skin (confetti macules, ash leaf macules, shagreen patches, facial angiofibromas), nervous system (seizures, intellectual disability, cortical dysplasias, subependymal nodules), heart (cardiac rhabdomyomas) and kidneys (angiomyolipomas, cysts and renal cell carcinoma).

Variable expressivity: In autosomal dominant disorders like autosomal dominant polycystic kidney disease, in the same family, different family members can have different severity of disease, in spite of carrying the same disease causing variation. This characteristic is known as variable expressivity. The variability may be intra-familial or inter-familial.

Penetrance: Penetrance of a disease is an index of the proportion of individuals with a mutant allele who manifest the disorder. Reduced penetrance may result in individuals with apparently no clinical symptoms and can lead to 'skipped generations' in pedigree. Reduced penetrance is caused due to interaction with environmental factors or the effects of other modifying genes. Treacher Collins syndrome is an autosomal dominant condition where reduced penetrance is seen.

Genetic counseling and recurrence risk: While interpreting the pedigree of a family with an autosomal dominant condition, variable expressivity, penetrance and pleiotropy should be taken into account. A person harboring a disease-causing variant for an autosomal dominant disorder has a 50% chance of transmitting the same variant to the offspring, regardless of sex, provided that the partner is normal. In certain disorders like achondroplasia, 80% of affected individuals have parents with normal stature. In such autosomal dominant conditions, new mutations or denovo mutations, which occur during gametogenesis, cause disease. New dominant mutations, which occur during gametogenesis, have negligible chance of recurrence for future siblings.

b. Autosomal recessive inheritance

In autosomal recessive disorders, the mutant alleles are recessive to the wild type alleles and hence cause disease manifestation only in homozygous state.

The features of a recessive disorder are (Fig.2):

- Both males and females are affected.
- Multiple members in a single generation are affected
- Consanguinity may be present in the family
- Parents of an affected individual are asymptomatic carriers of the disease-causing variants.

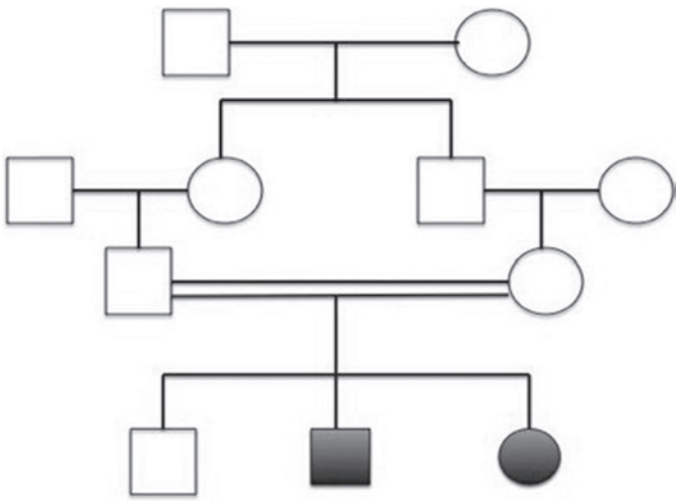

Fig.2. Autosomal recessive inheritance

Examples for autosomal recessive disorders include thalassemia, cystic fibrosis, spinal muscular atrophy etc.

Consanguinity: Consanguinity is defined as the union between two individuals who are as close or closer than second cousins. Random mating between carriers is the most common reason for autosomal recessive diseases like beta thalassemia, where the carrier frequency in a population is high. But consanguinity can be a cause for various rare autosomal recessive disorders.

Genetic counseling and recurrence risk: Partners who are carriers for a disease-causing variant of an autosomal recessive disorder have 25% chance of having a homozygous affected offspring, 25% chance of having a homozygous unaffected offspring and a 50% chance of having a heterozygous unaffected carrier offspring. If an affected homozygous individual has a partner who is a heterozygous carrier, then there is a 50% chance of having an affected offspring. This is known as pseudo dominance and is seen in common recessive conditions with a higher carrier frequency in population.

c. X linked dominant inheritance

This is an uncommon mode of inheritance and will affect both heterozygous females and hemizygous males (males have only one copy of X chromosome). Because females have two copies of X chromosomes and due to random X inactivation, females will be usually less severely affected than males.

The features of X linked dominant inheritance include (Fig.3):

- Usually there will be a female preponderance in pedigree.
- An affected male can pass on the disease to his daughters but not his sons.
- An affected female can pass on the disease to sons and daughters.

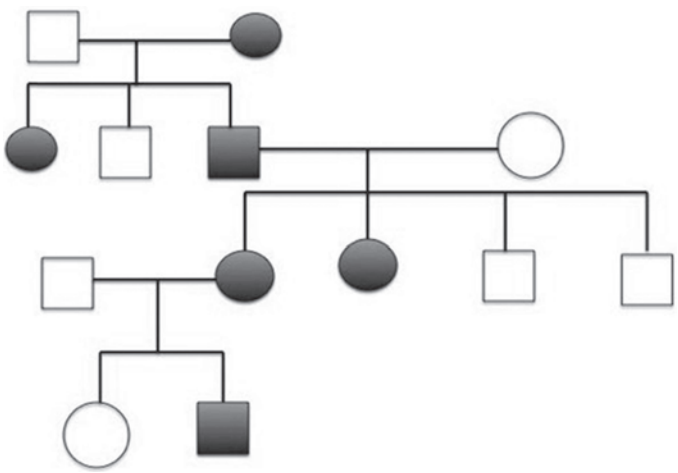

Fig.3. X linked dominant inheritance

Conditions which show X linked dominant inheritance are X linked hypophosphatemic rickets, incontinentia pigmenti, Goltz syndrome, etc.

Genetic counseling and risk of recurrence: Affected women have 50% chance of passing on the disease to all her offspring irrespective of sex. Affected male passes the disease to all his daughters but none of his sons. Some X linked dominant disorders like incontinentia pigmenti are lethal in males and hence affected males may not be seen on a pedigree.

d. X linked recessive inheritance

Conventionally the term 'sex linked disorders' indicate 'X linked recessive disorders'. In X linked recessive disorders, males are affected and females are generally carriers for the disease causing variant, because males are

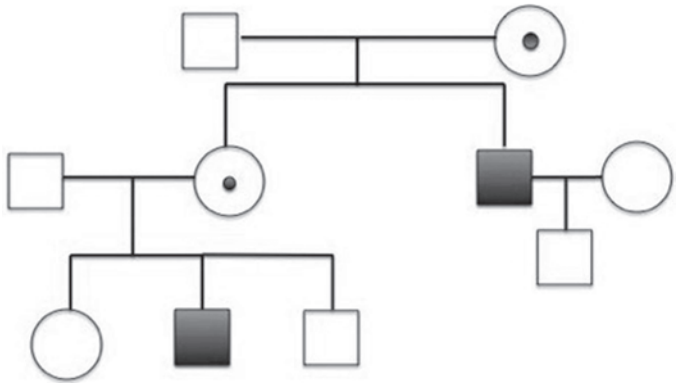

Fig.4. X linked recessive inheritance

'hemizygous', i.e. they have only one copy of X chromosome. Females are generally mildly affected or unaffected because females have two X chromosomes.

The following are the some of the characteristics of an X linked recessive disorder (Fig.4):

- Males are usually affected and females are mildly affected or unaffected carriers.
- There is no male to male transmission.
- Affected males can transmit the disease to their grandsons through their carrier female daughters. This is known as the 'knight's move' transmission pattern on a pedigree.

Examples of X linked recessive disorders include Duchenne muscular dystrophy, hemophilia A, hemophilia B, Hunter syndrome etc.

The following are the conditions where an X linked recessive disorder causes symptoms in a female:

- Turner syndrome (Only one X chromosome and a disease causing variant on that X chromosome)
- Skewed X inactivation.
- X- autosome translocation with disruption of a gene on X chromosome.
- Biallelic mutations in a gene on X chromosome.
- A phenotypic female (46, XY female) as in complete androgen insensitivity syndrome.

Genetic counseling and recurrence risk: A woman who is a carrier for an X linked disease has a 50% risk of having an affected male offspring and 50% risk of having a carrier daughter. If a family has only one affected male, the risk of recurrence could vary between 0 to 50% depending on whether the mutation is inherited or de novo and the

presence of gonadal mosaicism in mother. An affected male will never have affected sons but all his daughters will be carriers.

e. Y linked inheritance

No disease causing genes have been identified on Y chromosome till date. Certain traits like hairy pinna were thought to be Y linked, but evidence is still lacking. Y linked traits will be transmitted from a male to all his sons but none of his daughters.

Non-Mendelian inheritance patterns

Mechanisms of inheritance, which do not follow Mendelian laws of inheritance, are said to follow atypical or Non-Mendelian inheritance. The following are the non-Mendelian patterns of genetic transmission:

a. Mitochondrial transmission
b. Triplet repeat expansions/ trinucleotide repeat expansions
c. Genomic imprinting
d. Uniparental disomy
e. Mosaicism
f. Oligogenic inheritance
g. Multifactorial inheritance

a. Mitochondrial inheritance: Each cell has several mitochondria, which contain multiple copies of the mitochondrial genome. Mutations in mitochondrial genome may be inherited or acquired. Heteroplasmy refers to the state where a mixture of mutant and wild type of mitochondria is seen in every cell. Homoplasmy is the presence of only one type of mutant mitochondria in the majority of cells in an individual. Only when the number of mutated mitochondria crosses a threshold level in a tissue or an organ, a person manifests symptoms. This phenomenon is known as threshold expression.

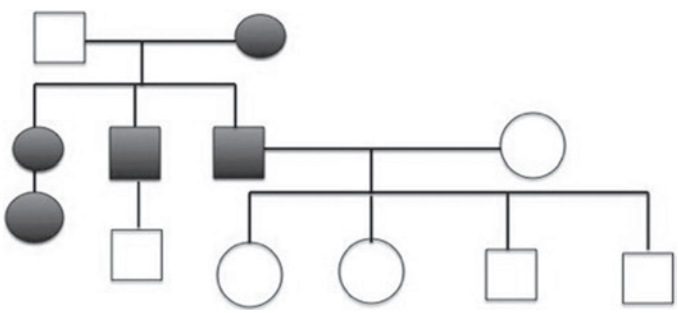

Fig.5. Mitochondrial inheritance

Since mitochondria are entirely contributed by the maternal oocyte, mitochondrial diseases are transmitted entirely from the maternal side. None of the affected males transmit the disease to offspring. There is a huge variability in the severity of symptoms of mitochondrial diseases among affected individuals in a family. Hence counseling for risk of recurrence in offspring and siblings and providing prenatal diagnosis, is a bit tricky in mitochondrial disorders. Some examples of disorders, which follow mitochondrial inheritance, are Leber hereditary optic neuropathy, Kearns- Sayre syndrome, mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS), mitochondrial encephalopathy with ragged-red fibers (MERRF) etc.,

b. Trinucleotide repeat disorders: Repeats of trinulceotides like CAG, CGG ,etc are present in the exon, intron, 5' or 3' untranslated regions of a gene. When the number of repeats increase beyond a threshold level, it results in manifestation of symptoms in trinucleotide repeat disorders. In successive generations, due to expansion of the repeats during gametogenesis, the severity of the disease increases and the disease manifest at an earlier age. This phenomenon is known as anticipation. Examples of these disorders include Huntington disease, Fragile X syndrome, Fredereich ataxia, myotonic dystrophy and spinocerebellar ataxia.

c. Genomic imprinting: This is an epigenetic phenomenon where the phenotypic expression of alleles of some genes depends upon the parent of origin. In imprinted genes, only one of the alleles is expressed depending on the parent of origin and the other allele is silenced through epigenetic mechanisms. Hence a mutation will cause disease only if it happens in the active allele. Examples of disorders with genomic imprinting are Prader Willi Syndrome, Angelman syndrome, Beckwith Wiedemann syndrome and Russel Silver syndrome.

d. Uniparental disomy: Normally an individual inherits one of a pair of homologous chromosomes from each parent. If both the chromosomes are derived from the same parent, it is known as uniparental disomy. Two types of uniparental disomy are recognized: uniparental isodisomy (when both chromosomes are derived from a single chromosome of a parent) and uniparental heterodisomy (when the two chromosomes are derived from both of the homologous pair of chromosomes of the parent). If uniparental disomy occurs in an imprinted locus, it can result in imprinting related disorders. Also uniparental isodisomy of a recessive mutation bearing allele in a carrier parent can cause bi-allelic mutation in the offspring

resulting in an autosomal recessive disorder, even if the other parent is not a carrier.

e. Mosaicism: Mosaicism is the presence of two or more genetically different cell lines in an individual, derived from the same zygote. It occurs due to a mutation, which happens during mitosis after a zygote is formed. Mosaicism can happen in somatic cells or germline cells causing a wide variety of phenotypic features in affected individuals. Mutations in somatic cells are not passed on to future generation. Examples of conditions where somatic mutations cause disease are Proteus syndrome and McCune Albright syndrome. Mutations in germline cells or gonadal cells can be passed on to offspring and can be one reason for recurrence of conditions like Duchenne muscular dystrophy or achondroplasia in offspring even when pathogenic mutations are not identified in both the parents.

f. Oligogenic inheritance: In some disorders, a simultaneous mutation in two or more loci causes disease and this is known as oligogenic inheritance. Bardet Biedl syndrome is conventionally thought to follow autosomal recessive inheritance, but now it has been shown that disease manifests only when an additional mutation in one allele of BBS6 gene occurs along with homozygous mutations in BBS2 gene. Other disorders with oligogenic inheritance are retinitis pigmentosa and Hirschsprung's disease.

g. Multifactorial inheritance: Here a complex interplay of different genes and environmental factors result in disease manifestations. Examples include conditions like cleft palate, neural tube defects, type 2 diabetes, etc. With the advent of advanced molecular diagnostic techniques, single gene basis for some multifactorial conditions has been identified. Many conditions, which were previously thought to be single gene disorders, are reclassified as oligogenic or multifactorial as newer modifier genes are identified.

Conclusion

A thorough knowledge of the patterns of inheritance is essential in genetic counseling of families with genetic disorders. A pedigree is a pictorial representation of family history and would aid in understanding the pattern of inheritance. Once the pattern of transmission is known, the family can be counseled about the risk of recurrence and appropriate preventive measures like prenatal diagnosis and presymptomatic genetic testing can be adopted.

Points to Remember

- ***Single gene disorders have a characteristic pattern of genetic transmission.***
- ***Mendelian patterns of inheritance depend on whether the phenotype is dominant or recessive and whether the gene is located on an autosome or sex chromosome.***
- ***Understanding the patterns of inheritance is essential in providing genetic counseling.***

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GENETICS

APPROACH TO A CHILD WITH DYSMORPHIC FEATURES

*Kalpana Gowrishankar
** Indhumathi N

Abstract: *Pediatricians often have to deal with a child having dysmorphic features. Though genetic testing has advanced in recent times, the first step is clinical evaluation. Making a diagnosis in such children can provide parents and professionals with important information which may not only influence the management but also understanding the natural history and prognosis.*

Keywords: *Malformation, Syndrome, Dysmorphic features, Counseling.*

The term "Dysmorphic" is used to describe children whose physical features, especially facial, are not usually found in a child of the same age or ethnic background.[1] "Dysmorphic" originates from a Greek word "dys" meaning disordered, "morph" meaning shape or form. The word was coined by Dr. David Smith in the 1960's. Dysmorphology is the study of structural defects, especially congenital anomalies. Making a diagnosis in a child with dysmorphic features will enable providers to recognize occult malformations and provide surveillance for complications that may develop over time. It will also provide information regarding prognosis for their child and recurrence risk estimation for future pregnancies.[2]

Congenital anomalies can either be isolated or multiple, minor or major. Major anomalies are those that interfere with the normal functioning of the individual organ systems and have significant medical, surgical or cosmetic implications. Minor anomalies are those that require no medical intervention.

Classification based on pathogenesis (Fig. 1)

- **Malformation:** Abnormal formation of a structure during organogenesis (Mostly <8 weeks of embryonic development; some > 8 weeks: brain, genitalia, teeth). e.g., Spina bifida, cleft lip and palate, polydactyly, hypospadias.
- **Deformation:** Abnormal form or shape caused by extrinsic forces. e.g., club foot, hip dislocation, craniostenosis, asymmetric face - all of them involve musculoskeletal tissues.
- **Disruption:** A structural abnormality resulting from a breakdown or destruction of a normally formed body part. e.g., Amniotic band disruption sequence.

Dysplasia: Disorganization of a cell structure itself or a disordered organization of cells within a tissue. e.g., Skeletal dysplasia, hemangioma.

Patterns of defects (Table I)

- **Sequence:** Cascade from a single known anomaly or mechanical factor. e.g. Pierre - Robin sequence → jaw constraint leading to micrognathia → glossoptosis → 'U' shaped cleft palate.
- **Syndrome:** Multiple anomalies thought to be pathogenetically related e.g. Down syndrome.
- **Association:** Non-random pathogenetically unrelated multiple anomalies. e.g. VACTERL, Mullerian duct aplasia, renal aplasia and cervicothoracic somite dysplasia (MURC).

Table I. Clinical classification of structural anomalies

Based on severity	Based on clinical phenotype
- Major	- Isolated
- Minor	- Multiple congenital anomalies
	- Syndrome
	- Sequence
	- Association
	- Development field defect

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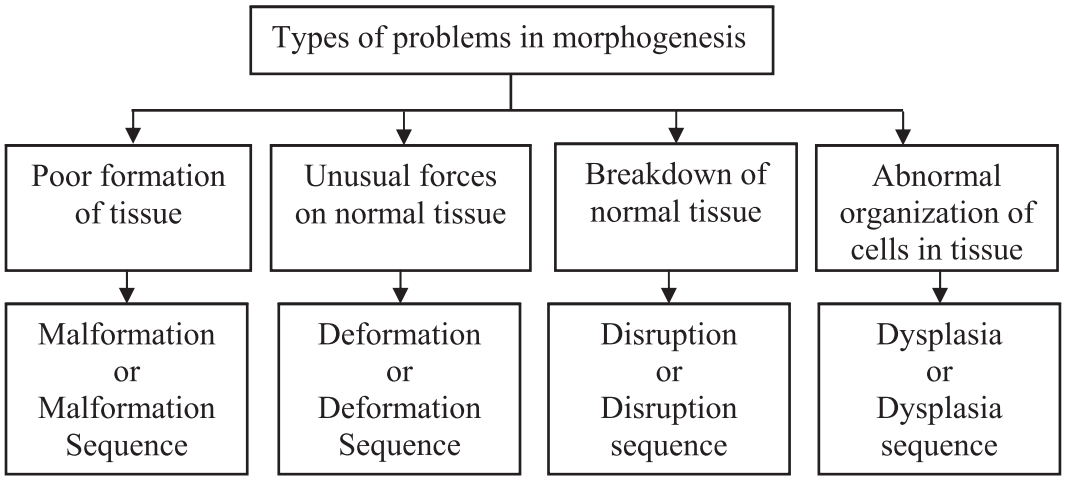

Fig.1. Approach to anomalies

- **Field defect:** Derived from disturbance of a single developmental field. e.g. Di-George syndrome (1st and 2nd branchial arch)

Every pediatrician encounters a child with dysmorphic features at some point in practice and becomes the dysmorphologist on call. In this situation, it is helpful to have a workable approach to the child with dysmorphic features. This includes a comprehensive history and physical examination which can be followed by laboratory testing as indicated.

History

Prenatal history may uncover specific exposures and etiologic factors. This should include medical and obstetric history such as duration of gestation, prenatal care, maternal illness like diabetes, bleeding and maternal exposures (e.g. alcohol, prescribed or illicit drugs, smoking, fever, chemicals, radiation). Severe hyperemesis has been linked with dysmorphic facial features and skeletal abnormalities. USG findings of nuchal edema or of choroid plexus cysts may raise the possibility of a chromosomal disorder. Severe oligohydramnios can predispose to congenital contractures and dysmorphic features consistent with oligohydramnios sequence. Some syndromes are associated with intrauterine growth retardation (IUGR) and others with overgrowth. Mechanical constraint cause uterine abnormalities e.g. bicornuate uterus can lead to fetal deformation and explain an unusual head shape or presence of talipes deformities.[3]

Family history

Drawing a three generation family pedigree may identify or demonstrate a clear Mendelian pattern on inheritance. A history of stillbirth and miscarriages could be related to a balanced chromosomal rearrangement in one of the parents. The age of the parents is important because the incidence of chromosomal aneuploidy is higher in older mothers. Likewise, de novo autosomal dominant mutations (e.g. achondroplasia) are more commonly seen in older parents with the effect being more marked for advanced paternal age. The parents should be asked for consanguinity which increases the incidence of autosomal recessive disorder. Delineating ethnic origin may be helpful because some diseases are more prevalent in certain ethnic groups e.g. post axial polydactyly is more often found in people of African descendants.[4]

Examination key points[5]

General examination beginning at the head and working downwards will reveal most of the major malformations. A specific search must be made for minor malformations and dysmorphic features. Examination of the parents for similar or related features is important. The following list highlights some of the most important dysmorphic features assessed during examination in the genetic clinic. Some of the abnormalities listed are paired with examples of common genetic syndromes in which these features are encountered.

- **Observation**
  - Body posture e.g. clenched hand posture of Trisomy 18
  - Abnormal body proportion e.g. achondroplasia
  - Facial appearance e.g. Down syndrome, Cornelia de Lange syndrome
- **Growth parameters**

Height, weight and occipitofrontal circumference should be the ones to start with. The further the

measurements deviate from the normal centile ranges, the greater the chance of making a genetic diagnosis. The measurements have to be plotted on a centile chart appropriate for age, gender and syndrome specific growth charts (Down syndrome, Turner syndrome, etc).

- **Cranium size and appearance**
  - Microcephaly (chromosomal and single gene disorders, fetal exposure to infections. e.g. toxoplasmos, rubella, zika virus)
  - Macrocephaly (Fragile X syndrome. Phosphatase and tensin (PTEN) mutation, achondroplasia)
  - Brachycephaly (Crouzon syndrome)
  - Plagiocephaly (Craniostenosis, deformations)
- **Craniofacial profile**

It is advisable to view the face both in the front and sides (lateral profile to identify nasal bridge and relation of mandible to maxilla). The face can be divided into forehead, mid part of face and perioral regions. The following measurements - inter pupillary and intercanthal distance, length of philtrum should be noted. For forehead - normal or broad, sloping, bitemporal narrowing, for eye brow –fused or not, for inter canthal distance – decreased (hypotelorism) or increased (hypertelorism), for palpebral fissures – upslanting (Downs syndrome) or downslanting (No nans syndrome), for palpebral fissures - short or long (the length of the palpebral fissure isusually equal to innercanthal distance), for eye size - equal on both sides, smaller or larger on one side,

For other features of eyes - ptosis or epicanthal fold, for philtrum- short, longer, or smooth, for nose - depressed bridge, upturned or not, for ears - low set, posteriorly rotated (1/3 of ear should be above the imaginary line connecting both outer canthi) and for mandible -micrognathia or retrognathia are noted. There may be mid face hypoplasia (Down syndrome), prognathism (Angelman syndrome) or asymmetry (Hemifacial microsomia, CHARGE Syndrome).

- **Eyes**
  - Hypertelorism (Cri -du- chat syndrome, Wolff-Hirschhorn syndrome)
  - Extraoccular movement abnormalities (Myopathy, neurologic abnormalities)
  - Opthalmoplagia in mitochondrial disorders
  - Esotropia (Angelman syndrome, Down syndrome)
  - Exotropia (Angelman syndrome)
  - Ptosis (Smith – Lemli Opitz syndrome, Kearns-Sayre syndrome)
- **Ears**
  - Shape and rotation (short, long, anterior or posterior rotation).
  - Malformed and posteriorly rotated ears which are often low set are seen in Trisomy 18, triploidy, Smith - Lemli Opitz syndrome
  - Microtia (Hemifacial microsomia, Treacher Collins syndrome, retinoic acid fetal exposure)
- **Nose**
  - Prominent bulbous tip (22q11.2 microdeletion)
  - Split appearance (Frontonasal dysplasia)
  - Anteverted nares (Cornelia de Lange syndrome, Smith-Lemli Opitz syndrome)
  - Philtrum
  - Length (long philtrum in William syndrome)
  - Smooth (fetal alcohol syndrome)
- **Mouth and throat**
  - Macrostomia (Oculo auriculo vertebral spectrum, Angelman syndrome)
  - Microstomia (Trisomy 18)
  - High arched palate (Marfan syndrome)
  - Cleft uvula (22q11.2 microdeletion syndrome)
- **Dentition**
  - Widely spaced teeth (Angelman syndrome)
  - Dental decay, enamel hypoplasia (Osteogenesis imperfecta, Dentinogenesis imperfecta)
- **Tongue**
  - Protrusion (due to macroglossia in disorders such as Beckwith - Weidemann syndrome, Pompe disease)
  - Thrusting (Down syndrome and other conditions with poor orofacial muscular tone)
- **Neck**
  - Broad (Turner syndrome, Noonan syndrome)
  - Short (Down syndrome)
  - Webbing (Turner syndrome, Noonan syndrome)

- **Chest**
  - Pectus excavatum (Noonan dystrophy)
  - Pectus carinatum (Marfan syndrome)
  - widely spaced nipples (Turner syndrome, Noonan syndrome)
- **Cardiovascular**
  - Cardiomyopathy (Noonan syndrome, Duchene muscular dystrophy, Pompe disease)
  - Arrhythmias (Myotonic dystrophy, multiple lentigenes syndrome)
  - Conotruncal defect (22q11.2 deletion syndrome, retinoic acid embryopathy)
  - Structural heart defects
  - Atrial septal defect (Holt -Oram syndrome),
  - AV canal defect or VSD (Trisomy 21)
  - Aortic hyperplasia (Turner syndrome), Supravalvular aortic stenosis (William syndrome)
  - Aortic dilatation (Marfan syndrome)
- **Abdomen**
  - Hepatomegaly with or without splenomegaly (Glycogen storage disease, Gaucher disease, Niemann- pick syndrome)
- **Genitourinary** examination includes evaluation of external genitalia, breast development and Tanner staging
  - Ambiguous genitalia (WAGR association, Robinow syndrome, Smith-Lemli Opitz syndrome)
  - Sex reversal (Campomelic dysplasia)
  - Micropenis (Prader-Willi syndrome, Robinow syndrome)
  - Cryptorchidism (Prader-Willi syndrome, trisomy 18, trisomy 13, trisomy 9 mosaic)
  - Hypoplasia of labia majora (Prader-Willi syndrome, Cornelia de Lange syndrome)
  - Bicornuate uterus with or without double vagina (Exstrophy of cloaca sequence, Fraser syndrome)
  - Vaginal atresia (MURC association)
- **Spine**
  - Thoracolumbar scoliosis (Neurofibromatosis type 1, Marfan syndrome, skeletal dysplasia)
  - Vertebral segmentation defects (Alagille syndrome, MURC, VACTERL associations)
  - Deep sacral dimple, sacral hair tufting, sacral tag (cord tethering)
- **Extremities**
  - Limited range of movement (Arthogryposis, storage disorder, contracture)
- **Hands and feet**
  - Polydactyly (Carpenter syndrome, Bardet - Biedl syndrome,trisomy13)
  - Syndactyly (Smith -Lemli Opitz Syndrome, Apert syndrome)
  - Brachydactyly (2q37 deletion syndrome, skeletal dysplasia)
  - Arachnodactyly (Marfan syndrome)
  - Broad thumbs and toes (Rubinstein -Taybi syndrome, Saethre - Chotzen syndrome)
  - Club foot (Potter syndrome, deformation sequence, distal arthrogryposis)
- **Skin, hair and nails**
  - Sparse hair (Ectodermal dysplasia)
  - Hair color lighter than expected (Albinism, Chediak- Higashi syndrome)
- **Skin pigmentation**
  - Hyperpigmentation (Somatic mosaicism)
  - Hypopigmentation (Tuberous sclerosis)
  - Skin color lighter than expected (Albinism, full/ partial)
  - Nail dystrophy (Ectodermal dysplasia)

Table II gives a detailed account of the checklist for the assessment of a dysmorphic child.

Laboratory evaluation[6]

It depends upon the history and physical examination. Diagnostic genetic testing is performed if a specific genetic defect is suspected based the initial evaluation. A broader genetic screening tool is used if no specific diagnosis has been identified with the history and physical evaluation.

Chromosomal analysis / karyotyping: Standard/ conventional Giemsa banded karyotyping of desired resolution 450-550 BPHS (bands per haploid set) is to be ordered if chromosome abnormality is suspected.

Table II. Assessment of dysmorphic child - Checklist[7]

1.General information	Name Sex Date of birth Ethnicity
2.Family history	Three generation pedigree Enquire about consanguinity Other family members with similar problem
3.Pregnancy history	Obstetric complications Maternal illness e.g., diabetes Exposures e.g., alcohol, medications Abnormal investigations (scan, serum screening, amniocentesis, chorionic villus sampling) Liquor volume, abnormal lie Fetal movements
4.Birth history	Gestation Mode of delivery Placenta and cord vessels Apgar Birth weight Malformations noted at birth Resuscitation Admission to special care unit
5.Neonatal period	Feeding complications e.g. jaundice, respiratory problems
6.General examination	Build, stature Skin pigmentary anomalies Edema, nuchal or general
7. Dysmorphic features	Skull shape, sutures, fontanelles Facial features Ear shape and position Eye spacing, red reflex,coloboma Body proportions and symmetry

	Chest shape and nipples Abdominal wall, spine, sacral anomalies or appendage Limbs - length, bowing, contractures, joint laxity Digits, number and shape Palmar creases - fetal pads Genitalia and anal anomalies
8. Development	Milestones Neurological signs SeizuresVision and hearing Behavior
9.Investigations	Ultrasound of abdomen, heart MRI brain, if indicated (abnormal head size, seizures) Skeletal survey if bone dysplasia suspected Routine hematology and biochemistry Metabolic Cytogenetic Molecular genetic
10. Photographs	Obtain parental consent Position main dysmorphic features against a plain background
11.Following assessment	Document findings in notes and for parents Discuss with parents Make clear plans for investigation and follow-up

Additional cytogenetic testing is indicated in the following circumstances.

FISH: Fluorescence In Situ Hybridization (FISH) is done in cases of known microdeletion syndromes.

Array comparative genomic hybridization (CGH): When the features are suggestive of a chromosomal anomaly, but the routine karyotype is normal, Array CGH is the test of choice. This will detect copy number variants (microdeletions and duplications).

Molecular genetic testing: Methylation analysis for Fragile X syndrome and imprinting disorders like Prader-Willi syndrome, Beckwith-Wiedemann Syndrome.

Specialized metabolic testing

Metabolic test done in a child with dysmorphic features are urine organic acids to rule out organic academia, peroxisomal studies if clinical features are suggestive of peroxisomal disorders, lactic acid and pyruvic acid in patients with mitochondrial disorders and metabolomics using mass spectrometry for inborn errors of metabolism.

Whole exome sequencing (WES)

Whole exome sequencing is a powerful genetic technology that is used for the diagnosis of children with multiple anomalies, intellectual disabilities and or seizures.

WES analyses the exons of approximately 19,000 - 20,000 genes simultaneously using next generation sequencing technology. This test is valuable for single gene disorders. By sequencing the exome of a patient and comparing with normal reference sequencing, variation on an individual DNA sequence can be identified. The analysis and interpretations of WES are rapidly improving. WES is not suitable for rare microdeletions, microduplications and triplet repeats expansions.

Whole genome sequencing (WGS)

WGS detects the variants present in regulatory regions in addition to those in coding regions. But WGS is a costlier screening tool that has its own limitations.

Imaging studies

Brain computed tomography (CT) and magnetic resonance imaging (MRI) scans, echocardiogram and appropriate radiography should be performed to help define abnormalities not apparent on physical examination. If skeletal dysplasia is suspected, skeletal survey should be ordered. If infant dies, postmortem pathology studies can be extremely useful to establish a diagnosis. USG evaluations are important to delineate renal, genitourinary and internal genitalia anomalies.

Prenatal diagnosis[7]

- Targeted mutation analysis/chromosome analysis/metabolic testing in fetal tissue depending on the diagnosis in the proband: Chorionic villus sampling/ amniocentesis/ preimplantation genetic diagnosis
- Fetal anomaly scan to look for the same/ associated malformations
- Fetal echocardiogram for fetal cardiac anomalies.

Unknown diagnosis[7]

In children with unknown diagnosis, regular follow up may lead to diagnosis. Taking help from literature search, databases and specialists in the field along with clinical photograph may be useful.

Surveillance and follow up[7]

Children with dysmorphic features should be followed up not only to offer any new available diagnostic tests or new therapeutic options but also to assess growth and development and observe natural history of the disorder. Anticipatory guidance and medical monitoring of patients for syndrome specific medical risks can improve quality of life.

Points to Remember

- ***Pediatricians are the first to identify congenital anomalies in a child.***
- ***Whenever a dysmorphic feature is recognized, a comprehensive evaluation for the presence of other dysmorphic features must be undertaken.***
- ***Malformation syndrome may have an underlying genetic etiology, may be multifactorial or may be due to an environmental cause.***
- ***Recognition of a syndrome may help in management, prognostication and enable recurrence risk estimation for future pregnancies.***

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NEWS AND NOTES

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GENETICS

MANAGEMENT OF GENETIC DISORDERS

Sankar VH

Abstract: *The treatment of inherited metabolic diseases and other genetic disorders have been limited primarily to symptomatic and supportive care. In the last two decades, advances in understanding the pathogenesis of the diseases and biotechnology has helped to develop novel therapies for genetic disorders like enzyme replacement therapy. Hemetopoietic stem cell transplantation is the state of the art treatment for hemoglobinopathies and some metabolic disorders. Enzyme replacement therapy is a reality for Gaucher disease, Fabry disease, mucopolysaccharidosis I and VI. Cost is prohibitive for clinical use especially in developing countries and enough facilities are not available. Bisphosphonates in osteogenesis imperfecta is the standard of care to prevent recurrent fractures. Gene therapy is envisioned as a potentially definitive treatment for a variety of diseases that have a genetic etiology. However, additional clinical and basic research is needed to determine the future role of gene therapy. This review discusses the various modalities of treatment of genetic disorders like metabolic correction, hematopoietic stem cell transplantation, enzyme replacement therapy, pharmacological therapy and gene therapy.*

Keywords: *Hematopoietic stem cell transplantation, Enzyme replacement therapy, Gene therapy, Metabolic correction.*

Genetic diseases result from inherited abnormalities in the body systems concerned with normal development and physiological homeostasis.[1] While "cure" of such diseases by correcting the primary cause is seldom achievable, alternative approaches are in place to control or prevent the effects of these illnesses. Furthermore, the provision of supportive care to the child as well as counseling and support to the family is also part of the management of these children.

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Therapy of genetic diseases can be viewed in a biological model starting from the clinical phenotype and working back to the molecular level (Fig.1).[2] Therapy of the clinical phenotype used in genetic diseases includes various medical and surgical measures to ameliorate the symptoms. Anticonvulsants for seizures in a patient with neurometabolic disorders and beta blockers in Marfan syndrome to prevent or delay the dilatation of aortic root are examples of this approach. In this review modalities like metabolic correction, enzyme replacement therapy (ERT), pharmacological therapy, hematopoietic stem cell transplantation (HSCT) and gene therapy in genetic disorders will be discussed.

Metabolic correction in metabolic disorders

Therapy at this level often involves nutritional and pharmacological approaches which will prevent toxicity due to metabolite excess or deficiency. Acute management of a newborn or child with suspected metabolic disorder should be protocol based.

Modalities of metabolic therapy

Substrate restriction: Dietary alterations designed to restrict intake of a particular substrate may be effective if the pathophysiology involves accumulation of a toxic precursor or metabolite. Dietary restriction of phenylalanine in phenylketonuria is an effective treatment to prevent mental retardation. In addition to substrate restriction, it is often necessary to replace the deficient end product like tyrosine in phenylketonuria. Early detection and treatment of galactosemia with restriction of galactose will reduce the mortality and lifelong restriction will prevent the development of cataract and cognitive impairment. But recent evidences suggest that those affected can develop mild learning disabilities and ovarian failure even though strict galactose restriction is practiced. Specific diets are available commercially for these metabolic disorders but is very expensive. Some modification of routine Indian diet may be tried based on content of different nutrients but the desirable fine control is very difficult to achieve.[3]

Alternative pathways to remove toxic metabolites: In some metabolic disorders the offending metabolite can be

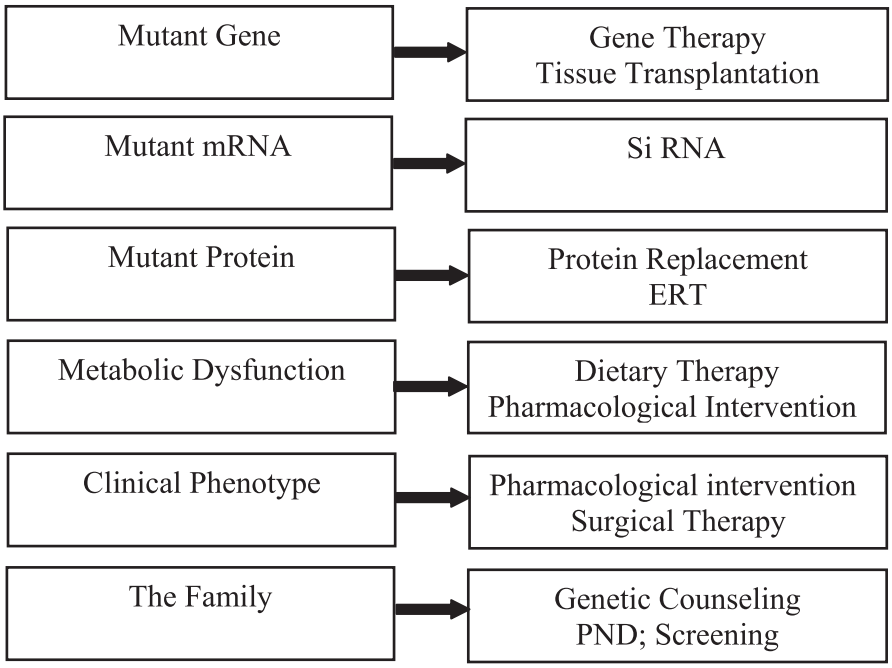

Fig.1.Different levels of therapy in genetic disorders considering the biological model.

converted to a less harmful substance which can be easily excreted. Classical example is urea cycle disorders where excess ammonia is central to the pathogenesis of various symptoms. Administration of benzoate, phenyl acetate or phenyl butyrate will lead to conjugation of these with glycine and glutamine respectively forming hippurate and phenyl acetyl glutamine. These conjugates are readily excreted in urine, thereby providing a way to eliminate excess nitrogen.

Replacement of deficient products: Replacement of the desired product is the most logical approach to the management of inherited metabolic diseases in which the symptoms of the diseases are due to deficiency of the product. This would apply to all hormone biosynthetic defects like hypothyroidism and adrenogenital syndrome. In glycogen storage disease (GSD I) the symptoms are due to hypoglycemia secondary to deficient hepatic glycogen conversion in fasting state. Cornstarch, a slowly digested glucose polymer acts as timed release source of glucose, will help these patients to prevent episodes of hypoglycemia.

Metabolic inhibitors: In disorders where alternate pathway overflows lead to formation of toxic metabolite, toxicity can be reduced by inhibiting a prior step in the pathway by some inhibitors. This may lead to accumulation of prior metabolite which may be better tolerated if this approach is to be successful. In gout, allopurinol is used which inhibits xanthine oxidase, thereby uric acid accumulation is prevented. The prior metabolite xanthine is better tolerated than uric acid since xanthine is water soluble. Another outstanding example of effective therapy with an enzyme inhibitor is use of 2-2-nitro 4 trifluromethyl benzoyl 1-3 cyclohexandione (NTBC) which inhibits 4-hydroxy phenyl pyruvate dehydrogenase in type I hereditary tyrosinemia, dramatically changing the prognosis in affected patients.

Management guidelines are available for common metabolic disorders like maple syrup urine diseases[4], methylmalonic acidemia[5], glutaric aciduria[6], phenylketonuria, galactosemia, etc.

Enzyme replacement therapy

The enzyme replacement therapy and enzyme enhancement therapy have witnessed remarkable advances in the last two decades.[7] The first successful treatment of Gaucher disease by enzyme replacement therapy was developed by Brady and colleagues in 1974. The breakthrough development in ERT is identification of mannose 6-phosphate receptor mediated pathway that helps in targeted delivery of the enzymes to lysosomes. Another crucial observation was that only a very small percentage increase in intracellular activity (1-5%) was required to correct the storage disorder. The main obstacles were a good animal model suitable for preclinical trials and purification of enzyme in sufficient quantities. Both hurdles were eventually overcome by technological advances.

At present, enzyme replacement therapy has become standard treatment for patients with type I (non neuronopathic) Gaucher disease.[8] The aim of ERT is to achieve reversal of the clinical symptoms and prevent irreversible organ damage. The current indications include the presence of symptomatic bone diseases, severe anemia, a tendency to bleed due to thrombocytopenia whether or not associated with coagulopathy, hepatic infiltration with liver dysfunction and pulmonary involvement.[9] Neurological manifestations associated with Gaucher disease type II will not respond to ERT and its use in these patients is deemed inappropriate. The response to ERT is generally excellent irrespective of the degree of the disease, although interindividual variability exists. However there are some bad prognostic factors like persistent hypersplenism, hepatic cirrhosis and pulmonary hypertension. The usual recommended dosage is 30-60 units/ kg/ two weeks IV with dose adjustments later. Dosage is to be determined on individual basis and optimum dosage frequency remains controversial. Several teams have reported interest in low dosages (5 IU/Kg, 2-3 times weekly or 15 IU/kg once in two week) which shows equal clinical and hematological improvement. The response will be evident in first year itself with correction of thrombocytopenia and anemia. Hepatomegaly will decrease by 30%-40% and splenomegaly will decrease by 50-60%. ERT is usually well tolerated except with immediate hypersensitivity reactions.[10] Even though during the last 10 years, worldwide experience with ERT in over 3000 patients with type I Gaucher disease has clearly documented its safety and effectiveness, the cost is prohibitive for common usage in clinical practice especially in developing country like India. However, cost effectiveness of this expensive therapy is well documented in some studies.[11] Gaucher disease task force of India has recently published its guidelines for management.[12]

In addition to Gaucher disease, ERT is approved for Fabry disease, Pompe's disease (GSD II), MPS type I, MPS type II, MPS type IV and MPS type VI. The results of ERT vary considerably from disease to disease. Important considerations are the age of onset, rapidity of progression and presence or absence of neurological involvement. There is no conclusive evidence that ERT crosses blood brain barrier. Even intrathecal administration of enzymes had no beneficial effects. Although there is experimental data demonstrating that enzyme given directly intracerebrally is taken up by neurons, such an approach is not logistically possible.

More recently, another potential therapeutic option chaperon mediated enzyme enhancement therapy (EET) has been tried in genetic disorders.[13] In EET, low molecular weight pharmacological chaperons are used to rescue misfolded or unstable proteins, thereby increasing protein function. In contrast to recombinant lysosomal enzymes, these hydrophobic molecules might cross blood brain barrier and diffuse through connective tissue matrices to reach the target site. The use of pharmacological chaperones like galactose to treat lysosomal storage disorders has been validated in the cardiac variant of Fabry disease. Limitation of this therapy is that it is likely to be effective only in those patients in whom the mutation does not inactivate the catalytic site.

Another novel approach to treatment is substrate reduction therapy.[14] The imino sugar N-butyldeoxynojirimycin (NB-DNJ) has been shown to inhibit ceramide specific glucosyltransferase, which catalyses the first step in glycosphingolipid biosynthesis. This results in inhibition of biosynthesis of all glucosylceramide based glycosphingolipid leading to reduction of storage material in different tissues. Since animal experiments showed significant reduction in storage material in CNS and clinical improvement, clinical trials in patients with Gaucher disease type I was done. The results showed a response in various disease parameters. However, it is unlikely that NB-DNJ will have a role in all sphingolipid disorders (e.g. Niemann-Pick disease type A). This type of therapy will deplete all glucosylceramide based glycosphingolipid, which may cause additional side effects.

There are several issues that should be answered in near future concerning ERT. Novel technologies need to be developed to deliver the therapeutic enzyme effectively to the target tissues like skeletal muscles in Pompe's disease and mesenchymal tissues in MPS. The development of severity score indices that can be used to quantify the benefit of ERT is also important. Most important concern regarding ERT in developing country like India is the prohibitive cost of the enzyme preparation for most of the affected population.[15]

Pharmacological therapy

1. Fetal hemoglobin (HbF) augmentation therapy in hemoglobinopathies: Augmentation of Hb F can inhibit sickle hemoglobin polymerization and red cell sickling, resulting in improved outcome for patients with sickle cell disease.[16] Augmentation of HbF synthesis can reduce the severity of beta thalassemia by improving the imbalance between alpha and non alpha globin chains. It has also been shown that good response is present in some specific genotype like Xmn1 (+) polymorphism and IVS2-1(G-A)

or IVS1-110(G-A) mutations. Hydroxyurea, an inducer of Hb F is the only currently approved agent for the treatment of moderate/severe sickle cell disease and thalassemia intermedia.[17] Hydroxyurea should be used in a dose of 10-15 mg/kg with constant monitoring of hematological, renal and liver profile. DNA demethylating agents like 5-azacytidine and decitabine are the most efficacious reactivators of HbF production but further studies to confirm the safety and effectiveness with chronic usage are going on because this agent is having potent myelosuppression and carcinogenicity.

2. Bisphosphonates in osteogenesis imperfecta: Osteogenesis imperfecta (OI) is a disorder of connective tissue characterized by recurrent fractures, blue sclera, dental abnormalities and progressive limb/vertebral deformities. Pamidronate, an analogue of pyrophosphate and a potent inhibitor of osteoclast activity has been shown to increase the bone mineral density (BMD), reduce the fracture rate and improve the functional status in OI.[18] Pamidronate is administered intravenously in the dose 1mg/kg/day for 2-3 days once in 3-4 months. Alendronate, an oral bisphosphonate, in a dose of 1mg/kg/day (max 20mg/day) is also found to be beneficial in OI. Oral therapy is recommended for older children as the preparation available in India is only in tablet forms. The tablet has to be swallowed with plenty of water and the patient has to be kept upright after taking the medicine. Recently zolendronate, a long acting bisphosphonate also has been tried in children with OI and found to be useful in preventing fractures.

3. Growth hormone therapy: It is well established that growth hormone(GH) therapy is effective in increasing the final adult height in children with Turner syndrome.[19] However, the magnitude of the benefit varied considerably in different patients. Factors predictive of taller adult stature include a relatively tall height at the time of initiation of therapy, tall parental heights, young age at initiation of therapy, a long duration of therapy and a high GH dose. The optimal age for initiation of GH treatment has not been established. However, this can be started as soon as the growth failure is demonstrated and its potential risks and benefits have been discussed with the family. The dosage for Turner syndrome is higher than for growth hormone deficiency. GH therapy is also tried and found to be useful in Prader Willi syndrome, Seckel syndrome, Russel Silver syndrome, hypochondroplasia and achondroplasia.

4. Lorenzo's oil in adrenoleukodystrophy: Lorenzo's oil was initially thought to be useful for treatment of adrenoleukodystrophy as it normalizes the level of very long chain fatty acids in blood. Later it was identified that even though the levels are normalized, symptoms did not improve as the drug will not cross the blood brain barrier. This therapy is now recommended in asymptomatic boys with X-linked adrenoleukodystrophy with normal MRI results.[20]

5. NTBC (2-2nitro-4-trifluromethy-benzoyl-1, 3 cyclo-hexanedione) in Tyrosinemia type 1: Tyrosinemia type I is a fatal liver disease caused by accumulation of toxic metabolite due to deficiency of fumaryl acetoacetate, which is the last enzyme in tyrosine catabolism pathway. NTBC is a potent inhibitor of 4-hydroxyphenylpyruvate dehydrogenase which prevents tyrosine degradation and accumulation of intermediate toxic substances. This drug was found to be useful in 90% cases if started sufficiently early and reduces the need of liver transplantation.[21] NTBC is available only through orphan drug net and the cost is prohibitively high.

Hematopoietic stem cell transplantation

For past two decades hemetopoietic stem cell transplantation (HSCT) has been used as an effective therapy for various genetic diseases.[22] HSCT acts in one of the three ways in treating genetic diseases: (i) By directly replacing diseased marrow or blood cells operating within the blood systems (e.g in hemoglobinopathies like thalassemia), (ii) By replacing phagocytic cells of the monocyte/ macrophage lineage which operate in solid organs (e.g. in osteopetrosis), (iii) By acting as a source of indwelling enzyme therapy in metabolic disease [e.g. in Hurler syndrome (MPS 1)].

HSCT was usually performed using donor bone marrow from siblings with identical HLA match. Major obstacle in HSCT is the availability of HLA matched donor. Recently the choice of donors has widened to include parents who are only half matched for their child's tissue type (haploidentical). However, risks remain substantially higher in the more mismatched transplants. Umbilical cord blood (UCB) is a viable alternative and determinant of outcome after UCB is the nucleated cell dose per kilogram of recipient weight.

Evaluation of the true long term effects of HSCT is very difficult to asses since most diseases have a wide spectrum of clinical phenotype. Moreover benefit varies between organ systems. Reticuloendothelial organs like liver and spleen often shrink quickly as enlarged macrophages take up the enzyme easily. However central nervous system improvement is slower because of the turnover of microglia and their replacement by donor

derived cells. Unfortunately the impact of HSCT on bone disease like dysostosis multiplex in mucopolysaccharidoses is little, presumably because of poor penetration into mesenchymal tissue. Timing of HSCT is also important for good results.[13] In metachromatic leukodystrophy, HSCT is recommended in presymptomatic patient where neurophysiologic functions and independence in activities of daily living remain good. In case of X-linked adrenoleukodystrophy, HSCT must be reserved for those who have early but definite evidence of cerebral disease as determined by MRI.

HSCT is a proven effective therapy for Hurler syndrome (MPS type 1).[23] Engraftment after HSCT in Hurler disease patients leads to rapid reduction in glycosaminoglycans (GAG) substrates in liver, tonsils, conjunctiva, CSF and urine. Obstructive airway symptoms, hepatosplenomegaly corneal clouding are dramatically reduced, hydrocephalus prevented or stabilized and hearing improves in many children. Dysostosis multiplex shows much poorer response because of poor penetration of enzyme into mesenchymal tissue. It is critical to perform the transplant as early as possible, ideally before 18 months of age when intellectual function is relatively well preserved. The optimal neurological outcome after HSCT in children with Hurler syndrome are likely to occur only when the child is less than 2 years of age and has a mental development index (MDI) of more than 70 before HSCT. In Gaucher disease, HSCT is not currently recommended as first line of treatment since enzyme replacement therapy (ERT) is effective with low morbidity. Other conditions where HSCT is the treatment of choice are thalassemia and malignant osteopetrosis. In malignant osteopetrosis, HSCT abolishes bone sclerosis, but has no impact on neurodegenerative disease.

Pre-transplant "conditioning therapy" both to eradicate patient's marrow and to suppress rejection reaction is an important determinant of successful HSCT. This carries many short and long term side effects like endocrine and growth problems. Recently reduced intensity nonmyeloablative preparatory regime have been used in some indolent form of metabolic diseases. Another major challenge during HSCT is intercurrent infections during immunosuppression. Graft versus host disease (GVHD) is a major concern after HSCT due to allogenic recognition of patient tissue by donor T-cells. The role of bone marrow graft engineering (T cell depletion) and cell dose of nucleated cells (CD34 cells) will reduce the rates of GVHD, but at the cost of increased infections and more frequent graft rejections. As a screening tool for graft acceptance, donor/recipient chimerism can be monitored accurately in most patients by PCR methods. Comprehensive multidisciplinary team approach is important in managing patient on HSCT.

Various innovative methods have been tried in HSCT to improve the outcome.[18] Insertion of genes for specific lysosomal hydrolases or for the ALD protein into autologous hematopoietic stem cells and transplantation of these cells is theoretically attractive. To correct skeletal manifestations of storage disorders co-transplantation of mesenchymal stem cells which may differentiate into chondrocytes and osteoblasts is a novel approach. In rapidly progressive infantile form of neurodegenerative disorders like Krabbe disease, intrauterine hematopoietic cell transplantation (HCT) may be worth exploring but the major limitation of this approach is the low levels of donor cell engraftment. Improvement in HCT techniques and the development of novel stem cells will significantly impact the safety and efficacy of therapy as well as expand the list of candidate diseases.

Therapy at the genetic level

Gene therapy is a potentially definitive treatment for diseases that have a genetic etiology.[24] Various forms of gene therapy are gene replacement therapy, gene expression alteration targeting mRNA and gene editing to introduce targeted changes in host genome.

Gene replacement therapy

This is a straightforward approach in monogenic disorders where reduced gene product is the pathology. This requires the targeted transfer of exogenous genetic material into human cells and the subsequent regulated expression of the corresponding gene product. Gene replacement can take place either directly in vivo or through ex vivo cell therapy. The gene therapy product available in the market is Glybera, a recombinant AAV vector for treating lipoprotein lipase deficiency. Gene therapy trials are going on for single gene disorders like cystic fibrosis, Hemophilia B, thalassemia, lysosomal storage disorders and primary immunodeficiency disorders.

Gene expression alteration targeting mRNA: RNA modification therapy targets mRNA, either to suppress mRNA levels or by correcting or adding functions to the mRNA. There are four basic approaches to modifying mRNA to treat monogenic disorders: antisense oligonucleotide, RNAi, trans-splicing and ribozymes. Antisense oligonucleotide (ASO) can be used to redirect splicing and induce exon skipping. Repeated administration of ASO has successfully produced widespread dystrophin

production in mouse models. Limitation of this therapy is the need for lifelong therapy and different deletions will require different ASOs. Read through of stop codons is another approach where read-through of premature stop codons will allow restoration of protein expression. PTC124 is a new orally administered investigative drug found to be useful in some cases of cystic fibrosis and DMD. RNA interference (RNAi) is a conserved biologic response to double-stranded RNA that results in sequence specific silencing of target gene expression. It has the potential to revolutionize the treatment of genetic disorders where gene expression has to be silenced like viral infections and malignancy.

Gene editing to introduce targeted changes in host genome (CRISPER technology): Target sequence specific, designer nucleases have become a powerful toolkit for genome engineering. Theses designer nucleases first induce double stranded DNA breaks in targeted DNA sequence and then realize a range of DNA modifications.

Despite its promise, gene therapy is not sufficiently developed for clinical use due to several reasons. Additional clinical and basic research is needed to evaluate the future role of gene therapy in clinical practice.

Summary

Technical experience for more than two decades has shown that HSCT may benefit some but not all patients with genetic disorders. Despite rapid technological improvements, there are still many short term risks and potential long term adverse effects secondary to HSCT. The rapid emergence of alternate therapies like enzyme replacement therapy (ERT) should be evaluated. ERT is a reality for Gaucher disease, Fabry disease, MPS I and MPS VI. Unfortunately these tend to be extremely expensive to be used in routine clinical practice especially in developing countries like India. Some specific targeted pharmacological therapies have also been identified. It is hoped that gene therapy can eventually replace all these modalities. However, additional clinical and basic research is needed to determine the future role of gene therapy. Even though rapid advances are occurring in treatment of genetic disorders, we should never forget preventive aspects like screening and prenatal diagnosis of genetic disorders.

Points to Remember

- ***Management of genetic disorders can be considered as a biological model viewed from clinical phenotype and working back to the molecular level.***
- ***Metabolic disorders can be treated with substrate limited therapy (dietary restriction) and pharmacological therapy to reduce the toxicity of the accumulated metabolite.***
- ***Enzyme replacement therapy and enzyme enhancement therapy is showing promising results in the management of lysosomal storage disorder.***
- ***Hematopoietic stem cell transplantation (HSCT) is an effective treatment for various genetic disorders like thalassemia, osteopetrosis and primary immunodeficiency disorders.***
- ***Gene therapy is a promising technology for the definitive treatment of genetic disorders.***

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CLIPPINGS

What is FPIES and how does it affect babies?

Food protein-induced enterocolitis syndrome is when food allergies affect the gastrointestinal tract of babies and young children. It is rare but may be more prevalent than doctors previously understood. Recurring, severe diarrhea and vomiting are the presentations. Vomiting typically occurs about 2 to 3 hours after eating a trigger food and is then followed by diarrhea. Infants who have FPIES may also show signs of failure to thrive, or FTT, which may result in delayed growth in many areas. Cows milk and soy protein in infant formulae and other infant foods are the identified culprits. 20 percent of children with FPIES have family members with food allergies, while 40 to 80 percent have family members with allergic diseases, such as hay fever, asthma, and eczema. Treatment usually involves eliminating all trigger foods from the diet. In the rare case that a breastfed baby reacts to breast milk, it may mean that the trigger food is in the mother's diet. As FPIES is not a typical allergic reaction, it usually does not require the use of epinephrine or an Epi-pen.

Jon Johnson. What is FPIES and how does it affect babies? Medical news today. https://www.medicalnewstoday.com/articles/323794.php on 15.12.2018.

GENETICS

DOWN SYNDROME – CURRENT PERSPECTIVES

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Abstract: *Down syndrome, identified as a cause of intellectual disability, still remains an area of research in view of the complexity and co-morbidities though molecular mechanisms causing phenotypic expression are being better understood. These patients require multidisciplinary care with significantly improved quality of life. This article discusses the newer concepts in pathogenesis, management of uncommon comorbidities, prevention and recent research in therapy of Down syndrome.*

Keywords: *Down syndrome, Neurological morbidities, Molecular mechanisms.*

Down syndrome (DS) is the most common genetic cause of intellectual disability worldwide. Incidence of DS in India is 1 in 825-900.[1] Down syndrome occurs due to an extra copy of chromosome 21. In 95% cases, the extra chromosome 21 occurs due to non-dysjunction during parental gamete meiosis.[2] In the remaining 4% cases it occurs due to translocation and up to 1% of cases are mosaics. Children with DS have a high prevalence of various comorbidities requiring management which include structural heart anomalies, hypothyroidism, celiac disease, leukemia, obesity, hearing and visual problems and early onset of Alzheimer's disease.[3] Researchers are trying hard to have a better understanding about the etiological aspects and management of co-morbid conditions. Molecular mechanisms that underlie the phenotypic expression and various comorbidities have been largely elucidated. Mouse models of segmental trisomy 21 have been extensively used to study the concept of gene dosage imbalances and relate it to understand the neuropathogenesis of this syndrome.[4] This article covers the recent advances in understanding the pathophysiology, management of selected comorbidities, newer preventive strategies and progress in therapeutics in DS.

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Pathophysiology

Sequence analysis of chromosome 21 was completed in 2000, almost 60 years after the discovery of trisomy 21 as a cause of DS.[5] Elucidating the functional aspects of genes located on long arm of chromosome 21 (HSA21), has helped in better understanding of phenotypic variability in DS.[4] There are about 240 protein coding genes, 144 pseudo genes and around 144 long non coding RNA (lncRNA) genes. HSA21 is among the richest chromosomes for lncRNA encoding genes.[6] Different phenotypic features in DS may be attributed to dosage imbalance of various coding or noncoding genes on HSA21, whose overexpression may alter various cellular and developmental processes. Letourneau, et al studied the genome wide differential gene expression and transcriptomic differences among trisomic cells and euploid cells in a pair of monozygotic twins, discordant for trisomy 21.[7] Monozygotic twins were selected to remove the genomic variability, so that, any difference at transcriptome (complete set of mRNA and noncoding RNA (ncRNA) transcripts produced by a cell) level was solely attributed to extra HSA21. They found specific domains along all chromosomes with genes having differential expression and were either up regulated or down regulated in T21. These were known as chromosomal domains of gene expression dysregulation (GEDD). They demonstrated that trisomy 21 cells had some chromatin modifications that may alter the overall transcriptome and GEDD, which may be responsible for some phenotypic variability in DS patients, although exact mechanism in relation to specific phenotype is still unclear.

Management

Children with Down syndrome have many associated comorbidities, which require multi-disciplinary approach for the management of associated problems.

Table I. Down Syndrome - Common comorbidities

System involved	Complications	Investigations	Management
Cardiac (40-63%)[8,9]	Most common AVSD followed by VSD, ASD, PDA, TOF	ECG, ECHO	Cardiologist consult and corrective cardiac surgeries if required
Thyroid abnormalities (28-40%)[10]	Subclinical hypothyroidism, congenital hypothyroidism, autoimmune thyroiditis, Graves disease	T3-T4-TSH, anti TPO, antibodies, USG- thyroid	Endocrinologist reference and to start drugs accordingly. Thyroid profile if normal at birth, to be repeated at 6 months and then annually.
Ear problems[11]	Hearing loss (75%), recurrent otitis media, obstructive sleep apnea,	BERA / OAE	ENT specialist opinion regarding the use of hearing aid and speech therapy.
Eye problems[12,10]	Refractive errors (43-70%), strabismus (20-47%), cataracts (15%), nystagmus (10%), nasolacrimal duct obstruction	Slit lamp and fundus examination	Ophthalmologist opinion for complete eye evaluation at birth and then follow annually.
GIT problems[10,11]	Congenital GI defects (4-10%) (duodenal atresia, esophageal atresia, pyloric stenosis).[10] Other problems include constipation, celiac disease, obesity, GERD	Screening for celiac disease and if suspecting any GIT defects, investigations to be planned accordingly.	Gluten free diet for celiac disease, anti reflux medicine for GERD, surgical intervention for any GI defect
Hematological[10,13]	Polycythemia, thrombocytopenia, transient myeloid dysplasia (<10%), AML and ALL (upto 1%)	Complete hemogram with peripheral smear, bone marrow biopsy if required.	CBC initially at 3 months, then yearly or according to need (iron deficiency / suspecting leukemia)
Skeletal[10,11]	Atlanto axial instability (10-30%), hypotonia, pes planus,	Cervical spine x rays	Avoid activities producing excessive jerks of body. Yearly neurologic examination

These children can have ear problems (recurrent ear infections, hearing loss), vision problems (cataracts, refractory error), structural heart defects [atrio-ventricular septal defects (AVSD), Tetralogy of Fallot, patent ductus arteriosus and others], thyroid dysfunction, neurological problems, gastrointestinal abnormalities (atresia, celiac disease) and myeloproliferative disorders. Proper and timely referral to various specialties, early intervention, stimulation and speech therapy, friendly environment and family support can significantly improve the functional capabilities of these children and also helps in early detection of complications and their treatment. Table I shows the common co-morbidities and their management. In this article, we will discuss few selected morbidities which are often neglected or missed but can have a significant impact unless helped by timely and early intervention. Additionally the current preventive strategies are also discussed.

Celiac disease

Celiac disease is an important co-morbidity that can be easily missed if proper screening is not done. Symptoms of celiac disease include diarrhea, bloating, large bulky stools, fatigue, growth failure, anemia, abdominal discomfort, excess flatus and irritability.[14] Children with DS may not be able to express some of these symptoms. Approximately 3.6-13.8% of DS children are reported to have celiac disease.[12,15,16] A recent meta analysis including more than 4000 children and adults with DS from 31 studies in literature reported the prevalence of biopsy verified CD as 5.8 %.[17] In an Indian study 7% of DS children were detected to have celiac disease and the major predictor was presence of anemia.[16] The mechanism of celiac disease in DS is probably autoimmune. AAP guidelines 2011, recommended serologic screening for celiac disease only in symptomatic individuals beginning at age 1 year for children on a diet containing gluten.[12] Standard screening of celiac disease includes tissue transglutaminase immunoglobulin A (tTG-IgA) level with simultaneous quantitative IgA. This is important, because low IgA will result in a false negative tTG-IgA result.[17] In contrast, European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) recommends routine screening for all children of DS who are >2 years with human leukocyte antigen HLA-DQ2 and HLA-DQ8 typing.[18] In patients who are HLA-DQ2 and HLA-DQ8 negative, further testing is not required., but if patient is positive for both or any of these markers, then an anti-TG2 IgA test and total IgA determination should be performed. If antibodies are negative, repeated testing for CD-specific antibodies is recommended every 2 to 3 years.[19]

Previously the screen positive tests were confirmed by intestinal biopsy. But ESPGHAN guidelines eliminated the need for a biopsy for a final diagnosis of CD in the presence of high serology tTG IgA titers (at least 10 times the upper limit of normal), positive anti endomysial antibodies and positive HLA DQ2 and HLA DQ8. We at our center still confirm the diagnosis by biopsy before initiating gluten free diet. The management of children of DS with celiac disease is similar to other children.

Dermatological problems

There is a wide spectrum of dermatological manifestations in Down syndrome. Seborrheic dermatitis is one of the commonest skin problem encountered in children with a prevalence of about 35%.[20,21] Atopic dermatitis, initially considered to be a common skin problem, has a prevalence of about 3-5 % according to Schepis, et al.[21] Other dermatological disorders include milia like calcinosis cutis, syringomas, vitiligo, onychomycosis, scabies, anetoderma (focal loss of dermal elastic tissue, resulting in localized areas of flaccid or herniated saclike skin mostly secondary to folliculitis).[19] Dermatological opinion may be required for recognizing these specific problems, so that their timely diagnosis can decrease the associated morbidities.

Autistic spectrum disorders (ASD)- Children with DS are historically known to be happy and socially outgoing, but this does not hold true for all. Some of these children may be socially conserved and have few autistic features that go unnoticed due to presence of DS per se. It has been a consistent observation that in comparison to only DS children, DS with autistic spectrum children have poor IQ score, less expressive language skill and more disruptive behaviour.[22,23] The diagnosis of autism in DS children is highly challenging. Concept of dual diagnosis has come into light recently due to the occurrence of autism in DS children. Various tools have been developed to diagnose autism including Autism Diagnostic Interview-Revised (ADI-R), Autism Diagnostic Observation Schedule-Generic (ADOS-G), social communication questionnaire (SCQ), Vineland Adaptive Behaviour Scales-Second Edition (Vineland II), DSM 4 & 5 Criteria.[24] Incidence of ASD in DS children ranges from 10 to 18%, depending on the screening method employed and population screened.[25-27] The prevalence of autism in DS patients may be attributed to various other genetic variations along with trisomy 21.[28] Early diagnosis of autism in DS children is very important for timely intervention and imparting educational services as is being done for other children with ASD.

Seizures: The incidence of seizure disorders in DS patients varies between 1%-17%.[12,29,30] Age wise distribution of epilepsy in DS patients include infantile onset, young adulthood epilepsy and seizures seen in older age group beyond 50-55 years. Young patients of DS typically have infantile spasms and tonic clonic seizures with myoclonus, however, in older patients, complex partial seizures and generalized tonic clonic seizures predominate.[30] Seizure predisposition in DS may be attributed to genetic differences in brain structure like overall small hippocampal and cerebellar volumes and decrease in GABAnergic neurons in brain matter and neuronal cell membrane channel dysfunction.[31] Other risk factors include prematurity, perinatal asphyxia, comorbidities like cardiovascular disease, recurrent infections.[32] Diana, et al have demonstrated a positive correlation between the presence of epilepsy and degree of intellectual disability. In this study, only around 20% of DS children with epilepsy had mild cognitive impairment, rest had moderate to severe cognitive impairment and there was subtle improvement in cognition after initiation of therapy and control of seizures.[33] Thus early recognition and prompt management of seizures in DS patients is recommended for better neurodevelopmental outcome in these patients.

Alzheimer's and dementia

Life expectancy of DS patients has increased in recent years due to better living standards, early diagnosis and management of associated complications like heart disease. But with advancing age, there is an upsurge in other age related health problems, one of them being early onset of Alzheimer's disease (AD) and dementia. In DS patients with age ranging from 50-60 years, up to 55% patients suffer from dementia.[34] Before onset of dementia, these patients may present with various other behavioral changes like decline of language and social skills, apathy, lack of motivation, anxiety, impulsiveness and other behavioral abnormalities.[35] There is excess deposition of A beta amyloid plaques and neurofibrillary tangles in the brain of DS - AD patients, earliest in the hippocampal area.[36] The gene coding protein – amyloid precursor protein (APP) is located on HSA21. It has been postulated that trisomy of APP may be responsible for early onset AD in DS patients. Triplication of even a small segment having APP gene may lead to early onset dementia even in the absence of DS.[37]

Effect of maternal choline intake in mouse model has proved to be beneficial in improving hippocampal function and choline acetyl transferase levels, though studies in human are still awaited.[38]

Malignancies

DS is associated with a wide spectrum of malignancies, most common being childhood leukemia (up to 40 folds relative risk). DS patients represent about 2% of all acute lymphoblastic leukemia (ALL) in pediatric patients and about 5-14% among all acute myeloid leukemia (AML).[39] About 10% of DS patients also have pre megakaryocytic leukemic stage known as "transient myeloproliferative disorder", which usually resolves by 3 months age. In up to 20% (TMD) patients, this may evolve into a unique subtype of leukemia known as acute megakaryocytic myeloid leukemia (AMKL).[40] Along with trisomy 21 as a contributory factor for leukemia, there is an additional mutation in specific X linked gene, GATA1, which is detected in nearly all DS patients with AMKL. GATA1 protein is a megakaryocytic X- linked transcription factor, present in megakaryoblasts and is essential for erythroid and megakaryocytic differentiation. GATA1 gene mutations are acquired somatic mutations limited to leukemic clones. It has been seen that during the remission phase of TMD, mutation in GATA1 gene disappears, which explains the evolution of AMKL from TMD and its clonal nature.[41,42] However, a study by Hesle, et al has paradoxically shown a low risk of solid tumors in DS patients.[43] Complete hemogram is advised initially within the first 3 months of birth and then yearly. Treatment remains same as for leukemia in other patients.[11]

Reproductive issues

Age of onset of pubertal changes in DS girls is almost similar as of other non DS females. They may be trained in maintaining menstrual hygiene but may be difficult in some cases and behavioral modifications; therapy to delay periods and contraceptive methods may help in such situations.[3]

Males have more fertility related problems such as ineffective spermatogenesis, hormonal insufficiency, testicular atrophy and erection/ejaculation difficulty and based on available literature, only 3 males with DS have fathered pregnancies.[44] Recurrence risk for offspring in case of trisomy 21 is about 50%, with partner being normal and optional prenatal testing should be offered during the pregnancy.

Research on cognitive improvement

DS patients require early intervention in the form of physical, occupational, and speech therapy and vocational training. Treatment in accordance with the associated comorbid condition should be also initiated timely soon after the diagnosis. But for intellectual disability, there is no definitive therapy presently.

According to the Cochrane review on pharmacological interventions on cognitive decline in people with Down syndrome (age range 20-55 years), effect of four drugs including donepezil, galantamine, memantine and rivastigmine was evaluated. 427 participants and nine studies met the inclusion criteria. Donepezil was the drug studied in 4 original communications including 192 participants, two studies evaluated for memantine in 139 patients, while one study each assessed simvastatin, antioxidants, and acetyl L-carnitine. Follow up period in studies ranged from four weeks to two years. Analyses indicated that there was no significant improvement on cognitive scores. However, participants who received donepezil were significantly more likely to experience adverse reactions. Due to the low quality of evidence in this review, it was difficult to draw conclusions.[45]

Chromosomal silencing

Various phenotypic features of DS children are attributed to genetic dose imbalance due to trisomy 21. A newer concept of silencing the whole extra chromosome has emerged to evolve trisomy into disomy. XIST, a long non coding RNA, has well proven role in X- chromosome inactivation. This property of XIST was exploited by inserting it into DYRK1A locus of chromosome 21 in induced pleuripotent stem cells, which resulted in production of chromosome silencing and barr body formation. However, chromosomal reactivation on long term follow up and partial silencing of autosome has been observed.[46] Other than XIST, a newer selected marker, TKNEO, has been used to target the specific gene, AAP, to induce loss of chromosome 21.[47] But more trials are required for practical application of these novel chromosome therapy in humans.

Role of megavitamins and nutritional therapies in improving cognition

Apart from drugs, nutritional therapy in DS patients has been a considerable area of on going research. Many studies have highlighted the increased oxidative stress in DS patients.[48] Antioxidants like high dose of vitamin E supplementation have been tried with promising results in both in utero mouse model Ts65Dn and adults. In-utero treatment improved neurogenesis, reduced lipid peroxidation and enhanced spatial memory in these animals.[49] Adult treatment, on the other hand, improved working memory, reduced reactive oxidative species production in the cortex and normalized the density of TrkA+ neurons.[50] Role of antioxidants and folinic acid in children with DS was confuted by Ellis JM, et al who showed no improvement in the developmental quotient of DS children after supplementation for 18 months.[51] Recently, in 2014, study by Parisotto, et al has proved the role of vitamin E and C in decreasing the oxidative stress and improving the glutathione levels in DS patients and thus can have putative role in future therapies.[52] But more structurally framed large scale studies are required for proving the definitive role of dietary modification and antioxidant supplementation in DS children.

Prevention of Down syndrome

Recurrence risk

95% DS occur due to an extra chromosome 21 i.e. trisomy 21 due to non-disjunction during meiosis and recurrence risk in this is almost 0.5-0.7%.[53] In case of parental Robertsonian translocations involving Group G/D (chromosomes 13,14,15 and 21,22) there are higher chances of recurrences depending on parental origin. In case of maternal carrier, the chances may be up to 15% and lesser in case of paternal carrier (<1%), possibly due to poor survival of sperms with an extra chromosome 21. In case of chromosome 21;21 translocation, there is 100% risk of recurrence.[54]

Prenatal screening and diagnosis

Screening for most common aneuploidies (trisomy 13,18,21) is now included as a part of routine antenatal care and offered to women of all ages.[55] First trimester combined screening includes measuring nuchal translucency by ultrasound and serum markers (PAPP-A and beta HCG) performed between 11-13+6 weeks. The sensitivity of this combined test ranges from 85-95% with false positive rate of 5-7%.[56,57,58] Second trimester screening is another option particularly for antenatal women presenting late which includes triple (HCG, uE3, AFP) and quadruple markers (HCG, uE3, AFP and inhibin A) done from 15-20th week of gestation. It has been found that quadruple test has more sensitivity (~83%) than triple test (65-77%) for screening aneuploidies in second trimester with false positive rate of 5%.[59,60] Although strategies like integrated test and sequential test (combined first and second trimester) are also available with results of around 95%, it is less favored as the results are available in 2nd trimester and requires multiple sampling and hence more visits to hospital. Consequently, first trimester combined screen is the preferred method due to more sensitivity and the availability of results in early gestation (Table II).[61,62] Screen positive patients are to be offered diagnostic tests with chorionic villus sampling (CVS) or amniocentesis. These tests carry a small risk of fetal loss i.e. 0.1%- 0.3% in amniocentesis and 0.22% for CVS

Table II. Screening options for Down syndrome and their detection rates[62]

Screening test	Detection rate (%)
First Trimester[a]	
• NT measurement alone	64-70
• NT, PAPP-A, nCG	82-87
Second Trimester[b] (Serum)	
• Triple screen (AFP, hCG, UE3)[c]	69
• Quad screen (AFP, hCG, UE3, inhibin-A)	81
Integrated screening options	
• First and second trimester serum only	85-88
• NT + first and second trimester serum	94-96
Other options	
• **Stepwise sequential screen**	
If first trimester positive screen, offer diagnostic test	95
If first trimester negative screen, continue as integrated screen	
• **Contingent/first trimester only**	
If first trimester positive, offer diagnostic test	88-94
If first trimester negative, no further screening	

[a]*First trimester: 11-14 weeks' gestation*

[b]*Second trimester: 15-22 weeks gestation*

[c]*No longer standard of care in United States*

AFP - Alpha fetoprotein; hCG - human chorionic gonadotrophin; NT - nuchal thickness; PAPP-A - pregnancy-associated plasma protein-A; UE3 - unconjugated estriol

though this would vary with the experience and expertise of the operator as any procedure will have a learning curve.[55]

Recently, non invasive prenatal testing/screening (NIPT/NIPS) using cell free fetal DNA (cffDNA) in maternal blood is introduced as a new screening tool for aneuploidies. Cell free fetal DNA constitutes about 10% of total cfDNA in maternal blood and can be detected as early as 10 weeks of pregnancy. Massive parallel shotgun sequencing is the preferred method used to diagnose aneuploidies in fetal DNA.[63]

At present NIPT is recommended to study only trisomy 13, 18 and 21 along with sex chromosome aneuploidy in high risk women. These include women aged 35 years and more, previous child with aneuploidy, positive serum marker screening test or high risk due to presence of soft markers on ultrasound.[64] According to a meta analysis done by Taylor-Phillips S, et al the sensitivity for detecting Down, Edward and Patau syndrome is 99.9%, 97.4% and 97.4% respectively, with same specificity of up to 99.9%.[65] Also research projects are going on to expand its applications and improve diagnostic yield using chromosomal microarray and whole genome sequencing.

Patients in whom NIPT fails to give any result due to low fetal fraction (called as "no calls"), must be confirmed by diagnostic tests, as repeat testing may not be beneficial in such cases. Such group of patients is at a high risk for aneuploidies (6.5%) and poor obstetrics outcome.[66] All positive cases after NIPT have to be confirmed by invasive testing. Detailed counseling of the couple explaining the availability of a battery of tests and sensitivity/specificity and predictive value of each test is essential so that they can take an informed decision.

Down syndrome though first described in 1866, its pathophysiology still remains elusive though newer concepts are emerging. No definitive therapies are available till now. With better understanding and management of co-morbidities, along with health supervision in children with Down syndrome (Table III)[67] individuals with DS can lead a purposeful and happy life.

Table III. Health supervision for children with down syndrome[67]

Condition	Time to screen	Comment
Congenital heart disease	Birth, by pediatric cardiologist Young adult for acquired valve disease	50% risk of congenital heart diseases increased risk for pulmonary hypertension
Strabismus, cataracts, nystagmus	Birth or by 6 mo; by pediatric ophthalmologist check vision annually	Cataracts occur in 15%, refractive errors in 50%
Hearing impairment or loss	Birth or by 3 mon with auditory brainsterm response or otoacoustic emission testing; check hearing q6 mon up to 3 yr if tympanic membrane is not visualized; annually thereafter	Risk for congenital hearing loss plus 50-70% risk of serous otitis media
Constipation	Birth	Increased risk for Hirschsprung disease
Celiac disease	At 2 yr or with symptoms	Screen with IgA and tissue transgiutaminase antibodies
Hematologic disease	At birth and in adolescence or if symptoms develop	Increased risk for neonatal polycythemia (18%) leukemoid reaction, leukemia (<1%)
Hypothyroidism	Birth; repeat at 6-12 mon and annually	Congenital (1%) and acquired (5%)
Growth and development	At each visit Use Down syndrome growth curves	Discuss school placement options Proper diet to avoid obesity
Obstructive sleep apnea	Start at ~1 yr and at each visit	Monitor for snoring, restless sleep
Atlantoaxial subluxation or instability (incidence 10-30%)	At each visit by history and physical exam Radiographs at 3-5 yr or when planning to participate in cantact sports Radiographs indicated wherever neurologic symptoms are present even if transient (neck pain, torticollis, gait disturbances, weakness) Many are asymptomatic	Special Olympics recommendations are to screen for high-risk sports, e.g., diving, swimming, contact sports
Gynecologic care	Adolescent girls	Menstruation and contraception issues
Recurrent infections	When present	Check IgG subclass and IgA levels
Psychiatric, behavioural disorders	At each visit	Depression, anxiety, obsessive compulsive disorder, schizophrenia seem in 10-17% Autism spectrum disorder in 5-10% Early-onset Alzheimer disease

Points to Remember

- ***Molecular mechanisms for phenotypic expression of DS.***
- ***Management of uncommon morbidities.***
- ***Current research in therapy.***

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CLIPPINGS

Selecting Appropriate Toys for Young Children in the Digital Era.

The report aims to address the evolving replacement of more traditional toys with digital media-based virtual "toys" and the lack of evidence for similar benefits in child development. Recent data presented in 2015 suggests that 96.9% of children have used mobile devices and most started using them before 1 year of age. For young children, the increase in screen time, is associated with a decrease in play, including both active play and play with toys. The best toys are those that support parents and children playing, pretending and interacting together. You just don't reap the same rewards from a tablet or screen. This is especially significant for young children's development because screen time directly interferes with both play activities and parent-child interactions, Following to be informed to the parents or caregivers to know while using toys for their kids. Most important purposes of play with toys is not educational at all but rather to facilitate warm, supportive interactions and relationships. Scientific studies supporting a developmental role for toys primarily come from studies of activities in which children play with caregivers rather than alone. Choose toys that will grow with the child, foster interactions with caregivers, encourage exploration and problem-solving, and spark the child's imagination. Keep in mind that toys are not a substitute for warm, loving, dependable relationships. Limit video game and computer game use. Total screen time, including television and computer use, should be less than 1 hour per day for children 2 years or older and avoided in children 18 to 24 months of age. Children younger than 5 years should play with computer or video games only if they are developmentally appropriate, and they should be accompanied by the parent or caregiver. Seek out toys that encourage the child to be both mentally and physically active.

Healey A, Mendelsohn A. APP COUNCIL ON EARLY CHILDHOOD. Selecting Appropriate Toys for Young Children in the Digital Era. Pediatrics 2018. Clinical report from The American Academy of Pediatrics. http://pediatrics.aappublications.org/content/early/2018/11/29/peds.2018-3348 on 15.12.2018.

GENETICS

FRAGILE X SYNDROME: WHAT A PEDIATRICIAN HAS TO KNOW?

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Abstract: *Fragile X syndrome is the most common cause of inherited intellectual disability caused due to a mutation in fragile X mental retardation 1 gene on the X chromosome. The clinical features of fragile X syndrome in affected males include severe intellectual disability, peculiar facial features, joint hypermobility, macroorchidism, seizures and neuropsychiatric abnormalities. Females with fragile X syndrome have milder intellectual disability and cognitive abnormalities because of one normal copy of the gene on X chromosome. Molecular diagnosis of this condition is possible by southern blot or triplet primed polymerase chain reaction and is essential for providing genetic counseling and prenatal diagnosis. This article is a brief review on clinical features, molecular diagnosis and genetic counseling issues in fragile X syndrome in pediatric practice.*

Keywords: *Intellectual disability, Fragile X, Southern blot, Prenatal diagnosis.*

Fragile X syndrome (FXS) is the second most common cause of intellectual disability after Down syndrome and the most common cause of intellectual disability in males.[1] The worldwide prevalence of FXS is 1 in 4000 to 5000 men and the prevalence in women is presumed to be half of the prevalence in men.[2]

The condition is more severe in males compared to females due to the presence of the normal copy of the fragile X mental retardation 1 (*FMR1*) gene in females. The mutation responsible for the condition in more than 99% of patients is a trinucleotide expansion of CGG in the 5' untranslated region of the *FMR1* gene on the X chromosome. This leads to hypermethylation in the promoter region of the gene, thereby silencing the gene with decreased production of FMR1 protein leading to the clinical symptoms. When a full mutation is present, it results in a cytogenetically detectable fragile site, named as FRAXA on X chromosome at Xq27.3 and hence the condition is named 'Fragile X syndrome'.

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Genetics

More than 99% of FXS is caused due to an expansion of unstable CGG repeats in the 5' UTR (untranslated region) of the *FMR1* gene on the X chromosome. A small percentage of FXS is caused due to deletions and single nucleotide variants in FMR1 gene. Depending on the number of repeats, the allelles are classified into normal, intermediate (gray zone), premutation and full mutation alleles. Table I shows the different effects of these alleles in males and females.

Normal alleles: These have approximately 5-44 CGG repeats and are stable during transmission from one generation to the other. At every 9 to 10 repeat blocks, CGG repeats are normally interrupted by AGG repeats which prevents the DNA segment from repeat expansion.[3]

Intermediate/gray zone: This constitutes approximately 45-54 repeats. Around 14% of these alleles are unstable and when transmitted by the mother, have the propensity to expand to premutation range.[4]

Premutation: The number of CGG repeats in premutation alleles is usually between 55 and 200. Premutation alleles are not associated with fragile X syndrome but may cause premature ovarian failure in females and fragile X tremor ataxia syndrome (FXTAS) in both males and females. Females who are carriers of premutation are at risk of having children with fragile X syndrome due to the chance for expansion of this allele. A premutation allele with 56 repeats is the smallest known repeat, which can expand to full mutation.[5]

Full mutation: The fully expanded allele has more than 200 repeats, which results in hypermethylation and thus silencing of the gene. This leads to the fragile X syndrome phenotype.

Table I. Types of FMR1 alleles and their effects

Type of allele	Effect in males	Effect in females	Risk to offspring
Full mutation (>200 repeats)	Severe intellectual disability in 100%	Intellectual disability in 50%; normal intellect in 50%	Males with full mutation have severe intellectual disability and generally do not reproduce. Offspring of females with full mutation have 50% risk of inheriting full mutation
Premutation (55-200)	At risk for fragile X tremor ataxia syndrome	At risk for premature ovarian insufficiency and fragile X tremor ataxia syndrome	Males with premutation are 'transmitting carriers' and transmit premutation to all daughters and none of the sons. Female premutation carriers have 50% risk of transmitting abnormal allele (premutation / full mutation)
Intermediate alleles (45-54)	None	None	14% can expand to premutation when transmitted by females

Fragile X syndrome - When to suspect

Newborn: It is difficult to diagnose fragile X syndrome because the babies do not have any distinct physical features at birth. In the newborn period, most of the physical characteristics of this condition become apparent as age advances.

Infancy and childhood: Affected children can have global developmental delay and behavioral abnormalities. FXS is the most common single gene cause for autism accounting for 2%-6% of all patients with autism.[6] Both male and females with FXS can have intellectual disability and a wide range of learning disabilities. Affected children can have hypotonia, recurrent otitis media and gastroesophageal reflux disease in infancy.

Prepubertal children: Affected males may have a large occipitofrontal circumference, which would become apparent in prepubertal period. Macroorchidism becomes apparent by 8 years of age and by mid adolescence, the testicular size becomes two to three times the normal.[7]

Post-pubertal children: The peculiar facial features like prominent jaw, large ears, long face, joint hyperextensibility and pes planus in affected males become apparent as age advances. Various behavioral abnormalities like poor eye contact, easy distractibility and poor impulse control become apparent. These individuals can also have joint laxity, soft skin, strabismus and mitral valve prolapse. Females who are heterozygous for full mutations can have milder clinical features when compared to males.

FMR1 related primary ovarian insufficiency: This should be suspected in a woman with cessation of menstruation before 40 years of age and one permutation FMR1 allele.

Fragile X associated tremor ataxia syndrome (FXTAS): Male and female premutation carriers, generally above 50 years of age, can present with ataxia, tremor, proximal muscle weakness, peripheral neuropathy, white matter signal changes on MRI brain and Parkinsonism, which is termed as FXTAS.

FMR1 mutations - Whom to test[8]

- Boys and girls with intellectual subnormality, developmental delay and behavioral disorders like autism, with or without family history of Fragile X syndrome.
- Women who have premature ovarian failure or a family history of premature ovarian failure, family history of Fragile X syndrome or undiagnosed mental subnormality in male or female relatives.
- Men or women with late onset of tremor, ataxia, cognitive impairment and features of Parkinsonism, especially if they have relatives with undiagnosed mental subnormality, movement disorders or fragile X syndrome.

Suspected fragile X syndrome - How to evaluate

An infant with fragile X syndrome should in particular be evaluated for feeding difficulties, hypotonia, seizures and recurrent ear infections. A detailed developmental evaluation is to be done along with psychological assessment to determine various behavioral abnormalities. A cardiac evaluation is warranted in patients with suspected mitral valve prolapse and an eye evaluation may be done in patients with strabismus.

Diagnostic testing

(i) Chromosome analysis: Chromosome analysis to check for fragile sites on X chromosomes has become an obsolete test with the advent of molecular diagnostic test.

(ii) Molecular testing

a. Southern blot analysis: This test can detect all the FMR1 alleles like normal, premutation and full mutation alleles. It can also determine the methylation status of the FMR1 promoter region. The major disadvantages are that it is labor intensive and difficult to interpret. Traditional PCR and Southern blot analysis were considered to be the gold standard of testing before the advent of newer diagnostic tests.

b. Triplet primed PCR (TP-PCR) (Fig.1): This technique was developed to overcome the limitations of PCR and Southern blot. It has become the gold standard for diagnosis and it can detect even mosaicism of expanded allele. This technique is most commonly used now for detection of triplet expansion. However, the disadvantage is that TP-PCR cannot detect the exact number of expanded repeats.

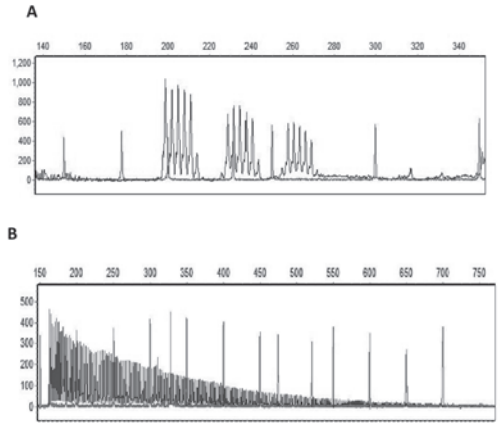

Fig.1.TP-PCR genotyping profile - A) normal person and B) Fragile X syndrome

c. Sanger sequencing: In rare instances when Fragile X syndrome is suspected and TP- PCR is normal, sequencing of the *FMR1* gene for identification of pathogenic variants can be done.

d. Multiplex ligation probe amplification (MLPA) for detection of deletion/duplications.

e. Methylation status by PCR based methods

Management

Though specific treatment is not yet available, supportive treatment in the form of behavioural therapy, management of seizures and other associated medical issues along with early intervention and special education may be done. Some novel targeted therapeutic strategies that are being tried are metabotropic glutamate receptors (mGluR) antagonists, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA receptor) positive modulators, γ-aminobutyric acid B (GABA B) agonists like lithium and baclofen, donepezil and minocycline.

Genetic counseling: Fragile X syndrome is inherited in an X linked dominant manner. Since the counseling is tricky and a bit more complicated, it is advisable to refer such families to a medical geneticist for counseling. Mothers of all affected males with full mutation are carriers of the same mutation or harbor a premutation in FMR1 gene and they can be affected, even though with a lesser severity. A 'transmitting male' is the one with a premutation whose daughters inherit the premutation but none of the sons do. When a male transmits a premutation, it never becomes an expanded full mutation and all his daughters become premutation carriers. Men who are carriers of premutation are at risk of developing FXTAS after 50 years of age. Women who are carriers of premutation are at risk for premature ovarian failure and FXTAS. A woman who is a premutation carrier has a 50% chance in transmitting an abnormal allele (premutation or full mutation) in every pregnancy. The risk of the premutation expanding to a full mutation depends on the number of CGG repeats and number of AGG repeats. It is difficult to predict the severity of involvement in case of a female offspring because of the wide variability in severity.

Carrier testing: This can be done in at-risk females in a family with an affected patient. Once carrier status is confirmed, prenatal diagnosis can be provided in subsequent pregnancies for at-risk individuals.

Points to Remember

- ***Fragile X syndrome is a common cause of inherited intellectual disability.***
- ***Males are severely affected than females.***
- ***Molecular diagnosis is essential for genetic counseling and providing prenatal diagnosis.***

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CLIPPINGS

Risk Stratification of Febrile Infants <60 Days Old Without Routine Lumbar Puncture.

Evaluation of the Rochester and modified Philadelphia criteria for the risk stratification of febrile infants with invasive bacterial infection (IBI) who do not appear ill without routine cerebrospinal fluid (CSF) testing was undertaken. A case-control study of febrile infants <60 days old presenting to the emergency departments from 2011 to 2016 was undertaken. For each infant with IBI (defined as a blood [bacteremia] and/or CSF [bacterial meningitis] culture with growth of a pathogen), controls without IBI were matched by site and date of visit. 135 infants with IBI (118 [87.4%] with bacteremia without meningitis and 17 [12.6%] with bacterial meningitis) and 249 controls were included. The sensitivity of the modified Philadelphia criteria was higher than that of the Rochester criteria (91.9% vs 81.5%; $P = .01$), but the specificity was lower (34.5% vs 59.8%; $P < .001$). Among 67 infants >28 days old with IBI, the sensitivity of both criteria was 83.6%; none of the 11 low-risk infants had bacterial meningitis. Of 68 infants <28 days old with IBI, 14 (20.6%) were low risk per the Rochester criteria, and 2 had meningitis. Study concluded the following. The modified Philadelphia criteria had high sensitivity for IBI without routine CSF testing and all infants >28 days old with bacterial meningitis were classified as high risk.

Aronson PL, Wang ME, Shapiro ED, Shah SS, DePorre AG, McCulloh RJ, et al, for the Febrile Young Infant Research Collaborative. Risk Stratification of Febrile Infants <60 Days Old Without Routine Lumbar Puncture. Pediatrics 2018; 142(6). Downloaded from http://pediatrics.aappublications.org/content/142/6/e20181879?download=true on 15.12.2018.

NEWS AND NOTES

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GENETICS

CONSTITUTIONAL CHROMOSOMES – FROM KARYOTYPING TO CHROMOSOME MICROARRAY IN PEDIATRIC PRACTICE

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Abstract: *Chromosomal abnormalities are one of the causes of multiple malformations with or without intellectual disability in children. Knowing and identifying underlying chromosomal abnormality requires prior knowledge of clinical chromosomal phenotype (indications to study chromosomes) and selection of appropriate chromosomal tests to diagnose. Definitive diagnosis forms the basis for proper management and genetic counselling. This article discusses briefly about the types of constitutional chromosomal abnormalities, methods to study chromosomes and management including genetic counselling.*

Keywords: *Karyotype, Chromosome analysis, Chromosome microarray, Congenital anomalies, Intellectual disability, Molecular cytogenetics.*

Specialised organizations of deoxyribonucleic acid (DNA) along with covering protein (DNA + Protein = Chromatin) during cell division are called chromosomes. Every cell of living organism contain distinct DNA, passed on from parent to offspring in certain way though meiosis. Abnormal meiosis result in chromosomal abnormalities. Chromosomal abnormalities can be constitutional (by birth) or acquired (in specific cancer tissue). Constitutional chromosomal abnormalities account for around 1 in 135 to 1 in 150 of live-births (includes all forms of balanced/ unbalanced chromosomal abnormalities detected by standard chromosome analyses - referred to as classic chromosomal disorders).[1,2] Chromosomal variants identified through molecular cytogenetics called as copy number variants (CNVs) may occur in many healthy individuals (benign) or in those with intellectual disability and/or congenital anomalies (pathogenic). In almost all the pathogenic chromosomal abnormalities (both classic chromosomal disorders and CNVs), many genes are either deleted or duplicated.

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Methods of chromosome analysis

Study of chromosomes can happen at different resolutions. Table I compares the different methods of studying chromosomes with 'increasing resolution', along after resolutions with use, limitations and time required to generate the report.

Standard chromosome analysis (SCA): Chromosomes are studied by different laboratory techniques (banding and staining) using microscope. The commonly used is Giemsa staining or G-banding. Here, the lymphocytes collected from the heparinised peripheral blood sample are stimulated by mitogen - phytohemagglutinin and arrested lymphocyte metaphases using colchicine are studied under the microscope after G - banding. Metaphase spread as seen under microscope is given in Fig.1. Chromosomes are arranged using computer tools and the systematised

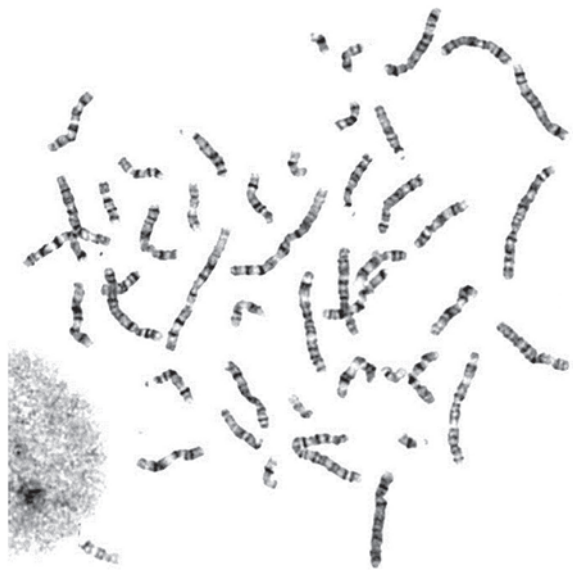

Fig.1. Metaphase spread as seen under microscope of 100X magnification

Table I. Comparison of various chromosomal tests

Characteristic	Standard chromosome analyses	Fluorescent in-situ hybridization	Chromosome microarray
Specimen required	Sodium heparinised blood	Sodium heparinised blood	Ethylene diamine tetraacetic acid blood
Cell culture	Required	Required	Not required
Type of chromosomal anomaly detected	Large deletion and duplications of >4 Mb size, mosaics, balanced chromosomal rearrangements	Targeted microdeletion / microduplications, cryptic balanced translocations	CNVs *- microdeletions/ microduplications of < 4 Mb size, uniparental disomy, region of homozygosity
Type of chromosomal anomaly which cannot be detected	CNVs, point mutations, epigenetic alterations	Point mutations, epigenetic alterations	Point mutations, epigenetic alterations, very low level mosaics, balanced chromosomal rearrangements
Chromosomal resolution	>4 Mb	>30 kb	>5-50 kb**
Turnaround time (TAT)	7-10 days	4-7 days	1-2 weeks
Examples of clinical scenario and preferred use of text	Recurrent spontaneous abortions, infertility, known obvious syndromes e.g. Down syndrome	Identify small deletion less than 4 Mb size with known deletion phenotype	Unexplained intellectual disability, autism spectrum disorders, congenital anomalies

**CNVs – Copy number variants , **Depends on the chromosomal microarray platform used*

array of chromosome arrangement is called karyogram (Fig.2). The nomenclature of writing the constitutional normal or abnormal chromosome of an individual is called karyotype. 46 XX is normal female and 46 XY is normal male constitutional karyotype. Though various numerical and structural chromosome abnormalities can be identified using SCA, structural chromosome abnormalities of size <4 Mb cannot be identified by SCA.

Fluorescent in-situ hybridisation (FISH): This test "maps" the genetic material in a person's cells, which can be used to visualize specific genes or portions of genes. Small deletions / duplications of less than <4 Mb size can be identified through molecular techniques – FISH or Multiple Ligation dependent Probe Amplification (MLPA). Fluorescent labelled locus-specific probe is hybridised on to the patient chromosome (within interphase cells or metaphase spreads) to identify clinically questioned deletion or duplication. FISH is commonly used in cytogenetic laboratory to identify small deletions.

Fig.2. Normal male karyogram

Since FISH is a targeted test, prior knowledge of deletion clinical phenotype is necessary. FISH also provides the option for the simultaneous use of one or more DNA probes by labeling different colors or color combinations (e.g. Whole chromosome paint probes in patients with complex chromosomal rearrangements or to trace origin of marker chromosome, etc).[3]

Chromosome microarray (CMA): Simultaneous genome-wide study of CNVs is done by CMA. Here, prior knowledge of clinical phenotype of precise pathogenic CNVs is not mandatory other than wide clinical indications to carry out CMA test as described below. Genomic imbalances (CNVs) are studied using multiple labelled (fluorophore) oligoneucleotide probes (with known genomic locations, probes are gridded on to the chip) with additional information of allelic genotype information [Single nucleotide polymorphism (SNP) arrays] or without allelic genotypic information [array-comparative genomic hybridization (CGH)] or combination of both. Patient's DNA is prepared and hybridized on to the chip and analysed for signal intensities using computer tools. Fig.3 shows heterozygous microdeletion at cytoband 17p11.2 identified through CMA (Illumina cytochip). Among available platforms of microarrays, SNP based arrays are widely used for the reason described later in the article.

Types of chromosome abnormalities

Chromosomal abnormalities could be those identified by standard chromosome analysis (numerical and structural abnormalities) and those identified by molecular cytogenetics techniques (micordeletions and microduplications, collectively called as copy number variations).

Chromosome abnormality identified by SCA

Numerical chromosomal abnormalities: Aneuploidy - one or more whole chromosome is extra or less. It could be autosomal aneuploidy or sex chromosome aneuploidy (Fig.4 Lane I). Polyploidy – complete sets of whole chromosomes are extra in single or multiple (Triploidy, Tetraploidy).

Structural chromosomal abnormalities: Translocations are defined as exchange of chromosome segment (either whole or part of arm) between two or more chromosomes. Fusion of two acrocentric chromosomes (long arms) is called Robertsonian translocation (Fig.4 - Lane II, d-f). Exchange of chromosome segments involving two chromosomes (other than acrocentric chromosomes) is called reciprocal translocation (Fig.4 - Lane II, a-c). Carrier frequency of autosomal translocations in general population ranges from 1 in 500 to 1 in 1000.[4,5,6] Carrier frequency of Robertsonian translocation in general population is 1 in 1000.[4,6] Individual with balanced (no gain or loss of genetic material) translocation does not show any clinical manifestation. However, they are at risk of infertility, recurrent spontaneous abortions, stillbirth and live born child with multiple malformation syndrome with or without intellectual disability (due to derived unbalanced

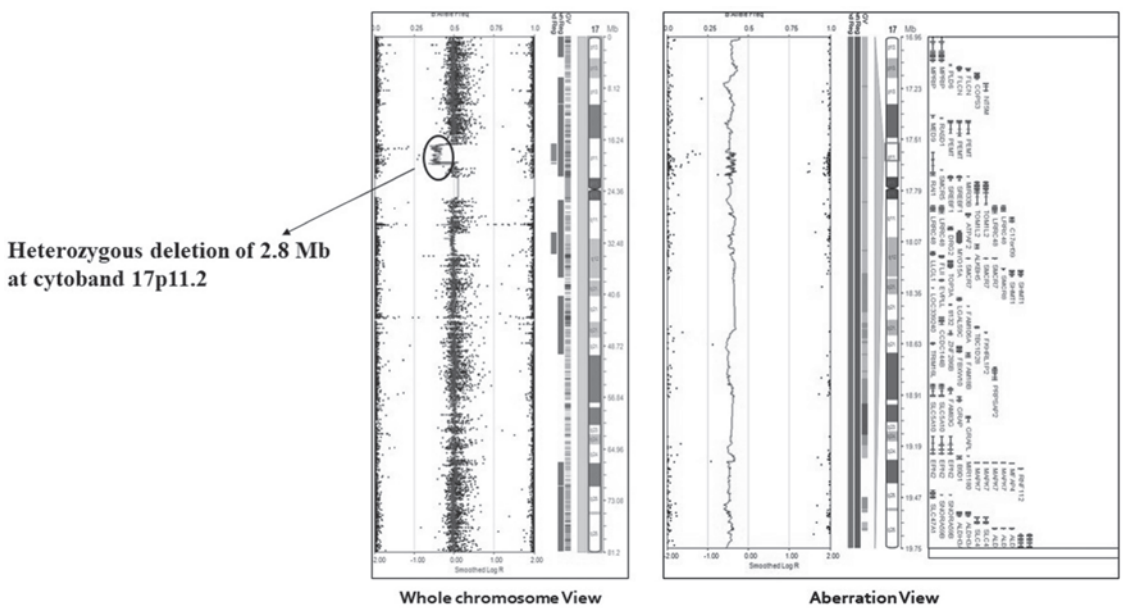

Fig.3. Chromosome microarray results showing heterozygous deletion of 2.8 Mb size at cytoband 17p11.2 (Illumina cytochip)

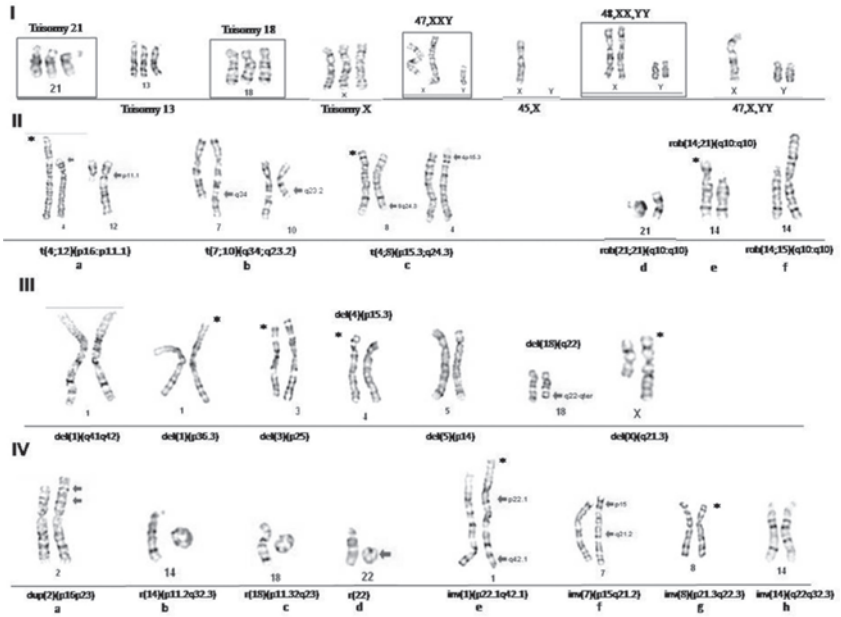

Fig.4. Partial karyotype of chromosomal abnormalities identified through standard chromosome analysis. Lane I – aneuploidies, Lane II – translocations, Lane III – deletions, Lane IV – duplication, ring chromosomes & inversions.

**Indicate abnormal chromosome is on the left side of the pair.*

chromosomal rearrangements – partial aneuploidies). In addition Robertsonian translocation might pose a risk of uniparental disomy. Rarely individuals with balanced translocations may manifest with clinical phenotype related to genes at the breakpoints [if breakpoints in the involved chromosomes cross through and/or disrupt functional gene(s)].

Deletion and duplications: Loss of a segment of chromosome is called deletion. When there is an extra copy of a segment of chromosome, it is called duplication. Large deletions and duplications of more than 4-5 Mb size can be visualised by SCA (Fig.3 – Lane III). Deletions could be terminal or interstitial (Fig.4 – Lane IV, a). Duplications could be tandem or an insertion. In general, deletions are more common and deleterious than duplications.

Ring chromosome: Fusion of ends (ends of p and q arm) of a chromosome due to breaks in both the arms results in formation of ring chromosome. Extent and content of loss of genetic material due to breaks at both ends and fusion, decides the clinical phenotype (Fig.4 – Lane IV, b - d).

Inversions: A segment of chromosome is inverted due to two breaks in a single chromosome. Inversions can be of two types (a) Pericentric inversion results in flip of chromosome segments across the centromere (Fig.4 - Lane IV, e.g.) and (b) Paracentric inversion results in flip within one arm of chromosome, not involving centromere (Fig.4 – Lane IV, h). Reported occurrence rates of pericentric inversions is 0.12 -0.7% and paracentric inversions is 0.1-0.5%.[5,6,7] Inversion carriers are asymptomatic like balanced translocation carriers. They are at risk of recurrent spontaneous abortions and offspring with multiple malformations.

Isochromosome: a unique type of chromosomal abnormality where one of the arm of a chromosome is deleted and the other arm gets duplicated. It could be isochromosome for short arm (p) or isochromosome for long arm (q).

Mosaic chromosomal abnormalities: Two or more cell lines with different constitutional chromosomes arising from the same zygote are referred as mosaic chromosomal abnormalities. Clinical phenotype in children with chromosomal mosaicism is highly variable as the phenotype depends on chromosome involved and percentage of cells carrying abnormal chromosome complement as against cells carrying normal chromosomes in different tissues.

Chromosome abnormalities identified through molecular cytogenetics

Copy number variants (CNVs): CNVs are defined as segments of DNA of size ranging from few hundred base pairs (usually >1kb) to millions of base pairs either

duplicated or deleted. CNVs result in copy number change of genes on a particular chromosome (gain or loss). CNVs could be inherited or de-novo (sporadic) and benign (normal variant) or pathogenic. Pathogenic CNVs have clinically recognizable phenotype. Some of the common pathogenic CNVs phenotype is relatively easy to recognize, wherein one uses targeted FISH or multiplex MLPA genetic tests to diagnose underlying microdeletion or microduplications. Since many of the pathogenic CNVs phenotypes are not easily recognizable due to subjective differences in diagnosis and overlapping features, genome-wide study of CNVs (Chromosome microarray) is suggested. In addition, CMA done using SNP based microarrays, would be useful in giving additional information about the size of CNV, gene content and regions of homozygosity (may indicate uniparental disomy, useful in getting clue to autosomal recessive genes in consanguineous families).

Chromosomal disorders and chromosome analysis

Chromosome analyses can be done in postnatal or prenatal clinical scenarios. Chromosomal disorders can be suspected in a child with developmental delay, intellectual disability, autism spectrum disorders, multiple malformations (major/minor), growth abnormalities (especially prenatal onset) and facial dysmorphism. Most of the chromosomal disorders have recognizable pattern of malformations, especially facial dysmorphism. In some of the chromosomal disorders (both classical and CNVs), phenotype is normal or show mild clinical manifestations (reduced penetrance and variable expression). Size of the genomic imbalances, mosaicism, gene content, second-hit (additional CNV and/or single gene mutation) and possible environmental factors have been suggested as causes for reduced penetrance and variable expression among chromosomal disorders.[8,9,10]

Facial phenotype (also commonly called as facial gestalt) of most chromosomal disorders is recognisable and is an important clinical clue to diagnose and investigate. Facial gestalt in chromosomal disorders might change with age and can show clinical variation [(severe/subtle) (interfamilial/intrafamilial variability)].[10,11] In some of the chromosomal disorders (especially those with pathogenic CNVs), facial phenotype might be near normal or subtle. Fig.5 depicts facial dysmorphism among chromosomal disorders. Gross chromosomal abnormalities identified through SCA, tend to have relatively more severe facial dysmorphism (Fig.5a-c,e) and CNVs identified through molecular cytogenetics tend to have relatively subtle facial dysmorphism (Fig.5d,f-i). Sometimes facial phenotype in the same type of CNVs tends to be variable [(interfamilial variablity in Fig. 5d/i with deletion 22q11.2 and f/h deletion 7q11.23) (relatively subtle facial gestalt in 4p deletion syndrome in spite of visible deletion by SCA in Fig.5j)].

Postnatal chromosome analyses

Postnatal chromosome analyses can be done using different methods (Table I). The choice of methods to study

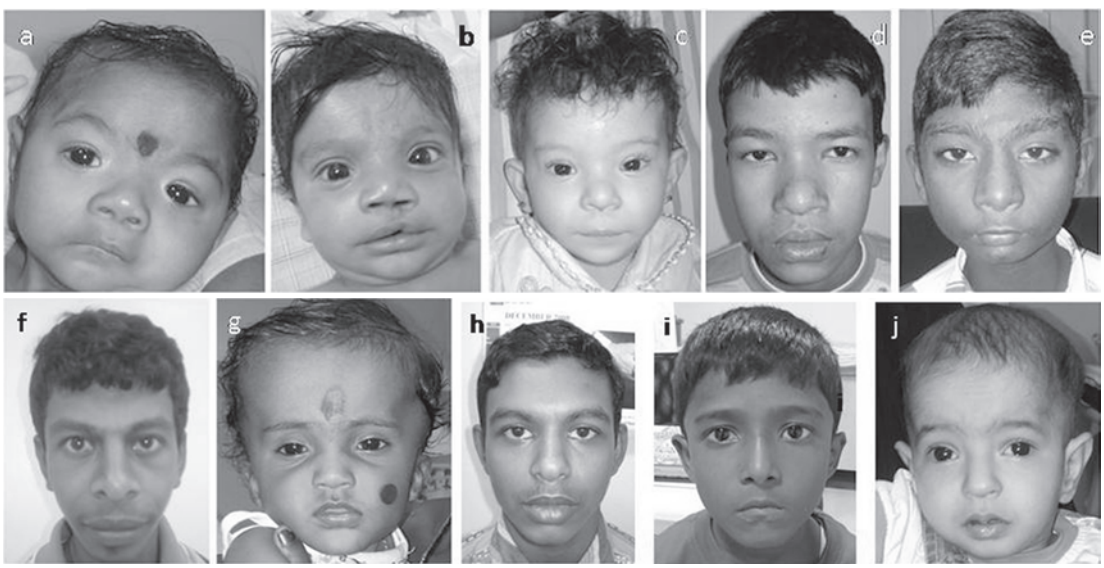

Fig.5. Facial phenotype of chromosomal disorders: a - del(5)(p14), b – partial trisomy 8q23/ partial monosomy 14q32.3, c - del(3)(p25), d – del(22)(q11.2), e – mos r(18) (p11.32q23) [55]/ 45,XY,-18[45], f – del(7)(q11.23), g – dup(7)(q11.23), h - del(7)(q11.23), i – del (22)(q11.2), j – del(4)(p16.3p16.2)

chromosomes depends on clinical impression. If clinical impression is of known obvious syndrome e.g. Down syndrome, Turner syndrome, then SCA should be suffice.

SCA is the preferred test in couples with recurrent spontaneous abortions, infertility and familial chromosomal rearrangements, as we expect balanced chromosomal rearrangements.

Most of the children with gross chromosomal abnormality [numerical (both non-mosaic and mosaic) and structural (partial aneuploidy)], will have multiple malformations (both major and minor), growth retardation and obvious/severe facial dysmorphism. In such patients SCA might be useful to begin with. The indications for chromosomal analysis are given in Box 1.

CMA is recommended as first tier test in patients with unexplained intellectual disability with or without malformations, autism spectrum disorders and congenital anomalies, as CMA is known to increase the diagnostic yield up to 20%. The selection of chromosomal tests depends on clinical impression, availability and mostly the cost (in places where patient pays for the tests).

Prenatal chromosome analyses

Prenatal chromosome analyses are indicated in following clinical scenarios: (a) fetus with malformations, (b) fetus at high risk for aneuploidy [(advanced maternal ages, positive for aneuploidy screening tests - maternal serum tests, noninvasive prenatal testing (NIPT)] and (c) parent with balanced chromosomal rearrangements. Prenatal chromosome analysis in high risk pregnancies using CMA increases diagnostic yield with concerns and challenges due to certain CNVs of unknown significance and phenotypic heterogeneity.

Box 1. Chromosome analyses - indications

1. Children with congenital malformations
2. Children with obvious known chromosomal syndromes likes Down syndrome, Turner syndrome, Klinefelter syndrome, etc.,
3. Children with unexplained intellectual disability/ developmental delay with or without malformations (minor or major)
4. Disorders of sexual development
5. Autism spectrum disorders
6. Parents of children with unbalanced chromosomal rearrangements, and extended family member screening in families with familial balanced chromosomal rearrangements.
7. Malignancy for diagnosis, prognosis and management.
8. In obstetric practice – couple with recurrent spontaneous abortions, male infertility, female infertility.

Management and genetic counselling

Children with congenital malformations need medical management and/or surgical correction. Those children with intellectual disability (ID) need referral to an intervention centre at an early age and school aged group children are referred to special schools for children with ID. In addition, children with chromosomal disorders are at risk of other co-morbidities (some are specific to underlying chromosomal disorder), which require periodic screening and follow up (e.g. Turner syndrome - short stature, congenital heart disease; Down syndrome - hypothyroidism, congenital heart disease, gastrointestinal anomalies; Williams syndrome - hypercalcemia, cardiovascular problems; Di-George syndrome - hypocalcemia, congenital heart disease, etc.). With medical and surgical managements, the numbers of adults with chromosomal disorders have increased and their follow up/management requires trained specialists.

Reproductive options to parents need to be discussed and is based on whether chromosomal disorder is de-novo or inherited. Inherited chromosomal disorders are an increased risk to the siblings of the affected child and extended family members. In most chromosomal disorders of de-novo origin, risk of recurrence is < 1% in view of gonadal mosaicism. In patients with inherited chromosomal disorders, risk of recurrence depends upon the type of chromosomal abnormalities. Various prenatal screening tests of aneuploidy (e.g. Trisomy 21, Trisomy 18 etc) are available for low risk pregnancies – maternal serum screening for biochemical markers, ultrasound markers and non-invasive prenatal testing using cell free fetal DNA in maternal plasma.

Points to Remember

- ***Common clinical presentation of chromosomal disorders in children includes congenital malformations, intellectual disability, growth abnormalities and facial dysmorphism.***
- ***Facial dysmorphism forms an important clue to diagnose and investigate for chromosomal disorder.***

- ***Resolution varies among the various chromosomal tests available to diagnose chromosomal disorders. Knowledge on use and limitation of each chromosomal test helps in selecting the appropriate test.***

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CLIPPINGS

Uninterrupted Infant Sleep, Development, and Maternal Mood.

Study was undertaken to investigate the proportion of infants who sleep through the night (6- or 8-hour sleep blocks) at ages 6 and 12 months in a longitudinal cohort and to explore associations between sleeping through the night, mental and psychomotor development, maternal mood and breastfeeding. At 6 and 12 months of age, maternal reports were used to assess the longest period of uninterrupted infant sleep and feeding method (n = 388). Two different criteria were used to determine if infants slept through the night: 6 and 8 hours of uninterrupted sleep. Mental and psychomotor developmental indices (Bayley Scales of Infant Development II) and maternal mood (Center for Epidemiologic Studies Depression Scale) were measured at 6, 12 and 36 months of age. 27.9% to 57.0% of 6- and 12-month-old infants did not sleep through the night uninterrupted for 6-8 hours. Linear regressions revealed no significant associations between sleeping through the night and concurrent or later mental development, psychomotor development, or maternal mood (P>.05). However, sleeping through the night was associated with a much lower rate of breastfeeding (P <.0001). The study concluded that considering that high proportions of infants did not sleep through the night and that no associations were found between uninterrupted sleep, mental or psychomotor development, and maternal mood, expectations for early sleep consolidation could be moderated.

Pennestri MH, Laganière C, Bouvette-Turcot AA, Pokhvisneva I, Steiner M, Meaney MJ, et al. Uninterrupted Infant Sleep, Development, and Maternal Mood. Pediatrics Dec 2018, 142 (6) e20174330; DOI: 10.1542/peds.2017-4330.

GENETICS

GENETIC COUNSELING AND PRENATAL DIAGNOSIS – A SYNOPSIS

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Abstract: *Genetic disorders are being diagnosed increasingly with the advent of novel molecular diagnostic methods. Hence, it becomes imperative for clinicians of all specialities to perfect the art of genetic counseling and plan and advice regarding antenatal testing as and when necessary. This article summarizes the fundamentals and methodology of genetic counseling and prenatal diagnosis.*

Keywords: *Prenatal diagnosis, Counseling, Genetic disorder, Antenatal testing.*

Genetic counseling

This term is used to refer to the two way communication process that happens between a genetic counselor and the person/persons who have come to seek advice for a genetic problem either for themselves, their children or some member in the family. Information about the nature of problems, mode of transmission, methods of prevention, treatment and support options need to be discussed. As the issues to be discussed are many, this can be done over 4 or more sessions. The indications for genetic counseling are given in Box 1.

There are certain terms to be familiarized while doing genetic counseling. A proband is a person with a genetic disorder in the family, a consultand is a person who has come to seek advice and an index case is a person with genetic problem presenting for workup.

Who can do genetic counseling?

A person who is trained in genetics - a doctor or a counselor is eligible. The person should be competent to handle complex genetic information with in-depth information, understanding of the latest developments, good communication skills and must be empathetic.

Box 1. Indications for genetic counseling

- A couple whose previous child/children have a genetic problem
- Siblings with a genetic problem
- Parent with a genetic problem
- Recurrent pregnancy losses
- Ongoing pregnancy with an anomalous fetus
- Screening tests for aneuploidy showing a high risk
- Advanced maternal age
- Teratogen exposure in pregnancy

Steps of genetic counseling

There are various time periods in which genetic counseling can be offered but the ideal time is pre-pregnancy period. It can be done during ongoing pregnancy and also done as premarital counseling. At the outset, the counselor introduces herself to the family who seeks advice. A clear understanding of the consultand's expectations should be understood and the exact outcome of a counseling session has to be clarified. The steps of genetic counseling are given in Box 2.

Information gathering

History taking: Starts with antenatal period of index, childbirth history, neonatal problems, current complaints, status of disease, clinical course, tests done so far and their results, medications - past and current including dosage

Box 2. Steps of genetic counseling

1. Information gathering
2. Giving information
3. Risk prediction
4. Reproductive options
5. Psychological support

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and response information, developmental milestones, education, speech, cognition, hearing, vision issues are all elicited and documented in a systematic way. A detailed three generation pedigree is also documented.

Physical examination: Examination is done with consent. Typically the index case is undressed and a detailed head to foot examination is carried out. Dysmorphic features if any are documented. This is followed by a thorough systemic examination. All findings are documented. Old photographs if available are reviewed. Fresh photographs and video if needed are done for documentation after informed consent is obtained.

Anthropometry: Every external structure in humans can be measured and there are nomograms according to age group. Usually head circumference, height and weight are recorded and plotted on standard growth charts.

III generation pedigree chart: This is a very essential part in genetic counseling. It is a visual diagram drawn using symbols, lines and abbreviations and can help reveal a pattern of inheritance, identify family members at risk and predict the risk of recurrence (Fig.1, 2 and 3).

Inheritance patterns and sample pedigrees

Understanding the basic laws of inheritance is important to appreciate how conditions are passed on in a family. Pedigree acts as a valuable tool to extract accurate family health history and illustrate how conditions are passed down through generations.

Autosomal dominant

In autosomal dominant pedigree both males and females are affected. The affected person has 50% chance

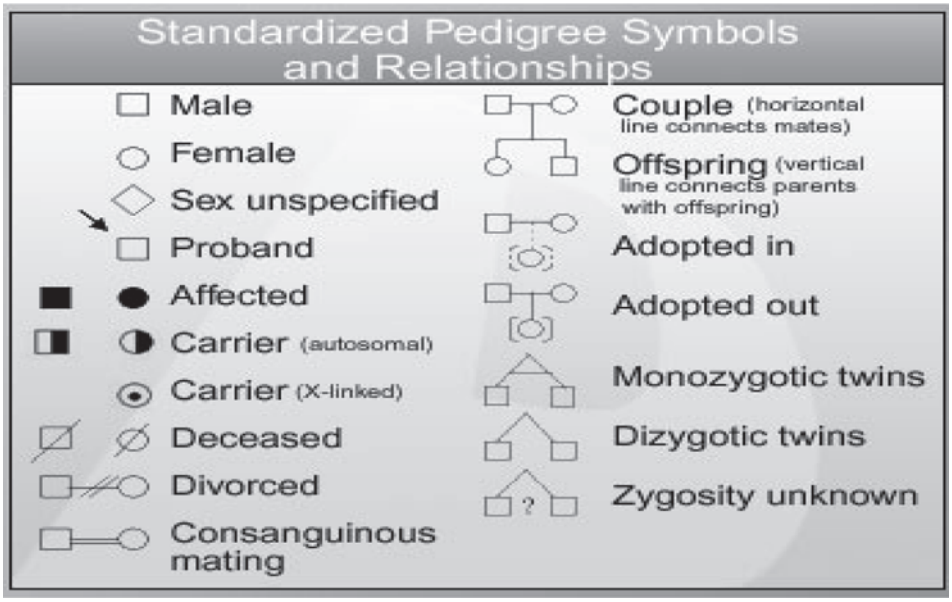

Fig.1. Standardized pedigree symbols used to represent individuals and relationships

	Male	Female	Gender not specified	Comments
1. Individual	b. 1925	30y	4 mo	Assign gender by phenotype (see text for disorders of sex development, etc.). Do not write age in symbol.
2. Affected individual				Key/legend used to define shading or other fill (e.g., hatches, dots, etc.). Use only when individual is clinically affected.
			With ≥2 conditions, the individual's symbol can be partitioned accordingly, each segment shaded with a different fill and defined in legend.	
3. Multiple individuals, number known	5	5	5	Number of siblings written inside symbol. (Affected individuals should not be grouped).
4. Multiple individuals, number unknown or unstated	n	n	n	"n" used in place of "?".
5. Deceased individual	d. 35	d. 4 mo	d. 60's	Indicate cause of death if known. Do not use a cross (†)to indicate death to avoid confusion with evaluation positive (+).

Fig.2. Symbols used to represent individuals in the pedigree

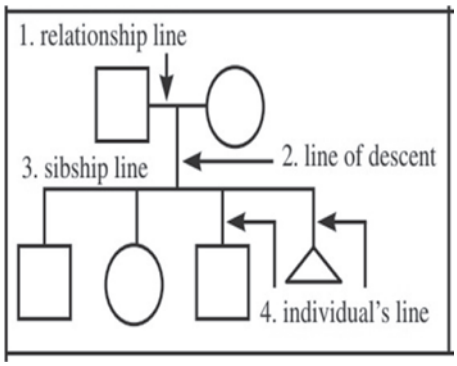

Fig.3. Definition of lines used to represent relationships in pedigree

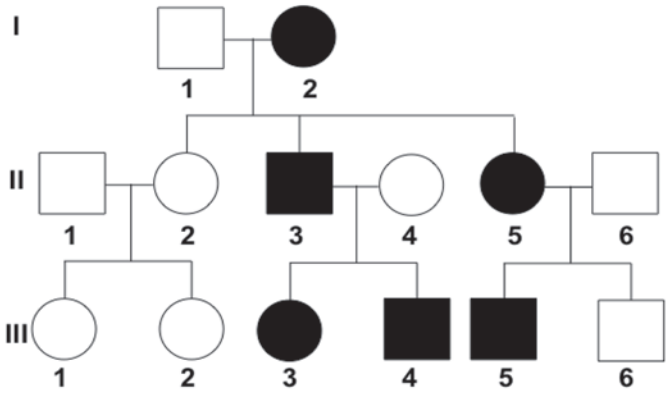

Fig.4. Autosomal dominant inheritance pattern

of transmitting the disease to each offspring. There is vertical transmission (Fig.4). (e.g. Achondroplasia, Marfan syndrome)

Certain unique features

- Incomplete penetrance - disease severity may not be the same down the generations. Some subtle features which may be noticed could be the only pointing feature toward autosomal dominant inheritance.
- Anticipation- Disease increases in severity and appear at an earlier age as it is transmitted down generations.

Autosomal recessive inheritance

In autosomal recessive inheritance, parents are carriers but are otherwise normal. As both parents are carriers, each offspring has a 25% chance of disease, 50 % chance of being carrier and 25% chance of being disease free (Fig.5). Examples are thalassemia, spinal muscular atrophy and most inborn errors of metabolism.

X-linked recessive inheritance

In X-linked recessive inheritance females alone

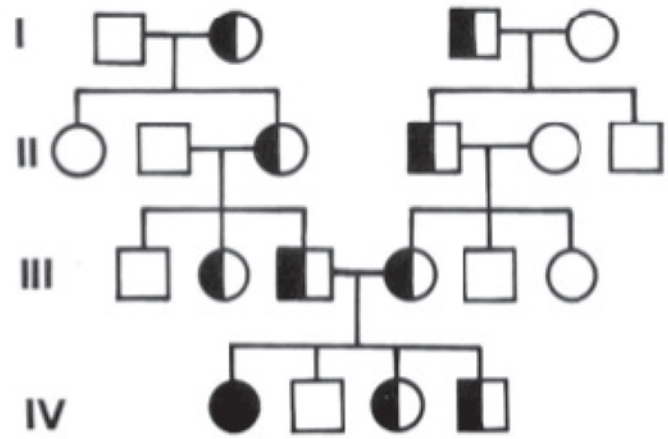

Fig.5. Autosomal recessive inheritance pattern

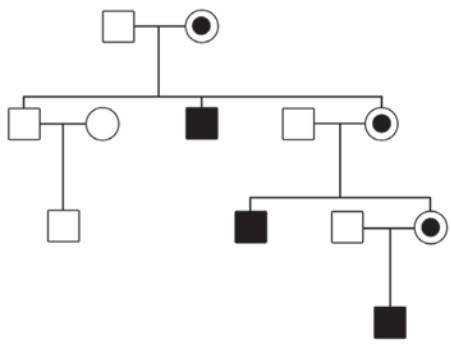

Fig.6. Pedigree representing X-linked recessive inheritance pattern

transmit disease. Carrier females do not manifest disease. Carrier female has a 50% risk of begetting an affected male and 50% risk of begetting a carrier female (Fig.6). Examples are Duchenne muscular dystrophy and hemophilia.

Mitochondrial inheritance

This refers to a mutation in mitochondrial DNA. In this inheritance affected female alone transmits the disease. All her male and female children will be affected.

Table I. Difference between autosomal dominant and X-linked recessive pattern of inheritance

Autosomal dominant	X-linked recessive
Males and females are affected equally	Males are affected and females are carriers
Occurs in every generation	Can skip generations

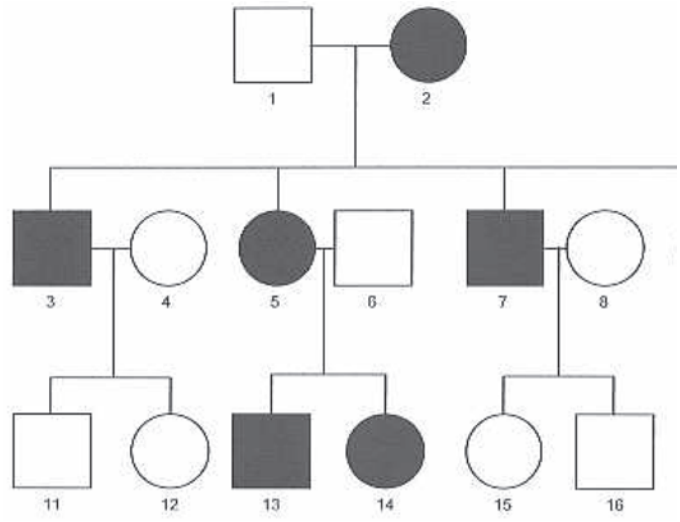

Fig.7. Mitochondrial inheritance pattern

The affected male does not transmit the disease (Fig.7). Please note that some of the mitochondrial functions are regulated by nuclear DNA and mutations of these genes behave like autosomal recessive inheritance.

Pedigree charting - Advantages

It helps to understand the degree of consanguinity and sharing of genes and predict the risk of recurrence for a consultand. Also one can observe the subtle manifestations of an autosomal dominant inheritance and other issues of concern in the family.

Investigations and cross-referrals

Investigations like MRI brain, EMG, echo, ophthalmic examination, ultrasound, radiology of bones, biochemical tests etc are done as the case may be and documented. Other subspecialty experts are consulted and their opinion about the diagnosis is sought. Finally based on all this information a provisional diagnosis is arrived at.

Giving information

This part is crucial in genetic counseling. The consultand without any prior information is easier to handle. But people with prior information from the internet or primary caregiver may or may not be having the right information. So first, we need to understand their prior knowledge. Complex medical jargon should be avoided and simplified information with illustration helps. Giving time for them to think about what they heard and providing a comfort level to discuss their doubts, fears and guilt helps a lot. In this session one puts forth information about the genetic condition, tests needed, cost, information about the implications of the results and how further plan of action will proceed after getting results. If the index child has a confirmed diagnosis, various treatment options and supportive therapy that can be offered to the child are also discussed. Absolute privacy and confidentiality have to be ensured throughout these sessions.

Risk prediction

This step is possible only after there is a confirmed diagnosis in the index case. Risk of recurrence in their offsprings, options of prenatal testing are discussed (Table II). At every point, ample time is given for family to think over and clear all their doubts and get over fears and guilt.

Reproductive options

Based on the etiology of genetic conditions the following options can be given

1. The couple can be reassured if they are not at risk
2. Natural pregnancy with an antenatal diagnosis if they are at risk
3. Alternate reproductive options with donor gametes depending on etiology of the condition
4. Natural pregnancy with a pre-implantation diagnosis
5. Adoption

Psychological support

Giving information about a genetic condition evokes a powerful emotional response in a couple. Adequate psychological support and confidentiality are to be provided

Table II. Risk predictions

Condition	Risk of transmission
Numerical chromosomal aberration	Low
Structural chromosomal aberration	5-15 % if one parent is carrier
Microdeletion	50% if inherited
Autosomal dominant	50% risk if inherited
Autosomal recessive	25% risk
X linked recessive	50% risk for affected males
Multifactorial	3-5%
Sporadic	Low

by the counselor. At times professional help has to be arranged for couples to overcome the agony.

Summarizing, genetic counseling is a process that happens over a period of time. The decisions will be quite varied as they will be based on multiple factors like family makeup, parity, socioeconomic status, religious beliefs, guidance provided by primary caregiver and individuals' thought process and intentions.

Prenatal diagnosis

It is a process whereby genetic normalcy of pregnancy is ensured. A fetal medicine specialist and geneticist have to work in close coordination to execute these tests. The methods used are (a) Screening (aneuploidy tests, ultrasound) and (b) diagnostic tests.

Screening tests: They are tests that are done to assess risk for aneuploidy in pregnancy (Table III). If the risk is found to be high, the next step would be definitive diagnostic testing.

Ultrasound: It is an excellent tool to view the fetus directly and look for structural anomalies. With the advent of modern technology in ultrasonography, most of the anomalies are picked up in the first trimester ultrasound between 11-13 weeks. The next gestational age for picking up anomalies is at 19-20 weeks. Some anomalies evolve with time and may be detected only in the third trimester. Some subtle anomalies may be evident only postnatally. If an anomaly is detected and a genetic etiology is suspected, suitable genetic tests are planned. The detection of anomalies depends on maternal habitus, liquor, fetal activity and position, equipment and above all skill of operator.

Genetic counseling for detection of fetal anomaly: If a fetal anomaly is diagnosed, the couple are explained about the nature of anomaly (lethal / non-lethal). If the anomaly is lethal, need for postnatal autopsy and storing samples for further genetic testing is explained. If the anomaly is non-lethal, but found to have genetic association, genetic tests are planned for the fetus. The mode and place of delivery including postnatal management options and prognosis are discussed with the couple. Cross consultation with pediatric subspecialists may be organized as the case may be for a better understanding.

Direct invasive tests

They include

(i) Chorionic villous sampling (CVS) - 11-14 weeks of gestation (trans-abdominal method is the preferred sampling technique)

(ii) Amniocentesis -16-20 weeks

(iii) Fetal blood sampling- beyond 20 weeks

Table III. Types of screening tests and its detection rates

Pregnancy Timing	Nature of test	Detection rate (DR) with a False positive rate (FPR) of 5%
First trimester	NT (nuchal translucency) ultrasound	64-70 %
First trimester	NT (nuchal translucency) ultrasound + Serum biochemistry	82-87%
Second trimester	Triple marker screening test	69%
Second trimester	Quadruple marker screening test	81%
Other screening tests		
Integrated screening	First trimester blood screening+ NT ultrasound + second trimester blood screening	94-96%
Serum integrated	First trimester blood screening + second trimester blood screening	85-88%
Non invasive screening (NIS) / Non invasive prenatal testing (NIPT)	Non-invasive screening using cell free fetal DNA	99% DR with 0.3% FPR

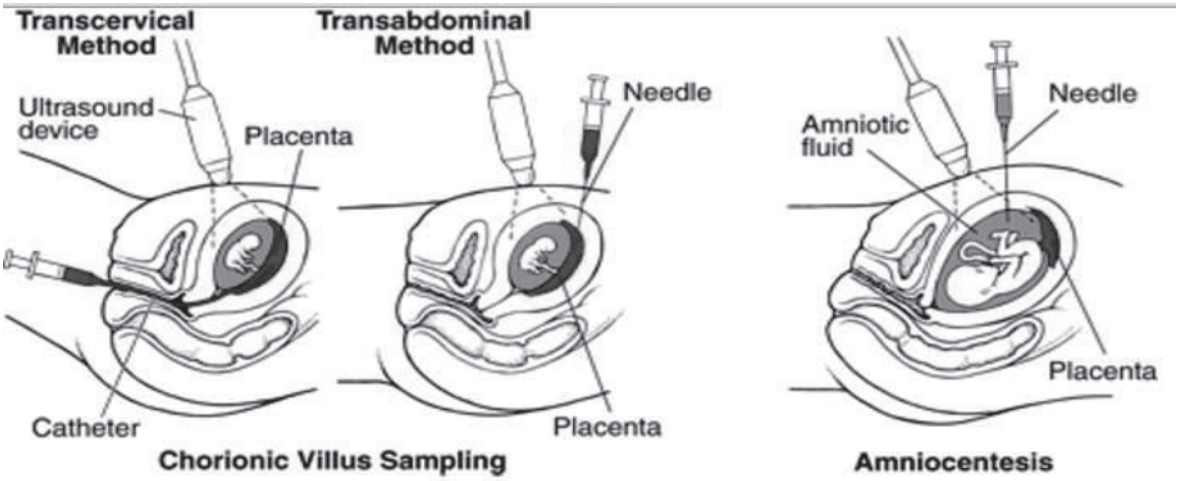

Fig.8. Illustration of types of direct invasive techniques

The tests that can be done from the samples are karyotype, fluorescence in situ hybridization (FISH), BACs-on-Beads (BoBs), microarray, Sanger sequencing and next-generation sequencing panels. DNA quantity yield is good in chorionic villus sample and can be used for karyotyping and molecular testing. In amniocentesis only 20 ml of fluid can be drawn and is used either for karyotyping or molecular testing.

Problems in prenatal samples: In CVS sampling there is a risk of interpretation error due to placental mosaicism. Also there is a risk of 0.3% miscarriage even in expert hands.

Caution: Maternal contamination studies are a must in all fetal sampling procedures. If a mother is Rh-negative anti D must be given after the procedure. Mothers with infectious diseases like HIV, hepatitis have a risk of transmitting the infection to the unborn fetus by invasive procedures. Necessary caution has to be exercised to avoid such mishaps.

Indications for prenatal testing

1. positive screening test
2. advanced maternal age
3. previous child with a known genetic problem
4. parent with a genetic problem
5. carrier parents of an autosomal recessive disorder
6. carrier mother of X linked recessive carrier

Note: for indications 3-6 the genetic error causing the disease in that particular family should be available on hand when antenatal testing is planned.

Index case if available has to be worked up in the pre-pregnancy period.

Stored samples of terminated pregnancy or deceased child should be tested in the pre-pregnancy period.

Late genetic testing - as termination is possible only before 20 weeks late answers may not help in the decision making of an affected pregnancy.

Pretest counseling: It is the most important step. In this session, the testing modalities, risks involved, cost, turn around time of results, issues in report interpretation, cultures failures, need for repeat testing (though chances are less) and plan after receiving results are all discussed so that eventually the process can be handled smoothly.

Informed consent

The couple has to have a detailed explanation of all the details. Their doubts and concerns have to be properly addressed and consent for test after complete understanding is procured. This has to be done before the procedure.

Pre-conception and pre-natal diagnostic techniques (PCPNDT) regulations

Any centre offering genetic counseling and prenatal testing should be registered under PCPNDT and the physicians involved should also be registered. The USG equipment also needs to be registered. Sign boards stating that sex determination will not be done at any cost should be displayed in all main areas. Monthly statistics of patients undergoing genetic counseling and prenatal testing should be submitted to PCPNDT office. The centre should be prepared to face audits done by PCPNDT authorities. Forms stipulated by PCPNDT have to be filled for each patient.

Conclusion

There is a paradigm shift in the etiology of infant and childhood mortality from infectious diseases to birth defects. Most of the birth defects have a genetic etiology. As a pediatrician, one should have the knowledge about genetic disorders, how genetic counseling should be offered and the available prenatal diagnostic modalities to help and guide the families who have a child with genetic problem. Thus genetic counseling helps in the preventive, supportive and curative care of children with birth defects. Prenatal diagnosis helps in early detection and optimal management of these children.

Points to Remember

- ***Genetic counseling has to be offered by a competent person which involves privacy and confidentiality.***
- ***Non-directive counseling - Facts are explained by the counselor but decisions are made by the family.***
- ***Index case work up and pre-pregnancy counseling is crucial and informed consent is necessary at different steps of genetic work up.***
- ***Genetic work up is an extensive and usually a very time-consuming process involving multiple sessions, hence ideal time for a referral is pre-pregnancy period.***
- ***Pedigree charting is an essential part in genetic counseling.***
- ***Prenatal diagnosis aids early detection and optimizes pregnancy management.***

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NEWS AND NOTES

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GENETICS

FETAL THERAPY: CURRENT SCENARIO IN INDIA

Shagun Aggarwal

Abstract: *Fetal therapy is a promising modality that makes it possible for fetal disorders to be treated in-utero. It also provides hope for successful pregnancy outcome in those cases wherein termination or fatalistic expectant management were the only possibilities. Both medical and surgical approaches to fetal therapy are possible. Recent years have seen significant developments in fetal therapy.*

Keywords: *Fetal therapy, Fetal surgery, Transplacental therapy, Minimally invasive fetal intervention, Intrauterine blood transfusion.*

Fetal therapy or in utero treatment of the developing fetus is an exciting treatment modality that provides the opportunity to correct or ameliorate fetal disease states, thereby preventing morbidity and/or mortality and facilitating the successful culmination of pregnancy. This involves administration of pharmacologic agents i.e. medical therapy or operative interventions i.e. surgical therapy.

The decision for embarking on any intervention should be carefully weighed against alternative options as fetal therapy is often associated with risk to both mother and fetus. International Fetal Medicine and Surgery Society has proposed certain guidelines which act as a benchmark in this regard: 1) The natural history of the disease should be at least partly understood, 2) the condition should be lethal or could result in severe morbidity if not treated in utero and 3) the fetal intervention should be at least partly corrective and the results should compare favourably with those obtained following postnatal treatment.[1]

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Medical therapies have been widely utilized at many obstetric units since a long time, in view of their widespread availability, ease of administration, low risk profile and robust results. On the other hand, surgical therapies are presently in infancy, with few centres in the country having the expertise and the means to perform these interventions. This article provides overview of this exciting realm of fetal therapy and attempts to capture its current status in our country.

Medical therapy

Medical therapy involve administration of a drug or pharmacologic agent either to the mother leading on to trans-placental passage to the fetus or directly to the fetus by minimally invasive routes like trans-amniotic, intravascular or intra-peritoneal administration. Some of the conventional medical therapies, which are also available and used in India, are as follows[2]:

a) ***Steroids for fetal lung maturation***: The administration of betamethasone (two doses of 12 mg, 24 hours apart) or dexamethasone (four doses of 6 mg at 12 hour intervals) for accelerating fetal lung maturation in pregnancies complicated by spontaneous or induced preterm delivery reduces respiratory distress syndrome, comorbidity and mortality in neonates up to 48 hours after birth.

b) ***Non-steroidal anti-inflammatory drug (NSAIDs) for polyhydramnios***: Polyhydramnios can be associated with fetal and maternal morbidities: Treatment with NSAIDs has been shown to reduce the amniotic fluid volume and provide symptomatic relief as well as prevent complications. Sulindac, which has a better safety profile in comparison to indomethacin, can be used in doses of 400 mg per day for severe polyhydramnios causing maternal distress or threatened preterm labour and in cases of monoamnniotic twins to prevent cord entanglement.

c) ***Fetal thyroid disorders***: Both fetal hypothyroidism and thyrotoxicosis are amenable to in utero therapy. Fetal hypothyroidism usually results due to maternal anti-thyroid medication, but can also be due to maternal iodine deficiency related hypothyroidism or

primary thyroid dyshormogenesis. The diagnosis is by cordocentesis followed by estimation of TSH levels. Treatment involves administration of intra-amniotic levothyroxine 150-600µg weekly, resulting in fetal euthyroid status. Fetal thyrotoxicosis results from radio-iodine ablated maternal Grave's disease with persistent antibodies that cross transplacentally to the fetus. Treatment involves administration of propylthiouracil to the mother in dose of 100-600mg/day, which crosses transplacentally to the fetus and exerts therapeutic effect.

d) ***Congenital adrenal hyperplasia***: Maternal administration of dexamethasone prevents genital ambiguity in a female fetus affected with congenital adrenal hyperplasia. The drug is given in a dose of 20µg/kg weight empirically, starting by 6-8 weeks of gestation. Subsequently at 11-12 weeks the fetal gender and disease status is confirmed by chorionic villus sampling and targeted molecular testing. If the fetus is unaffected or male, the treatment is stopped, else it is continued till term. This has shown 85% efficacy for prevention of genital ambiguity, although recent reports have raised concerns regarding the teratogenic effects and long term outcome of this treatment for the fetus.[3]

e) ***Cardiac rhythm abnormalities***: Sustained fetal tachycardia can progress to congestive heart failure and fetal hydrops resulting in fetal demise. To prevent the progression of these events, antiarrhythmic drugs can be used by either transplacental route through maternal administration or by direct fetal administration. Some of the drugs used for this purpose are digoxin (drug of choice for supraventricular tachycardia without hydrops, dose 0.25-0.50mg/day), flecainide (primarily for supraventricular tachycardia with hydrops, dose 200-400mg/day), sotalol (primarily for atrial flutter with our without hydrops, dose 80-160mg 2-3 times a day), and in refractory cases amiodarone or a combination therapy during pregnancy. The conversion rates to sinus rhythm with these drugs ranges from 15-90% with lower rates being observed in fetuses with hydrops. In refractory cases and fetuses with hydrops, a direct instillation of the drug to the fetus by intra-amniotic, intramuscular, intraperitoneal or intravascular administration is shown to be of efficacy. On the other hand, fetal bradycardia due to congenital heart block is more refractory to therapy. In cases with maternal anti-Ro and La antibodies, administration of steroid to the mother have been shown to halt the progression of second degree block to 3rd degree block in some cases. In refractory cases, maternal intravenous immunoglobulin (IVIG) administration has been used, but the preliminary results have not been favourable.

f) ***Novel medical therapies***: Congenital pulmonary airway malformation is a fetal lung lesion, which has shown response to administration of maternal corticosteroids. A recent case series of 43 patients showed 82% reduction in lesion size and 88% hydrops resolution rate following betamethasone intramuscular administration of to mother.[4] Recently there were few case reports on the use of maternally administered drugs for some rare fetal conditions, e.g. biotin for fetal biotinidase deficiency, vitamin B12 for fetal methylmalonic acidemia and pyridoxine for pyridoxine dependent seizures. Some case reports of intravascular or intraperitoneal administration of fresh frozen plasma have shown to improve in utero growth for fetuses with Smith Lemli Opitz syndrome. However, most of these treatment approaches still remain experimental and await larger studies.

g) ***Intrauterine fetal blood transfusion (IUT***): This was the first minimally invasive fetal therapy procedure used by Liley, et al in 1963 for management of Rh hemolytic disease. Initially transfusions were given intra-peritoneally under fetoscopic guidance. Later, USG-guided intravascular transfusion became the preferred technique. Besides Rh hemolytic disease, this approach can be used for other causes of fetal anemia like parvovirus infection and feto-maternal hemorrhage. Presently, management of Rh hemolytic disease involves serial monitoring of middle cerebral artery peak systolic velocity for evidence of fetal anemia [anemia requiring transfusion defined as MoM (multiples of median) >1.5], followed by ultrasound-guided fetal umbilical vein puncture, estimation of fetal hematocrit and subsequent infusion of packed PRBCs if hematocrit is <30%. O negative, maternally cross-matched, washed, filtered, gamma irradiated PRBCs with a hematocrit >80% are used for IUT. The volume of blood to be transfused is calculated on the basis of fetal weight, fetal hematocrit and the hematocrit of the blood to be transfused. The target hematocrit is usually 40-50%. Ultrasound guided puncture of the umbilical vein is done, followed by confirmation of fetal blood, infusion of a fetal paralytic agent and then slow infusion of the PRBC under continuous ultrasound visualisation. At the end of infusion of the calculated volume, fetal blood is collected and the hematocrit checked to confirm that the transfusion has been successful. Subsequent transfusions are usually done at intervals of 15-20 days

and delivery can be planned after lung maturity is attained. Various procedural complications can arise like fetal demise, bradycardia, cord bleeding, preterm labour and infection. The fetal survival rate is 88% and in the absence of hydrops has been reported to be as high as 96%.[5] IUT has been used in various fetal medicine centres across India since many years, with success rates equivalent to western literature.[6,7] The team from AIIMS, New Delhi has also reported concomitant use of maternal IVIG administration, which reduces the rate of fall of hematocrit and frequency of transfusions.[8]

Surgical therapy

These are usually definitive and potentially curative therapies involving invasive means and hence associated with significant fetal and maternal risks in the form of preterm membrane rupture, chorioamnionitis and anesthetic hazards. In view of the heightened risk, prior to intervention it is essential to ensure that the defect is isolated and not part of a multiple malformations syndrome. Hence, fetal MRI and fetal karyotyping/chromosomal microarray/ exome sequencing are important pre-requisites before performing fetal surgery. In addition, the following principles need to be followed: a) Condition should be severe enough to warrant intervention, b) Prenatal intervention is associated with better prognosis compared to postnatal treatment and c) Condition should not be severe enough to be irreversible already. The various routes by which these therapies can be carried out are as follows:[9]

a) ***Open surgery***: This is performed through a hysterotomy incision under general anaesthesia. It has been used for correction of lesions like congenital diaphragmatic hernia, congenital pulmonary airway malformation of lung, myelomeningocoele and sacrococcygeal teratoma. However, this approach is limited by the highly invasive nature and associated high incidence of complications including the need for operative delivery in the present as well as subsequent pregnancies. Very few centres in the world have the facilities and expertise to perform open fetal surgeries, and presently this modality is not available in India.

b) ***Fetoscopic approach***: This involves introduction of a thin calibre endoscope into the uterine cavity through an abdominal incision, usually under ultrasound guidance. General or regional anesthesia and uterine tocolysis are needed for this procedure. The various therapeutic procedures which can be conducted using a fetoscope are laser ablation of communicating vessels in twin-twin transfusion syndrome, selective fetal reduction, intratracheal balloon insertion for diaphragmatic hernia, valvotomy for posterior urethral valves and amniotic band resection. Few centres in bigger cities of India presently offer fetoscopic procedures like laser ablation for twin to twin transfusion syndrome and selective fetal reduction.[10-15] Although initial period involved a significant learning curve for these intricate procedures, presently the intervention outcomes of these centres are comparable to the western world.

c) ***Ultrasound guided:*** Many minimally invasive fetal procedures can be conducted under ultrasound guidance, a modality available in most tertiary level obstetric centres. These include placement of shunts for drainage of obstructed bladder (vesico-amniotic shunts) and for draining thoracic collections like pleural effusion, cystic lung lesions (thoraco-amniotic shunts), radiofrequency ablation of umbilical cord for fetal reduction, valvuloplasty for obstructive cardiac outflow tract lesions and intracardiac potassium chloride administration or cord occlusion for fetal reduction in multiple order pregnancies. Most of these procedures can be performed under local or regional anesthesia, and many of the fetal medicine centres are routinely practicing fetal reduction procedures with success and complication rates comparable to literature reports. Vesico-amniotic shunting has previously been performed for a large number of cases at a premier centre in Chennai, but in view of concerns regarding postnatal renal function in large trials across the globe, this approach is no longer favoured. Placement of thoraco-amniotic shunt is also available at some maternal-fetal medicine centers in the country.

Table I provides an overview of the surgical approaches for various fetal abnormalities and Table II provides status of fetal therapeutic modalities at various maternal-fetal medicine centres in India. Besides the centres mentioned in Table II, maternal fetal medicine units at Sir Ganga Ram Hospital, New Delhi; Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow; Jawaharlal Institute of Postgraduate Medical Education and Research, Pondicherry and Centre for Infertility Management & Assisted Reproduction, Cochin are few other places where fetal therapies are available.

Novel therapies in the pipeline

1. In utero stem cell therapy: This is based on the premise that due to the relative immunodeficient state of the fetus, introduction of stem cells may lead to better engraftment.

Table I. Fetal surgical therapy

Fetal abnormality	Pathophysiology	Rationale	Approach	Evidence of use and current status
Congenital diaphragmatic hernia	Pulmonary hypoplasia and pulmonary hypertension	Timely reversal of pulmonary hypoplasia and prevention of pulmonary hypertension	Open surgery followed by closure of diaphragmatic defect	Not useful
			Fetoscopic occlusion of trachea using a balloon leading to pulmonary inflation	Large trials show survival benefit, randomised trial ongoing[15]
Lower urinary tract obstruction	Progressive renal damage by obstruction Pulmonary hypoplasia by oligohydramnios	Urinary diversion prevents obstructive uropathy and restores amniotic fluid volume	Vesico-amniotic shunt-placement of pigtail catheter communicating bladder to amniotic cavity	Variable results, concerns regarding pre-existing renal damage, randomised trial stopped midway[22]
			Cystoscopic posterior urethral valve ablation	Novel technique, requires further study[22]
Neural tube defects	Damage to exposed neural tube; CSF leak, leading to Chiari malformation and hydrocephalus	Covering exposed spinal cord, cessation of leakage preventing hydrocephalus and reversing cerebellar herniation	Open surgery-Closure of spinal defect	Large randomised trial showed survival and long term benefit[16,17]
Twin-to-twin transfusion	Intertwin transfusion leads to oligo-polyhydramnios sequence, hemodynamic changes; obstetric complications	Bichorionization stops intertwin transfusion, reverses cardiac failure, delays delivery	Fetoscopic selective laser fulgration of placental vessels	Large trials have shown definite survival benefit, treatment of choice for 18-26 weeks, >stage I TTTS[18]
Sacrococcyge-al teratoma	High output cardiac failure by arteriovenous shunting Fetal anemia by tumor growth and/or bleeding within a tumor	Cessation of steal phenomenon Reversal of cardiac failure; Prevention of polyhydramnios	Open or fetoscopic excision of tumor	Few case reports
			Ablation or embolisation of feeding vessels	

Thoracic space-occupying lesions	Pulmonary hypoplasia (space-occupying mass) Hydrops by impaired venous return (mediastinal compression)	Prevention of pulmonary hypoplasia and cardiac failure	Open surgery-Excision of solid lesions	Few case reports 60% survival rate[21]
			Ultrasound guided placement of thoraco-amniotic shunts for cystic lesions	Few case series 70-85% survival rate[21]
Cardiac malformations	Critical lesions causing irreversible hypoplasia or damage	Prevention of hypoplasia or arrest of progression of damage	Ultrasound guided valvuloplasty	Technical success of 60-70% in aortic valvuloplasty. Further trials required
Amniotic bands	Progressive constrictions causing irreversible neurological or vascular damage	Prevention of limb deformities and function loss	Fetoscope guided excision of bands-scissors or laser or electric current	Few case reports
Fetus acardiacus (parasitic twin without a heart, using the circulation of its twin) and discordant anomalies	Discordant anomalies: where one fetus can be a threat to the other, or to avoid termination of entire pregnancy	Selective feticide to improve chances of the other fetus; avoidance of termination of entire pregnancy	Selective feticide of anomalous fetus-Intracardiac saline/potassium chloride injection under ultrasound guidance for multichorionic gestations Coagulation / ablation / ligation of umbilical cord vessels for monochorionic gestations	Many case series show survival benefit for co-twin Results better for dichorionic twins
High order (>=3) Multiple pregnancy	Increased risk of prematurity, pregnancy loss, pre-eclampsia	Reduction of number of fetuses reduces the complication rates inherent in multiple pregnancy	Same as above	Reduction in preterm delivery rates and perinatal mortality Approved for clinical use

Table II. Fetal therapeutic interventions available in India

Fetal intervention	Mediscan systems, Chennai	AIIMS, New Delhi[6]	Apollo hospitals, New Delhi	BFMC, Bangalore	Fernandez Hospitals, Hyderabad
Medical Therapies					
IUT	Yes(307)	Yes(300+)	Yes(80)	Yes(150+)	Yes(43)
NSAID treatment for Polyhydramnios	-	Yes	-	Yes(100+)	-
Arrhythmias	Yes	Yes	Yes(9)	Yes(10)	Yes
Thyroid disorders	Yes	Yes	-	Yes(3)	Yes(19)
Others	-	-	Amniodrainage (54)	-	IVIG intraperitoneal for CHB
Surgical Therapies					
Fetscopic laser for TTTS	Yes(163)	Yes	Yes(46)	Yes(88)#	-
Fetal reduction	Yes(4532)	Yes	Yes(206)	Yes(300+)	-
LUTO- shunt	Yes(11)	Yes	Yes(4)	Yes(1)	-
Thoraco-amniotic shunt	-	Yes	Yes(7)	Yes(22)	-
Others	Embolisation	Yes: RFA and Laser	-	-	-
	RFA for TRAP				
	Cord occlusion				

IUT: Intrauterine PRBC transfusion, TTTS: Twin to twin transfusion syndrome, LUTO: Lower urinary tract obstruction, RFA: Radiofrequency ablation, TRAP: Twin reverse arterial perfusion

This formed the basis of various IUHSCT (In Utero Hematopoietic Stem Cell Transplantation) trials in mouse models. Few human trials showed benefit in Severe Combined Immunodeficiency syndrome. However, presently, this approach is in the pre-clinical stage.

2. In utero gene therapy - The introduction or correction of a defective gene in the fetal life has the potential to prevent disease prior to the onset of irreversible organ damage. The procedure involves ultrasound guided introduction of the transgene into a fetal cavity or organ of interest. This approach has been tried in rodent models of various metabolic, central nervous system and musculoskeletal diseases. Recent report also showed use of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) technology for correction of a defective gene in a human embryo. However, due to the potential for teratogenic effects and germline transmission, presently, human trials for fetal gene therapy are not considered ethical.

Conclusion

The development of various fetal treatment strategies offer hope for in utero management of fetal disorders and birth of a healthy baby for families who are confronted with the scenario of an anomalous fetus. Although there have been rapid strides in this direction over the last few years, most of the studies have been retrospective trials with historical controls or small case series. Long term outcomes for many of these interventions need to be studied, and there are also ethical concerns especially with regards to the fetal surgical therapies. Additional challenges concerning availability of trained experts and centres offering these therapies are especially relevant for India. These limitations indicate that although many fetal therapies are now within the realm of patient care, the field is still in its infancy, and there is a long way to go before these interventions translate to routine clinical practice.

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Points to Remember

- ***Various fetal disorders are amenable to medical and surgical therapies.***
- ***Decision to perform a fetal intervention needs to be carefully made considering the risk-benefit involved.***
- ***Rapid advances have been made in recent years in fetal surgical therapies and some minimally invasive interventions are now approved for clinical use.***
- ***Availability, cost, lack of randomised studies and long term follow up studies remain a limitation.***

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IAP WHITE PAPER ON NUTRIENT GAPS AND MANAGEMENT

NUTRITIONAL GAPS AND MANAGEMENT IN CATCH-UP GROWTH AND IMMUNITY IN INDIAN SCENARIO

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Abstract: *Malnutrition during pregnancy may have deleterious effect not only on the health and wellbeing of mother and baby in the early life but is also linked to many adulthood diseases. The Infant and Young Child Feeding (IYCF) practices, especially breast feeding is the cornerstone for child survival. According to recent estimates, 38% of children in India below 5 years are stunted. Suboptimal feeding, late initiation or insufficient quantity and quality of complementary feeds and rise in infectious morbidity are the major drivers in increasing the prevalence of undernutrition and stunting. Nutrition and immunity are closely inter-related. Type 1 nutrients are functional nutrients, that are important in immunity and convalescence and Type 2 nutrients are growth nutrients, which are essential for optimal growth. Catch up growth happens in children, when the cause of growth faltering is removed, in those who have experienced growth faltering before. Proportionate and balanced nutrition during this period is crucial to build up lean body mass and prevent obesity and other complications in childhood and adulthood. Both quantity and quality of protein plays a crucial role in optimal growth of children. Oral nutritional supplementation (ONS) is considered as a nutritionally complete supplement, which benefits, nutritional status of children with faltering growth and immunity. Anticipatory nutrition guidance (ANG) is a key factor in addressing stunting, wasting and catch up growth.*

Keywords: *Catch up growth, Immunity, Type 1 & Type 2 Nutrients, Protein digestibility corrected amino acid score, Average/Acceptable Macronutrient Distribution Range, Anticipatory Nutrition Guidance, Oral Nutrition Supplementation, IAP's Malnutrition Proactive Assessment-A Comprehensive Tool- IMPACT*

Executive summary

Proper nutrition is a prerequisite for optimal growth, development, immunity and overall wellbeing of a child

and future health. Stunting is the most common disorder seen in children from developing countries like India, either due to exposure of fetus or infant to nutritional deficiency, suboptimal feeding, insufficient amount of complementary feeding, infectious diseases or maternal malnutrition during or after delivery.[1,2] However, these influential contributors of stunting in the early childhood can lead to various conditions like diabetes, high blood pressure, and coronary heart diseases developing in the middle age.[2,3] India continues to grapple with the problem of inadequate nutrition in mothers and children, with greater than one third of the world's wasted children living in India.[4] During nutrient deficiency, there can be either reduction in immunity or the baby may stop growing with or without weight loss. Nutrition from the time of conception to two years of age, that constitutes the first 1000 days of life, is considered the foundation of health of an individual. Prevention of maternal malnutrition is not only beneficial for the health of the mother and child in early life but is also beneficial for future health during adulthood.[5]

IYCF Practices

The Infant and young child feeding (IYCF) practices, especially breast feeding is the cornerstone for child survival**.** Breast milk has the ideal composition for a newborn baby as it contains all essential nutrients, antibodies, hormones and antioxidants.[6] Timely initiation and age specific breast feeding practices are recommended to promote physical as well as neurological growth and development. This also reduces infection and thus prevents irreversible stunting, as well as acute undernutrition.[6,7] Optimal breastfeeding practices include encouraging the use of only breastmilk to feed infants upto 6 months of age. Breast feeding should be continued till at least 2 years of age along with complementary foods, starting from 6 months, to meet the nutritional gaps.[8] Prolonged duration of breastfeeding is also linked to reduction in the risk of a child being either overweight or obese.[9] The Revised Baby Friendly Hospital Initiative (BFHI) Guidelines 2018 recommends 10 steps to successful breastfeeding.[10]

As per the recent survey, only 54.9% of Indian children under the age of 6 months are exclusivelybreastfed[8] and a total of 38% of the Indian children under five years are stunted.[11] Hence, there is an urgent need to reinforce the BFHI and the Mother's Absolute Affection (MAA) programs, for successful initiation and sustenance of optimal breastfeeding practices.[8] Deviation from breastfeeding recommendations result in growth faltering, which results from early introduction of poor-quality food when compared to the breast milk and increased chances of infections. For older children, it is imperative to follow the '5210 health initiative' for prevention of obesity and for promoting healthy life style; 5 serving of fruits and vegetables per day, limiting daily recreation screen time to 2 hours or less, a minimum of 1 hour or more of active play and zero use of sugar sweetened beverages.[12]

Type 1 and Type 2 Nutrients

Normal growth in children depends on various factors like intracellular proteins, hormones, paracrine factors and extracellular matrix molecules that regulate the activity of growth plate chondrocytes. There is also a notable correlation between nutrition and immunity. Different types of nutrients are needed to overcome growth and immunity lag. Nutrients are broadly classified into 2 categories; Type 1/functional nutrients, which helps in optimal immune function and Type 2/growth nutrients, which helps in optimal growth. Type 1 nutrients that are essential for developing immunity and convalescence include Iron, Iodine, Copper, Calcium, Selenium, Thiamine, Riboflavin, Pyridoxine, Niacin, Folate, Cobalamin and Vitamin A, C, D, E and K. Iron improvesoxygen carrying capacity to the tissues leading to reduced formation of lactic acid. This improves mobility and decreases pain during convalescence. It is also required for phagocytic activity and maintenance of immunoglobulin levels.[13,14] Copper is essential for differentiation, maturation and proliferation of leucocytes, hat have a role in immunity.[15] Selenium exerts its action through selenoproteins which initiates and enhances immunity. Thus, Selenium helps in immunoregulation and enhances cytokine production.[16,17] Vitamin A helps in cell division and communication between T-helper cells,[18] increases CD4 count and helps in proliferation of natural killer cells.[15,19] Vitamin D helps in the regeneration of blood vessels after inflammation of the vessel linings,[14] modulates innate and adaptive immunity and has effect on both B and T cells.[20,21,22] Vitamin E modulates cytokine production, stimulates T cell proliferation, increases cytotoxic activity and optimal macrophage activationand modulates host immune response as a potent anti oxidant.[15,23] Vitamin C is effective in bringing relief of the symptoms with upper respiratory tract infections, especially common cold.[24] Riboflavin contributes to proper functioning of immune system andinfluences neutrophil and macrophage migration.[25,26] Pyridoxine influences both humoral and cell mediated immune response and its supplementation increases immune response in critically ill patients.[27,28] Type 1 nutrient deficiency result in specific deficiency and these nutrients can be supplemented and stored up.

Type 2 nutrients required for growth include protein-essential amino acids, nitrogen, potassium, magnesium, phosphorus, sulphur, sodium, chloride and zinc.[29] Zinc, in addition to promoting growth, also stimulates the immune system, helps in antibody formation, speedy recovery from viral infection, improves wound healing capacity[30] and increases CD4/CD8 ratio in children.[31] Type 2 nutrients have limited body stores and need to be supplemented on a daily basis to promote growth.

Catch up Growth

Infants and young children who had an episode of retarded growth exhibit a period of rapid growth called catch-up (CU) growth period once the cause of growth deficit is removed. During this period, the increase in weight and height of the child is at a higher velocity than normal.[32,33] Supplementation with proportional and balanced Type 2 nutrients may help to fill the gap of height or weight deficits. However, if the nutrients are not optimum, itmay result in imbalance in growth, with a higher proportion of fat and a lower proportion of lean tissue. It also leads to an increased risk of adulthood diseases like certain cardiovascular diseases, along with the functional alteration of pulmonary, renal and cerebral systems, metabolic syndrome and obesity. Hence, during CU growth, the nutrient density must be sufficient to allow the child to regain physiological, anatomical and immunological normalcy, while taking care not to deposit excess adipose tissue. There is an increased demand and requirement of protein and energy during the period of rapid catch up growth.[34,35,36]

Nutritional intervention which is a combination of dietary counselling and use of oral nutritional supplementation promotes an initial catch-up growth related to the weight and also improves linear growth during maintenance phase of growth, which helps to maintain proportional growth in children.[37] The age group of 3-9 years being a critical growth phase for children, timely interventions may help in child's growth and development in a healthy way.

Adequate proteins are required not only for tissue development, but also for the maintenance of normal body functions.[38] Inadequate intake of protein is one of the prime causes of growth faltering.[39] A normally growing child (up to 9 years of age) has a protein requirement of 1.17 - 1.29 g/kg/day,[40] whereas during CU growth (for >5 years of age), the protein requirement is 1.82-2.82 g/kg/day.[41] Favourable CU growth not only depends on sufficient delivery of energy, but also on adequate proteins.[41] Protein is necessary for building up the lean mass during CU growth and a deficiency in the quantity and/or quality of the same may result in obesity. Children from low- and middle-income countries (LMICs) thrive on plant-based diets, which provide only about 15% of dietary proteins.[42] Proteins from animal sources are complete and provide nutrients that are easily absorbed, thus facilitating CU growth.[43] Plant proteins are incomplete, with one or more limiting amino acids. Because of lower digestibility of plant proteins, protein intake may have to be increased by 15% to 20% for children > 6 years of age.[44] Digestibility, comparative content and metabolic availability of indispensable amino acids present in the proteins are the major factors determining the quality of protein. If the content of an essential amino acid is less in a protein, it can result in limited utilization of the other amino acids for protein synthesis, irrespective of adequate total nitrogen intake. The dietary protein's nutritional value is determined by the limiting amino acid.[45]

Protein Digestibility Corrected Amino Acid Score (PDCAAS)

The various types of protein are whey, casein soy and milk protein. Whey protein is one of the best proteins as it has one of the highest biological values with protein digestibility corrected amino acid score (PDCAAS) of 1 and is easily digested and has faster absorption.[46] Soy protein is not an ideal protein as it has low concentration ofmethionine, lysine and tryptophan as well as absorption of minerals and trace elements are lower from soy protein-based formulas due to high phytate content.[47,48] Casein protein which is less water soluble and digested slowly, causes a slower and more sustained release of amino acids in circulation and decreases protein catabolism.[49] Milk protein has high biological value due to the presence all crucial amino acids and nitrogen.[50] The quality as well as the quantity of crucial amino acid profile determines whether protein food strength is strong or weak, which then can result in healthy growth and development. The protein quality is classified by PDCAAS based on the amount of amino acid required by the most demanding age group of 2-to-5-years. The three-protein based amino acid profilesthat have received the ideal PDCAAS score of 1.0 include whey, soy and egg whites.[51]

Arginine

Similarly, arginine is a crucial amino acid involved in the development of various prime physiologic factors and growth hormone release. It also contributes in protein synthesis. However, arginine cannot be produced in

sufficient quantities by the natural biosynthetic pathway; hence it should be consumed through the dietary source.[52] It has been found that the serum concentrations of crucial amino acids like arginine were significantly lower in stunted children.[53]

Oral Nutritional Supplementation (ONS)

All these nutrients are considered vital for healthy growth and development. Oral nutritional supplementation (ONS) is considered as a nutritionally complete supplement containing a blend of macro as well as micronutrients, that was reported to be an effective method which benefits, nutritional status of children with faltering growth and immunity.[54,55] Hence, good nutritional intake is crucial for healthy physical growth, development and enhancement of immunity.[55]

Average/Acceptable Macronutrient Distribution Range (AMDR)

There is arecent shift seen in the focus of nutritional management, from supplementation with energy only to both adequate energy and protein, post the recent WHO guidelines on CU growth.[31] It is advisable that the children are fed diet as per the Average/Acceptable Macronutrient Distribution Range (AMDR). AMDR refers to the predetermined ranges of intake for specific macronutrient energy nutrients in the diet. An AMDR which is in the suggested range is linked with a reduced risk of chronic adulthood diseases and can provide appropriate amounts of crucial nutrients. Carbohydrates should make up 45-65% of the energy the child eats, proteins up to 10-35% and fats should constitute upto 20-35% of the calories, that the child eats.[56] Dietary composition of fat supply for children > 2 years includes <10% saturated fatty acids, poly unsaturated fatty acid 10 -15%, n6 4-13%, n3 1-2% with n6 to n3 ratio of 5:1 to 10:1, cholesterol<300mg/day and no restriction for monosaturated fatty acids within the limits of total fat. Evidence recommends that the intake of proteins linked with higher end of the AMDR have greater chances to benefit from variety of health-relatedoutcomes as opposed to simply preventing protein deficiency, i.e., helping to maintain nitrogen balance.[57]

Anticipatory Nutrition Guidance (ANG)

Only one in five (22%) children have been found to consume diets adequate in protein and energy, thus leading to undernutrition, underweight and stunting.[31,58] Picky eating is a common childhood habit characterised by strong food preferences, consumption oflimited number of foods, restricted consumption of vegetables and other food groups and unwillingness to try new foods. This frequently leads to nutritional deficiency and inadequate weight gain. Severe undernutrition is generally characterized by the occurrence of fat degeneration in different organs of the body like GIT, that triggers loss of both absorption and digestion capacity. Children with picky eating habits have inadequate nutrition and negative effect on immunity, thus leading to infectious illnesses. Repeated infections that worsen malnutrition through repeated cycles are related to poor nutritional intake problems faced in childhood.[54,59]

A situation analysis of the possible reasons behind childhood stunting and their applicable interventions is a very helpful method to address the situation. The inadequate quality of foods can be addressed by way of macro and micronutrient supplementation, anticipatory nutrition guidance (ANG) and multiple macro and micronutrient powders for home fortification of complementary food.[60] Similarly, chronic or significant food shortages can be curbed by social protection program, community management of acute malnutrition program, nutrition surveillance system, or promotion of linkages with agriculture. Many measures can be taken for inadequate breastfeeding such as breastfeeding counselling and support through community, capacity building for health and nutrition workers, responsive feeding as per the cues flagged of by the infant or child, control of the marketing of breast milk substitutes, baby friendly hospital initiative, IYCF practices and maternity protection in work place.

IAP's Malnutrition Proactive Assessment-A Comprehensive Tool – IMPACT

As part of the Nutrition Education Program (NEP) in India, a holistic 'ABCDEFG assessment scale' has been proposed, for assessment of growth, with the aspects covering anthropometry, biochemical/lab tests, clinical features, dietary evaluation, ecological / epidemiological / environmental data, functional parameters like bone age, developmental mile stones, supported by continued growth monitoring. In the evaluation of quality of life and care plan, a tool called 'IMPACT' (IAP's Malnutrition Proactive Assessment-A Comprehensive Tool) has also been developed, incorporating the above.[61]

Conclusion

Catch-up growth period should be used for introducing appropriate supplementation with proportional and balanced Type 2 nutrients, to fill the gap of any height or weight deficits. Of all the nutrients, protein is of prime importance for building up the lean mass during catch-up growth. Pediatricians should not wait till obvious signs of

nutrient deficiencies appear or growth faltering sets in. It is essential to take various anthropometric measurements and plot them on age-appropriate charts. Corrective actions should be taken by dietary improvement and if necessary, by appropriate oral nutrition supplements at the right time.

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NEWS AND NOTES

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DRUG PROFILE

ANTIFUNGALS IN CHILDEN

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Abstract: *Over the past few years the antifungal armamentarium against invasive mycoses has expanded greatly. Most studies evaluating the safety and efficacy of antifungal agents are limited to adults and the dosage guidelines for newer antifungal agents lack adequate pediatric studies and hence their use in pediatrics is often off label. Antifungal studies specifically designed for children are necessary as changes in pharmacokinetics of these drugs occur throughout childhood.*

Keywords: *Antifungals, Children, Fluconazole, Itraconazole, Amphotericin, Caspofungin*

Antimicrobial agents are among the most commonly prescribed drugs in neonates and children. Though widespread use of broad-spectrum antimicrobials is known to contribute to antimicrobial resistance, failure to initiate appropriate treatment is associated with significantly increased mortality. The appropriate use of antifungal drugs is of particular importance in the prevention and treatment of infection in the presence of severe intercurrent illness, prematurity and immunosuppression. Invasive fungal infections (IFI) continue to be associated with an unacceptably high mortality rate in these vulnerable populations. In low and extremely low birth weight neonates, IFI is associated with a mortality rate of 30 to 40%.[1] In the setting of hematopoietic stem cell transplantation (HSCT), the mortality rates from IFI are 30 to 40% for yeast infections and up to 70% for mould infections.[2,3]

Classification according to the mechanism of action

According to their target sites, the principal antifungal drugs are classified as antifungal agents acting on a) plasma membranes (azoles, polyenes), b) synthesis of nucleic acids (5-flucytosine), and c)fungal cell walls (echinocandins) (Box 1).[4] The commonly used antifungals in systemic and superficial infections are given in Table I, while Box 2 shows the specific antifungals for specific infection.

Box 1. Antifungals - Classification

1. Drugs altering membrane permeability
   - a. Azoles
     - i. Triazoles: Fluconazole, itraconazole, voriconazole, terconazole, posaconozole
     - ii. Imidazoles: Ketoconazole, miconazole, clotrimazole, econazole, butoconazole, oxiconazole, sertaconazole, sulconazole
   - b. Terbinafine, butenafine, naftifine
   - c. Polyenes: Amphotericin B, nystatin, hamycin
2. Drugs blocking nucleic acid synthesis: Flucytosine
3. Drugs disrupting microtubule function: Griseofulvin
4. Drugs inhibiting cell wall synthesis: Caspofungin, nikkomycin

Azole antifungals

Azole antifungal agents are heterocyclic synthetic compounds and are subdivided into 2 groups: imidazoles and triazoles. The imidazoles are an older group consisting of miconazole, ketoconazole and clotrimazole. The triazoles include fluconazole and itraconazole and the second generation of triazoles includes voriconazole, ravuconazole and posaconazole. Isavuconazole, albaconazole and E-1224, a prodrug of ravuconazole are the 3 main azole antifungal agents currently under development.[7]

Fluconazole

This continues to be one of the most frequently prescribed triazoles because of its excellent bioavailability, tolerability and side-effect profile. It is fungistatic and activity is concentration independent. It is available in oral or intravenous forms. The bioavailability of the oral form is approximately 90%. Drug concentrations in the

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Table I. Drugs useful in systemic and superficial infections[5]

Systemic fungal infections	Superficial fungal infections
Amphotericin B Flucytosine Triazoles Ketoconazole Echinocandins	• Systemic drugs: Griseofulvin, allylamines (terbinafine, naftifine, butenafine), azoles • Topical drugs: Polyenes (nystatin), imidazoles, allylamines, ciclopirox olamine, benzoic acid with salicyclic acid (Whitfield's ointment), tolnaftate, undecyclinic acid, haloprogin

cerebrospinal fluid (CSF) and vitreous humor are around 80% of those found in blood.[8]

Fluconazole is active against Candida species with the exception of C. krusei and C. glabrata.[9] There is no appreciable activity against aspergillus, fusarium, pseudoallescheria, or zygomycetes. Fluconazole remains the drug of choice in oropharyngeal candidiasis (not C. krusei, C. glabrata), superficial mycoses as in genital candidiasis and tinea capitis. Tinea capitis always requires systemic treatment because topical antifungal agents do not penetrate the hair follicle.[10] Fluconazole can be used alone or as part of a combined antifungal treatment against invasive and disseminated candidiasis, cryptococcosis and cryptococcal meningitis, or histoplasmosis.[11] Fluconazole is very well absorbed after oral administration, excreted largely unchanged in the urine and can be used to treat candiduria.

Indications and dose[12] (Table II)

Oral / IV infusion

Neonate up to 13 days: 3-6 mg/kg, dose to be given on first day, then 3 mg/kg every 72 hours.

Neonate 14-28 days: 3-6 mg/kg, dose to be given on first day, then 3 mg/kg every 48 hours.

Table II. Mucosal candidiasis (genital)

Conditions	Oral dose
Candidal balanitis	Child 16-17 years: 150 mg for 1 dose
Vaginal candidiasis	Child: 150 mg for 1 dose, for use in patients who are post-puberty
Vulvovaginal candidiasis (recurrent)	Initially 150 mg every 72 hours for 3 doses, then 150 mg once weekly for 6 months, for use in patients who are post-puberty.

Child 1 month-11 years: 3-6 mg/kg, dose to be given on first day, then 3 mg/kg daily (max. per dose 100 mg) for 7-14 days in oropharyngeal candidiasis (max. 14 days except in severely immunocompromised patients); for 14-30 days in other mucosal infections (e.g. oesophagitis, candiduria, non-invasive bronchopulmonary infections)

Child 12-17 years: 50 mg for 7-14 days in oropharyngeal candidiasis (max. 14 days except in severely immunocompromised patients); for 14-30 days in other mucosal infections (e.g. oesophagitis, candiduria, non-invasive bronchopulmonary infections); increased to 100 mg daily, increased dose only for unusually difficult infections.

Tinea capitis

Child 1-17 years: 6 mg/kg daily (max. per dose 300 mg) orally for 2-4 weeks

Tinea pedis, corporis, cruris, pityriasis versicolor Dermal candidiasis

Child: 3 mg/kg daily (max. per dose 50 mg) orally for 2-4 weeks (for up to 6 weeks in tinea pedis); maximum duration of treatment 6 weeks.

Invasive candidal infections (including candidemia and disseminated candidiasis) and cryptococcal infections (including meningitis)

Oral / IV infusion

Neonate up to 13 days: 6-12 mg/kg every 72 hours treatment continued according to response (at least 8 weeks for cryptococcal meningitis).

Neonate 14-28 days: 6-12 mg/kg every 48 hours treatment continued according to response (at least 8 weeks for cryptococcal meningitis).

Child: 6-12 mg/kg daily (max. per dose 800 mg) treatment continued according to response (at least 8 weeks for cryptococcal meningitis)

Box 2. Treatment of specific infections[6]

Aspergillosis

Voriconazole (drug of choice)

Liposomal amphotericin B as alternative if voriconazole cannot be used

Second line or refractory cases: Caspofungin / itraconazole

Candidiasis

Superficial candidal infections of skin: topical imidazoles (clotrimazole, econazole, ketoconazole, miconazole). In refractory cases, systemic fluconazole.

Oropharyngeal candidiasis: Topical nystatin or miconazole. If not responding / if topical therapy cannot be used: systemic treatment with fluconazole or itraconazole in fluconazole resistant infections.

Vaginal candidiasis: Topical imidazoles or oral fluconazole

Invasive / disseminated candidiasis: Amphotericin B as IV infusion / echinocandin. Fluconazole is an alternative for Candida albicans infection in clinically stable children who have not received an azole antifungal recently. Amphotericin B should be considered for the initial treatment of CNS candidiasis. Voriconazole can be used for infections caused by fluconazole-resistant Candida spp. when oral therapy is required, or in children intolerant to amphotericin or an echinocandin. In refractory cases, flucytosine can be used with intravenous amphotericin.

Cryptococcosis

Cryptococcal meningitis: IV amphotericin and IV flucytosine for 2 weeks, followed by fluconazole orally for 8 weeks or until cultures are negative.

In children with HIV, fluconazole prophylaxis may be continued until immunity improves

Histoplasmosis

Immunocompetent children with indolent non-meningeal infection like chronic pulmonary histoplasmosis: Itraconazole

Severe / fulminant infections: IV amphotericin

Skin and nail infections

Tinia corporis, Tinea cruris, Tinea pedis - Mild and localized - Topical therapy with imidazoles / terbinafine cream

If large areas affected/ topical therapy fails/ difficult to treat (nails- onychomycosis, scalp- tinea capitis) - Systemic therapy with oral imidazole / triazole antifungals (itraconazole) and griseofulvin

Tinea capitis: Topical and systemic treatment is required.

Griseofulvin: Effective against trichophyton tonsurans and microsporum spp.

Tinea capitis caused by T. Tonsurans: Terbinafine.

Fluconazole/itraconazole are good alternatives.

Pityriasis versicolor: Oral itraconazole if topical therapy fails. Oral fluconazole is an alternative. Oral terbinafine is not effective.

Onychomycosis: If asymptomatic-, treatment may not be necessary. If symptomatic - systemic antifungals- Terbinafine and itraconazole -more effective than topical therapy but used with caution. Itraconazole can be administered as intermittent ‘pulse’ therapy.

Topical treatment of onychomycosis: early onychomycosis or when the involvement is limited to mild distal disease: topical tioconazole. Chronic infection may be treated with topical clotrimazole or nystatin but used with caution in children who suck their fingers. Chronic infection of the toes can be treated with topical terbinafine.

Prevention of fungal infections in immunocompromised patients

Oral / IV infusion

Neonate up to 13 days: 3-12 mg/kg every 72 hours, dose given according to extent and duration of neutropenia.

Neonate 14-28 days: 3-12 mg/kg every 48 hours, dose given according to extent and duration of neutropenia.

Child: 3-12 mg/kg daily (max. per dose 400 mg), commence treatment before anticipated onset of neutropenia and continue for 7 days after neutrophil count in desirable range, dose given according to extent and duration of neutropenia.

Prevention of fungal infections in immunocompromised patients (for patients with high risk of systemic infections e.g. following bone-marrow transplantation)

Oral / IV infusion

Child: 12 mg/kg daily (max. per dose 400 mg), commence treatment before anticipated onset of neutropenia and continue for 7 days after neutrophil count in desirable range.

Prevention of relapse of cryptococcal meningitis (HIV infected patients after completion of primary therapy)

Oral / IV infusion

Child: 6 mg/kg daily (max. per dose 200 mg)

Contra-indications: Cardiac arrythmias may occur when used with cisapride; concomitant use is contraindicated. May cause nausea, headache, rash, vomiting, abdominal pain, hepatitis, cholestasis and diarrhea. To be avoided in acute porphyrias.

Caution: Susceptibility to QT interval prolongation

Side-effects: Common side effects are abdominal discomfort, diarrhea, flatulence, headache, nausea and rash. Rare side effects include alopecia, anaphylaxis, angioedema, dizziness, dyspepsia, hepatic disorders, hyperlipidemia, pruritus, seizures, Stevens-Johnson syndrome, taste disturbance and toxic epidermal necrolysis.

Monitoring requirements: Monitor liver function with high doses or extended courses—discontinue if signs or symptoms of hepatic disease (risk of hepatic necrosis).

Preparations

Capsule: 50mg, 150mg, 200mg, Tablets: 50mg, 100mg, 400mg

Oral suspension: 50mg/5ml, 200mg/5ml

Itraconazole

This drug is active against a wide range of dermatophytes. There is limited information available on use in children. Itraconazole capsules require an acid environment in the stomach for optimal absorption. Concomitant administration of H2-receptor antagonists, proton pump inhibitors or antacids causes erratic and unpredictable drug absorption. Thus, it has been recommended that itraconazole capsules must be taken with food, but the oral suspension is better absorbed on an empty stomach. Itraconazole has been associated with liver damage and should be avoided or used with caution in patients with liver disease. No need for dosage adjustment in patients who have renal function impairment.[13]

Itraconazole is used for salvage therapy of invasive aspergillosis and for allergic bronchopulmonary aspergillosis.[14] It is also indicated in other invasive mycosis such as cryptococcal meningitis, histoplasmosis, non-meningeal coccidioidomycosis and infections caused by Blastomycosis, Sporothrix schenckii, Paracoccidioides brasiliensis and Blastomyces dermatitidis.[15,16] Itraconazole remains the drug of choice for prophylaxis in children affected by HIV with a history of histoplasmosis before immune reconstitution with an antiretroviral drug.

Indications and dose[12]

Oropharyngeal candidiasis

Child 1 month-11 years: 3-5 mg/kg once daily orally for 15 days; maximum 100 mg per day

Child 12-17 years: 100 mg once daily orally for 15 days

Oropharyngeal candidiasis in patients with AIDS or neutropenia

Child 1 month-11 years: 3-5 mg/kg daily orally for 15 days; maximum 200 mg per day

Child 12-17 years: 200 mg once daily orally for 15 days

Systemic candidiasis where other antifungal drugs are inappropriate or ineffective

Child: 5 mg/kg once daily (max. per dose 200 mg) orally, dose increased in invasive or disseminated disease and in cryptococcal meningitis, increased to 5 mg/kg twice daily (max. per dose 200 mg) oral.

Intravenous infusion dose

Child: 2.5 mg/kg every 12 hours (max. per dose 200 mg) for 2 days, then 2.5 mg/kg once daily (max. per dose 200 mg) for max. 12 days

Pityriasis versicolor

Child 1 month-12 years: 3-5 mg/kg once daily (max. per dose 200 mg) orally for 7 days

Child 12-17 years: 200 mg once daily orally for 7 days

Tinea pedis / Tinea manuum

Child 1 month-11 years: 35 mg/kg once daily (max. per dose 100 mg) orally for 30 days

Child 12-17 years: 100 mg once daily oral for 30 days, alternatively 200 mg twice daily orally for 7 days

Tinea corporis / Tinea cruris

Child 1 month-11 years: 3-5 mg/kg daily (max. per dose 100 mg) orally for 15 days

Child 12-17 years: 100 mg once daily oral for 15 days, alternatively 200 mg once daily orally for 7 days

Tinea capitis

Child 1-17 years: 3-5 mg/kg daily (max. per dose 200 mg) orally for 2-6 weeks

Onychomycosis

Child 1-11 years: 5 mg/kg daily (max. per dose 200 mg) orally for 7 days, subsequent courses repeated after 21-day intervals; fingernails 2 courses, toenails 3 courses

Child 12-17 years: 200 mg once daily oral for 3 months, alternatively 200 mg twice daily for 7 days, subsequent courses repeated after 21-day intervals; fingernails 2 courses, toenails 3 courses

Systemic aspergillosis where other antifungal drugs are inappropriate or ineffective

Intravenous infusion dose

Child: 2.5 mg/kg every 12 hours (max. per dose 200 mg) for 2 days, then 2.5 mg/kg once daily (max. per dose 200 mg) for max. 12 days

Child: 5 mg/kg once daily (max. per dose 200 mg), increased to 5 mg/kg twice daily (max. per dose 200 mg) orally, dose increased in invasive or disseminated disease and in cryptococcal meningitis

Histoplasmosis

Child: 5 mg/kg 1-2 times a day (max. per dose 200 mg) orally.

Systemic cryptococcosis including cryptococcal meningitis where other antifungal drugs inappropriate or ineffective.

Child: 5 mg/kg once daily (max. per dose 200 mg) orally, dose increased in invasive or disseminated disease and in cryptococcal meningitis, increased to 5 mg/kg twice daily (max. per dose 200 mg) orally.

Intravenous infusion

Child: 2.5 mg/kg every 12 hours (max. per dose 200 mg) for 2 days, then 2.5 mg/kg once daily (max. per dose 200 mg) for max. 12 days

Maintenance in HIV-infected patients to prevent relapse of underlying fungal infection and prophylaxis in neutropenia when standard therapy inappropriate

Child: 5 mg/kg once daily (max. per dose 200 mg) orally, then increased to 5 mg/kg twice daily (max. per dose 200 mg) orally, dose increased only if low plasma itraconazole concentration.

Prophylaxis of deep fungal infections (when standard therapy inappropriate) in patients with hematological malignancy or undergoing bone-marrow transplantation who are expected to become neutropenic

Child: 2.5 mg/kg twice daily orally, to be started before transplantation or before chemotherapy (taking care to avoid interaction with cytotoxic drugs) and continued until neutrophil count recovers. Safety and efficacy not established.

Contra-indications: Acute porphyrias, liver disease.

Caution: Evidence of heart failure

Side-effects: Common side effects are abdominal pain, blood pressure changes, cough, diarrhea, dyspnea, headache, hepatitis, hypokalemia, nausea, rash, taste disturbances and vomiting. Uncommon side effects are constipation, dizziness, dyspepsia, flatulence, menstrual disorder, myalgia, oedema and peripheral neuropathy.

Monitoring requirements: Absorption reduced in AIDS and neutropenia (monitor plasma-itraconazole concentration and increase dose if necessary). Monitor liver function if treatment continues for longer than one month, if receiving other hepatotoxic drugs, if history of hepatotoxicity with other drugs, or in hepatic impairment.

Preparations

Capsule: 100mg, 200mg

Oral solution: 10mg/1ml, 50mg/5ml

Infusion: 250mg/25ml

Voriconazole

This is a second-generation triazole, derivative of fluconazole, which combines the broad spectrum of antifungal activity of itraconazole with increased bioavailability of fluconazole. It is available as both an intravenous and oral formulation. The bioavailability of oral voriconazole is 96% and is distributed through the lungs, kidneys, liver, spleen, eyes, as well as the central nervous system. Metabolism occurs via hepatic CYP isoenzymes and hence drug interactions are common.[17]

Indications and dose: Invasive aspergillosis | Serious infections caused by Scedosporium spp., Fusarium spp., or invasive fluconazole-resistant Candida spp. (including C. krusei)

Child 2-11 years: Initially 9 mg/kg orally every 12 hours, then reduced in steps of 1 mg/kg, reduce dose if not tolerated, alternatively increased in steps of 1 mg/kg (max. per dose 350 mg every 12 hours), increase dose if response inadequate, maximum dose adjustment in steps of 50 mg.

Intravenous infusion dose

Child 2-11 years: Initially 9 mg/kg every 12 hours for 2 doses, then 8 mg/kg every 12 hours for max. 6 months; reduced in steps of 1 mg/kg, reduce dose if not tolerated, alternatively increased in steps of 1 mg/kg, increased dose if response inadequate

Contra-indications: Acute porphyrias

Cautions: Avoid exposure to sunlight, bradycardia, cardiomyopathy, electrolyte disturbances, history of QT interval prolongation, patients at risk of pancreatitis, symptomatic arrhythmias

Side-effects: Common side effects are abdominal pain, acute renal failure, agitation, alopecia, altered perception, anemia, anxiety, asthenia, blood disorders, blurred vision, cheilitis, chest pain, confusion, depression, diarrhea, dizziness, hematuria, hallucinations, headache, hypoglycemia, hypokalemia, hypotension, influenza like symptoms, jaundice, leucopenia, nausea, oedema, pancytopenia, paresthesia, photophobia, photosensitivity, pruritus, rash, respiratory distress syndrome, sinusitis, thrombocytopenia, tremor, visual disturbances and vomiting. Uncommon side effects are adrenocortical insufficiency, arrhythmias, arthritis, ataxia, blepharitis, cholecystitis, constipation, duodenitis, dyspepsia, flushing, fulminant hepatic failure, gingivitis, glossitis, hepatitis, hypersensitivity reactions. hypoaesthesia. hyponatremia, nystagmus, optic neuritis, pancreatitis, psoriasis, QT interval prolongation. raised serum cholesterol, scleritis, Stevens-Johnson syndrome, syncope.

Monitoring requirements: Monitor renal function, liver function before starting treatment, then at least weekly for 1 month and then monthly during treatment.

Preparations

Tablets: 50mg, 200mg

Oral suspension: 40mg/ml

Infusion: 200mg

Imidazole antifungals

The imidazole antifungals include clotrimazole, econazole nitrate, ketoconazole and tioconazole. They are used for the local treatment of vaginal candidiasis and for dermatophyte infections. Miconazole can be used locally for oral infections; it is also effective in intestinal infections. Systemic absorption may follow use of miconazole oral gel and may result in significant drug interactions.

Ketoconazole

Indications and dose[12]

Dermatophytosis and Malassezia folliculitis (either resistant to fluconazole, terbinafine or itraconazole or in patients intolerant of these antifungals, chronic mucocutaneous, cutaneous and oropharyngeal candidiasis either resistant to fluconazole or itraconazole or in patients intolerant of these antifungals).

Child with body weight 15-30kg: 100mg once daily orally

Child with body weight >30kg: 200mg once daily orally, increased if response inadequate to 400mg once daily.

Seborrhoeic dermatitis and dandruff

Treatment: Child 12-17 years - Apply twice weekly for 2-4 weeks, leave preparation on for 3-5 minutes before rinsing.

Prophylaxis: Child 12-17 years - Apply every 1-2 weeks, leave preparation on for 3-5 minutes before rinsing.

Pityriasis versicolor

Treatment: Child 12-17 years - Apply once daily for maximum 5 days, leave preparation on for 3-5 minutes before rinsing.

Prophylaxis: Child 12-17 years - Apply once daily for up to 3 days before sun exposure, leave preparation on for 3–5 minutes before rinsing.

Cautions: Avoid contact with eyes. Avoid contact with mucous membranes

Side effects: Erythema, hypersensitivity reactions, itching, mild burning sensation, occasional local irritation

Econazole

Indications and dose

Fungal skin infections

Child: Apply twice daily

Fungal nail infections

Child: Transungual application once daily, applied under occlusive dressing.

Cautions: Avoid contact with eyes and mucous membranes.

Side effects: Burning sensation, erythema, hypersensitivity reactions, itching, occasional local irritation.

Miconazole

Indications and dose

Skin infections

Neonate: Apply twice daily continuing for 10 days after lesions have healed.

Child: Apply twice daily continuing for 10 days after lesions have healed

Nail infections

Child: Apply 1-2 times a day

Cautions: Avoid in acute porphyrias. Contact with eyes and mucous membranes should be avoided.

Side effects: Burning sensation, erythema, hypersensitivity reactions, itching, occasional local irritation

Polyene antifungals

The polyene antifungals are the oldest antifungal class which includes amphotericin and nystatin. Neither drug is absorbed when given by mouth. Nystatin is used for oral, oropharyngeal and perioral infections by local application in the mouth. Nystatin is also used for Candida albicans infection of the skin.[18] Amphotericin by intravenous infusion is used for the treatment of systemic fungal infections and is active against most fungi and yeasts. It is highly protein bound and penetrates poorly into body fluids and tissues. When given parenterally amphotericin is toxic and side-effects are common. Lipid formulations of amphotericin (Abelcet and AmBisome) are significantly less toxic and are recommended when the conventional formulation of amphotericin is contra-indicated because of toxicity, especially nephrotoxicity or when response to conventional amphotericin is inadequate; lipid formulations are more expensive.[19]

Amphotericin (Amphotericin B)

Indications and dose[20,21]

Disseminated candidemia, invasive aspergillosis, cryptococcal infections and meningitis (AMB + flucytosine is the gold standard), blastomycoses, coccidioidomycoses, histoplasmosis (lipid formulation has greater efficacy), zygomycetes (not Scedosporium, Fusarium, Trichosporum) and endemic mycoses.

Intravenous infusion

Neonate: 1 mg/kg once daily, increased if necessary to 1.5 mg/kg daily for 7 days, then reduced to 1-1.5 mg/kg once daily on alternate days if required.

Child: Test dose 100 micrograms/kg (max. per dose 1 mg), included as part of first dose of 250 micrograms/kg daily, then increased if tolerated to 1 mg/kg daily, dose is gradually increased over 2-4 days; in severe infection max. 1.5 mg/kg daily or on alternate days. Prolonged treatment usually necessary; if interrupted for longer than 7 days recommence at 250 micrograms/kg daily and increase gradually.

Lipid formulations

Ambisome

Severe systemic or deep mycoses [where toxicity (particularly nephrotoxicity) precludes use of conventional amphotericin or in suspected or proven infection in febrile neutropenic patients unresponsive to broad-spectrum antibacterials].

Intravenous infusion

Neonate: 1 mg/kg once daily, increased if necessary to 3 mg/kg once daily; maximum 5 mg/kg per day.

Child: Test dose 100 micrograms/kg (max. per dose 1 mg), to be given over 10 minutes, then 3 mg/kg once daily; maximum 5 mg/kg per day.

Visceral leishmaniasis (unresponsive to the antimonial alone)

Intravenous infusion

Child: 1-3 mg/kg daily for 10-21 days to a cumulative dose of 21-30 mg/kg, alternatively 3 mg/kg for 5 consecutive days, followed by 3 mg/kg after 6 days for 1 dose.

Abelcet

Indications

Severe invasive candidiasis, Severe systemic fungal infections in patients not responding to conventional amphotericin or to other antifungal drugs or where toxicity or renal impairment precludes conventional amphotericin, including invasive aspergillosis, cryptococcal meningitis and disseminated cryptococcosis in HIV patients.

Intravenous infusion

Child: Test dose 100 micrograms/kg (max. per dose 1 mg), then 5 mg/kg once daily

Cautions: Anaphylaxis can occur with any intravenous amphotericin product and a test dose is advisable before the first infusion in children over 1 month of age; the patient should be carefully observed for at least 30 minutes after the test dose.

Side-effects: Common side effects are abdominal pain, abnormal liver function (discontinue treatment), anaemia, arrhythmias, blood disorders, blood pressure changes, cardiovascular effects, chest pain, diarrhea, disturbances in renal function, dyspnea, electrolyte disturbances, febrile reactions, headache, hypokalaemia, hypomagnesaemia, nausea, rash, renal tubular acidosis.

Monitoring requirements: Hepatic and renal function tests, blood counts, and plasma electrolyte (including plasma-potassium and magnesium concentration) monitoring required.

Echinocandin antifungals

The echinocandin antifungals include caspofungin and micafungin. The echinocandins have a unique mechanism of action, inhibiting beta-(1,3)-D-glucan synthase, an enzyme that is necessary for the synthesis of an essential component of the cell wall of several fungi. They are only active against Aspergillus (fungistatic) and Candida (fungicidal). The echinocandins have been shown to be efficacious for the treatment of esophageal candidiasis, candidemia, and invasive candidiasis However, micafungin is not used for the treatment of aspergillosis. Echinocandins are not effective against fungal infections of the CNS.

Caspofungin

Caspofungin is only available as an IV formulation, due to limited oral bioavailability. It is the first echinocandin to be approved for the treatment of fungal infections in pediatric patients. Caspofungin is fungicidal in vitro against Candida species and is fungistatic against Aspergillus species, but has little or no fungicidal or fungistatic activity against Cryptococcus neoformans, Zygomycetes, Fusarium species, or Trichosporon beigelii.[22]

Indications and dose[23]

Invasive aspergillosis, invasive candidiasis (empirical treatment of systemic fungal infections in patients with neutropenia)

Intravenous infusion

Neonate: 25 mg/M2 once daily.

Child 1-2 months: 25 mg/M2 once daily

Child 3-11 months: 50 mg/M2 once daily

Child 1-17 years: 70 mg/M2 once daily (max. per dose 70 mg) for 1 day, then 50 mg/M2 once daily (max. per dose 70 mg); increased to 70 mg/M2 once daily (max. per dose 70 mg), dose may be increased if lower dose tolerated but inadequate response

Side-effects: Common side effects are arthralgia, diarrhea, dyspnea, flushing, headache, hypokalemia, hypomagnesemia, hypotension, injection-site reactions, nausea, pruritus, rash, sweating, tachycardia, vomiting. Uncommon side effects are abdominal pain, anemia, anorexia, anxiety, arrhythmia, ascites, blurred vision, bronchospasm, chest pain, cholestasis, constipation, cough, disorientation, dizziness, dry mouth, dyspepsia, dysphagia, erythema multiforme, fatigue, flatulence, heart failure, hepatic dysfunction, hyperglycemia, hypertension, hypoesthesia, hypocalcemia, leucopenia, metabolic acidosis, muscular weakness, myalgia, palpitation, paresthesia, renal failure, sleep disturbances, taste disturbances, thrombocytopenia, thrombophlebitis, tremor.

Preperations

Injection: 50mg, 70mg

Micafungin

Micafungin is the only echinocandin evaluated for use in children for the treatment of invasive candidiasis, and for prophylaxis of Candida infections in patients undergoing allogenic HSCT. But its use in children is not yet globally licensed.[24]

Indications and dose[25]

Invasive candidiasis

Intravenous infusion

Neonate (administered on expert advice): 2 mg/kg once daily for at least 14 days; increased if necessary to 4 mg/kg once daily, increase dose if response inadequate.

Child (body-weight up to 40 kg): 2 mg/kg once daily for atleast 14 days; increased if necessary to 4 mg/kg once daily, increase dose if response inadequate.

Child (body-weight 40 kg and above): 100 mg once daily for at least 14 days; increased if necessary to 200 mg once daily, increase dose if response inadequate.

Oesophageal candidiasis

Intravenous infusion

Child 16-17 years (body-weight up to 40 kg): 3 mg/kg once daily

Child 16-17 years (body-weight 40 kg and above): 150 mg once daily

Prophylaxis of candidiasis (patients undergoing bonemarrow transplantation or who are expected to become neutropenic for over 10 days).

Intravenous infusion

Neonate: 1 mg/kg once daily continue for at least 7 days after neutrophil count in desirable range.

Child (body-weight up to 40 kg): 1 mg/kg once daily continue for at least 7 days after neutrophil count is desirable range

Child (body-weight 40 kg and above): 50 mg once daily continue for at least 7 days after neutrophil count is desirable range

Side-effects: Common side effects are abdominal pain, anaemia, blood pressure changes, diarrhea, fever, headache, hepatomegaly, hypocalcaemia, hypokalaemia, hypomagnesaemia, leucopenia, nausea, phlebitis, rash, renal failure, tachycardia, thrombocytopenia, vomiting. Uncommon side effects include anorexia, anxiety, bradycardia, cholestasis, confusion, constipation, dizziness, dyspepsia, dyspnea, eosinophilia, flushing, hepatitis, hyperhidrosis, hyperkalaemia, hyponatraemia, hypophosphataemia, palpitation, pancytopenia, pruritus, sleep disturbances, tachycardia, taste disturbances, tremor.

Monitoring requirements: Monitor renal function and liver function and discontinue if significant and persistent abnormalities in liver function tests develop.

Other antifungals

Flucytosine is used with amphotericin in a synergistic combination. Bone marrow depression can occur which limits its use, particularly in HIV-positive patients; weekly blood counts are necessary during prolonged therapy. Resistance to flucytosine can develop during therapy and sensitivity testing is essential before and during treatment. Flucytosine has a role in the treatment of systemic candidiasis and cryptococcal meningitis.

Griseofulvin is effective for widespread or intractable dermatophyte infections but has been superseded by newer antifungals, particularly for nail infections. Griseofulvin is used in the treatment of tinea capitis. It is the drug of choice for trichophyton infections in children. Duration of therapy is dependent on the site of the infection and may extend to a number of months.

Terbinafine is the drug of choice for fungal nail infections and is also used for ringworm infections where oral treatment is considered appropriate.

Flucytosine

Flucytosine is primarily used only in the treatment of Cryptococcus (combined with amphotericin B) and chromoblastomycosis.[26] Amphotericin B and flucytosine are also suggested for use in patients with candidal meningitis.[27]

Indications and dose

Systemic yeast and fungal infections (adjunct to amphotericin in severe systemic candidiasis and in other severe or long-standing infections).

Oral / IV infusion

Neonate: 50 mg/kg every 12 hours.

Child: Usual dose 50 mg/kg every 6 hours usually for not more than 7 days, alternatively 25–37.5 mg/kg every 6 hours usually for not more than 7 days, lower dose may be sufficient for sensitive organisms.

Cryptococcal meningitis (adjunct to amphotericin)

Orally or by IV infusion

Neonate: 50 mg/kg every 12 hours.

Child: 25 mg/kg every 6 hours for 2 weeks

Side-effects: Common side effects are diarrhea, nausea, rashes and vomiting. Uncommon side effects are alterations in liver function tests, cardiotoxicity, confusion, convulsions, hallucinations, headache, sedation, toxic epidermal necrolysis and vertigo.

Griseofulvin

In view of better efficacy, safety, cost, and a long track record of use, oral griseofulvin is the presently the treatment of choice for tinea capitis.[28]

Indications and dose

Dermatophyte infections (where topical therapy has failed or is inappropriate).

Child 1 month-11 years: Usual dose 10 mg/kg daily (max. per dose 500 mg) orally, alternatively 20 mg/kg daily orally, higher dose for severe infections; reduce dose when response occurs, daily dose may be taken once daily or in divided doses.

Child 12-17 years: 500 mg daily orally, alternatively 1 g daily, higher dose for severe infections; reduce dose when response occurs, daily dose may be taken once daily or in divided doses.

Tinea capitis (caused by Trichophyton tonsurans)

Child 1 month-11 years: 15-20 mg/kg once daily (max. per dose 1 g) orally, alternatively 15-20 mg/kg daily orally in divided doses.

Child 12-17 years: 1 g once daily orally, alternatively 1 g daily in divided doses

Terbinafine

Terbinafine is widely used in the pediatric population to treat superficial fungal infections, in particular tinea capitis. It is found to be effective and safe using treatment regimens that involve short duration therapy, leading to an increased compliance and providing a cost-effective means of treating pediatric superficial fungal infections such as tinea capitis. Terbinafine has been approved for the treatment of tinea capitis and provides good efficacy rates for Trichophyton tinea capitis using shorter regimens than the gold standard griseofulvin.[29] It is shown to be well tolerated in children aged between 2 and 17 years. The recommended duration of treatment for tinea capitis is 4 weeks.[30]

Indications and dose

Tinea pedis

Child 1-17 years (body-weight 10–19 kg): 62.5 mg once daily orally for 2-6 weeks

Child 1-17 years (body-weight 20–39 kg): 125 mg once daily orally for 2-6 weeks

Child 1-17 years (body-weight 40 kg and above): 250 mg once daily orally for 2-6 weeks

Tinea corporis

Child 1-17 years (body-weight 10–19 kg): 62.5 mg once daily orally for 4 weeks

Child 1-17 years (body-weight 20–39 kg): 125 mg once daily orally for 4 weeks

Child 1-17 years (body-weight 40 kg and above): 250 mg once daily orally for 4 weeks

Tinea cruris

Child 1-17 years (body-weight 10–19 kg): 62.5 mg once daily orally for 2-4 weeks

Child 1-17 years (body-weight 20–39 kg): 125 mg once daily orally for 2-4 weeks

Child 1-17 years (body-weight 40 kg and above): 250 mg once daily orally for 2-4 weeks

Tinea capitis

Child 1-17 years (body-weight 10-19 kg): 62.5 mg once daily orally for 4 weeks

Child 1-17 years (body-weight 20-39 kg): 125 mg once daily orally for 4 weeks

Child 1-17 years (body-weight 40 kg and above): 250 mg once daily orally for 4 weeks

Dermatophyte infections of the nails

Child 1-17 years (body-weight 10–19 kg): 62.5 mg once daily orally for 6 weeks-3 months (occasionally longer in toenail infections)

Child 1-17 years (body-weight 20–39 kg): 125 mg once daily orally for 6 weeks-3 months (occasionally longer in toenail infections)

Child 1-17 years (body-weight 40 kg and above): 250 mg once daily orally for 6 weeks-3 months (occasionally longer in toenail infections)

Side-effects: Common side effects include abdominal discomfort, anorexia, arthralgia, diarrhea, dyspepsia, headache, myalgia, nausea, rash, urticarial.

Monitoring requirements: Monitor hepatic function before treatment and then every 4-6 weeks during treatment-discontinue if abnormalities in liver function tests.

Conclusion

Neonatologists and pediatricians need to be aware of judicious and appropriate use of topical and systemic antifungals as we are now increasingly dealing with sick children and their survival in intensive care settings have markedly increased the recognition, and therefore, the incidence of fungal sepsis. The guidelines for indications, dosing, and duration of therapy in neonates, children and adolescents need to be formulated. Improper collection of specimen for cultures, non-availablity of local anti-fungal sensitivity patterns, delay in reporting of fungal cultures, sub-optimal dosing, lack of awareness of specific antifungal for each fungal infection and non-availability of antifungal agents are significant problems in neonates and children. Well-designed clinical studies in neonates, children and adolescents as well as the incorporation of antifungal stewardship are the need of the hour to improve prescribing practices and clinical outcomes.

Points to Remember

- ***Aspergillosis is best treated with voriconazole.***
- ***Superficial candidiasis is often treated with topical imidazoles whereas in invasive infections amphotericin B or caspofungin is used as first line.***
- ***Superficial skin and nail infections often require only topical therapy but in tinea capitis, topical and systemic therapy is warranted.***
- ***Many promising new antifungal agents are in the pipeline for treatment of childhood mycoses but clinical trials are limited in pediatric population and hence, judicious use of these medications is necessary.***

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CLIPPINGS

One Month of Rifapentine plus Isoniazid to Prevent HIV-Related Tuberculosis.

Tuberculosis is the leading killer of patients with human immunodeficiency virus (HIV) infection. Preventive therapy is effective, but current regimens are limited by poor implementation and low completion rates.

A randomized, open-label, phase 3 noninferiority trial comparing the efficacy and safety of a 1-month regimen of daily rifapentine plus isoniazid (1-month group) with 9 months of isoniazid alone (9-month group) in HIV-infected patients who were living in areas of high tuberculosis prevalence or who had evidence of latent tuberculosis infection was conducted. The primary end point was the first diagnosis of tuberculosis or death from tuberculosis or an unknown cause. Noninferiority would be shown if the upper limit of the 95% confidence interval for the between-group difference in the number of events per 100 person-years was less than 1.25.

A total of 3000 patients were enrolled and followed for a median of 3.3 years. Of these patients, 54% were women; the median CD4+ count was 470 cells per cubic millimeter, and half the patients were receiving antiretroviral therapy. The primary end point was reported in 32 of 1488 patients (2%) in the 1-month group and in 33 of 1498 (2%) in the 9-month group, for an incidence rate of 0.65 per 100 person-years and 0.67 per 100 person-years, respectively (rate difference in the 1-month group, “0.02 per 100 person-years; upper limit of the 95% confidence interval, 0.30). Serious adverse events occurred in 6% of the patients in the 1-month group and in 7% of those in the 9-month group (P=0.07). The percentage of treatment completion was significantly higher in the 1-month group than in the 9-month group (97% vs. 90%, P<0.001).

CONCLUSIONS: A 1-month regimen of rifapentine plus isoniazid was noninferior to 9 months of isoniazid alone for preventing tuberculosis in HIV-infected patients. The percentage of patients who completed treatment was significantly higher in the 1-month group.

Swindells S, Ramchandani R, Gupta A, et al. One month of rifapentine plus isoniazid to prevent HIV-related tuberculosis. N Engl J Med 2019;380:1001-1011.

ADOLESCENT MEDICINE

ADOLESCENT POLYCYSTIC OVARY SYNDROME

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Abstract: *Polycystic ovary syndrome is the most frequent endocrinopathy in women and adolescents. Insulin resistance and compensatory hyperinsulinaemia are seen in most cases of polycystic ovary syndrome. This article focuses on the clinical features, differential diagnosis, the current recommendations for diagnosis, management strategies, the importance of screening for PCOS in adolescence to prevent future problems and long-term sequelae, especially, the metabolic syndrome.*

Keywords: *Polycystic ovary syndrome, Adolescence, Insulin resistance, Metabolic syndrome*

Polycystic ovary syndrome (PCOS) is now being increasingly seen among adolescents. The prevalence of PCOS has been shown to be 5-10% in women of reproductive age.[1] The disorder was initially described by Stein and Leventhal in 1935.[2] PCOS, traditionally thought of as a triad of oligomenorrhea, hirsutism and obesity, is now recognized as a heterogeneous disorder that results in overproduction of androgens, primarily from the ovary and is associated with insulin resistance.

Definition

Rotterdam criteria: According to the Rotterdam consensus it has been recommended that PCOS be defined when at least two of the following three features are present; irregular menses, clinical and/or biochemical signs of hyperandrogenism and polycystic ovarian morphology on ultrasound in the absence of another etiology for the above symptoms (Box 1).[3] Using menstrual irregularity to diagnose PCOS in the adolescent age group is difficult as menstrual irregularity is considered normal in the first few years following menarche.[4] Trans abdominal ultrasound, the only option available in young girls is technically difficult in obese girls. Also multicystic ovaries are usual in adolescence.

Recommendations of International Guidelines for PCOS: The International Guidelines for the assessment and management of PCOS brought out recently endorse the Rotterdam criteria in adults with specific recommendations to diagnose PCOS in adolescence.[5] Adolescent PCOS should only be diagnosed more than 2 years after menarche and if both irregular periods and hyperandrogenism are present. Irregular periods are defined as cycles more than 35 days or less than 21 days. Androgen excess can be assessed clinically and when required biochemically. Polycystic ovaries on ultrasound are not required for the diagnosis.

Pathogenesis

Insulin resistance and compensatory hyperinsulinemia are seen in most cases of polycystic ovary syndrome.[6] Hyperinsulinemia is responsible for the hyperandrogenism, which in turn causes dyslipidemia with an increase in triglycerides and LDL and a decrease in HDL. The theca cells of the ovaries have a generalised overactive steroidogenesis in PCOS. There is excess of oestradiol also

Box 1. Rotterdam diagnostic criteria - Diagnosis of polycystic ovary syndrome

Two of the following th ree criteria are required

- oligo/anovulation
- hyperandrogenism
  - clinical (hirsutism or less commonly male pattern alopecia) or
  - biochemical (raised FAI or free testosterone)
- polycystic ovaries on ultrasound

Other aetiologies such as congenital adrenal hyperplasia, androgen secreting tumours, Cushing syndrome, thyroid dysfunction and hyperprolactinaemia must be excluded

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in addition to the excess androgens. In the ovary, inhibin is increased, which causes low FSH concentrations compared with LH, in girls with PCOS. Since inhibin stimulates androgen production which in turn stimulate inhibin secretion, the result is a vicious cycle in the ovary that inhibits follicular development and ovulation. There is a genetic component as most cases of PCOS show a familial clustering in female siblings and there may be a family history of diabetes. A defect in the insulin receptor gene has been demonstrated. Both X linked and autosomal genes are thought to be responsible.

Clinical presentation

PCOS usually presents for the first time at puberty along with weight gain. Menstrual disorders along with hirsutism is the most common presentation.

Menstrual disorders: The menstrual disorder may be anovulation or oligo-ovulation and ranges from amenorrhoea to oligomenorrhoea. Amenorrhoea is usually secondary but may rarely be primary. Irregular or infrequent menstrual cycles are common in the first few years following menarche but are usually self-limiting, once ovulation is established. If it persists or is associated with signs of hyperandrogenism like hirsutism, evaluation is necessary. The current recommendations are that the diagnosis of PCOS should be made when irregular periods /amenorrhoea persists for two years after menarche.[7]

Hyperandrogenism: Progressive hirsutism is a manifestation of hyperandrogenism and should be assessed by the Ferriman Galwey scale.[8] Acne is common in adolescence and hence not to be used as a marker of hyperandrogenism in adolescence. In the absence of clinical hyper androgenism, biochemical evaluation is recommended to confirm the diagnosis of adolescent PCOS.[7] Free testosterone or free androgen index should be used. Overt signs of virilisation like male pattern baldness, increased muscle mass, clitoromegaly and deepening of voice and very high androgen levels usually indicate the presence of an androgen producing tumour.

Obesity: It is seen in 50-70% of girls with PCOS. The South Asian guidelines consider a BMI of 23 or more as overweight and 25 or more as obese. A waist circumference more than 80 cm is considered significant in the South Asian context. Obesity is not essential for the diagnosis of PCOS, but should be considered as 'at risk' for the metabolic syndrome.

Acanthosis nigricans: This is the presence of dark, velvety patches in the armpits, nape of neck and under the breasts. This is a definite sign of insulin resistance.

Differential diagnosis

The differential diagnosis includes hypothyroidism, hyperprolactinaemia, androgen secreting tumours of ovary and adrenal, late onset congenital adrenal hyperplasia and Cushing's syndrome.

Investigations

Ultrasound abdomen: It may show the presence of polycystic ovaries.[9] However, in adolescence ultrasound evidence of PCOS is not necessary as multifollicular ovaries are common in adolescence. Ultrasound helps to rule out androgen secreting tumours in cases of severe hyperandrogenism.

Hormonal investigations

It includes thyroid function tests and prolactin. Free testosterone or free androgen index is the best marker of ovarian androgen production. Dehydroepiandrosterone (DHEA) is increased if there is an adrenal tumour. 17 α OH progesterone is increased in late onset congenital adrenal hyperplasia.

Tests to detect metabolic problems

Fasting blood glucose and a 75 gram OGTT may reveal overt diabetes or impaired glucose tolerance while lipid profile may show increased total cholesterol, triglycerides and LDL and low levels of HDL. At present there is no reliable test to detect insulin resistance. Routine testing of insulin level is not mandatory for detecting insulin resistance. The best markers of insulin resistance are BMI, waist circumference more than 80 cm, acanthosis nigricans and impaired glucose tolerance on OGTT.

Why diagnose PCOS in adolescence?

The problems in adolescent PCOS are menstrual disorders, cosmetic problems, obesity and psychosocial problems. Menstrual problems include irregular or excessive bleeding and amenorrhoea. Cosmetic problems are hirsutism and acne which are very distressing to the adolescent. Psychological problems like anxiety and depressive symptoms are common in adolescent PCOS. Obesity and hirsutism may have a negative impact on body image, which will contribute to loss of self-esteem resulting in psychological problems. The natural history of PCOS suggest that after a gap of 2 years, among adolescent girls with confirmed menstrual irregularity with or without ultra sound diagnosed polycystic ovaries, percentage of those with menstrual irregularities have reduced but those with hirsutism have increased.[10]

Table I. Clinical characteristics of PCOS

Presenting problems	Pregnancy related problems	Future problems
Menstrual disorders	Infertility	Type 2 diabetes
Hirsutism	Recurrent miscarriage	Hyperlipidaemia
Acne	Gestational diabetes	Hypertension
Obesity	Preeclampsia	Coronary artery disease
		Non-alcoholic fatty liver disease
		Adenocarcinoma uterus

Detecting PCOS in the late adolescence or early adulthood is essential to identify a target population at risk of infertility and metabolic syndrome and institute preventive measures like life style changes. This is of public health importance in preventing the metabolic and cardiovascular sequelae of PCOS and the disease burden (Table I).[11]

Long-term problems

Pregnancy related problems: The most widely known long-term problem is infertility. PCOS is responsible for about 30-40% of the overall infertility. Even if the woman becomes pregnant, there is increased morbidity due to an increased chance of miscarriage, gestational diabetes, preeclampsia and all the associated problems of obesity.

Long-term sequelae: PCOS is a forerunner of the metabolic syndrome. Indeed, many adolescent girls with PCOS already exhibit features of the metabolic syndrome. This is even more relevant as the prevalence of the metabolic syndrome especially type 2 diabetes is much more in India compared to the West. The metabolic syndrome is a constellation of cardiovascular disease risk factors associated with insulin resistance; glucose intolerance, dyslipidemia, hypertension and central obesity.[12] Analogous to the situation in adults, the prevalence of the metabolic syndrome increases with obesity, reaching as high as 50% among morbidly obese adolescents. There is intriguing evidence that this increased risk may be conferred not only by insulin resistance but also by hyperandrogenemia. There is an increased risk of the metabolic syndrome in girls and women with PCOS associated with increased androgen levels and independent of obesity.[13] PCOS also predisposes to endometrial cancer, due to unopposed estrogenic stimulation of the endometrium.

Pediatricians are increasingly concerned about the long-term health effects of childhood and adolescent metabolic syndrome and they rightly believe that it may be associated with early cardiovascular disease in adulthood.[7] Cardiovascular event endpoints are difficult to target because of the long latency period between the onset of atherosclerosis and the first cardiovascular event. However, there is evidence from autopsy studies that atherosclerosis starts in childhood.[7]

Management

This includes management of presenting complaint and prevention of long term sequelae. The presenting complaint may be menstrual problems like menorrhagia, irregular periods or amenorrhoea and cosmetic problems like hirsutism. Adolescents with these symptoms should not be denied treatment.[14]

Prevention of the metabolic syndrome

Lifestyle modification: Lifestyle modification by diet and regular moderate intensity aerobic exercise for a minimum of 30 minutes five days a week remains the first line of treatment especially in obese PCOS. Weight loss can cause spontaneous resumption of menstrual cycle and lower androgen levels. Even a 5% reduction in weight can result in these changes. Weight loss results in lowered insulin levels leading to an increased sex hormone binding globulin and thereby a decrease in the free testosterone levels. This approach is ideal in the adolescent group as this is the period when lifestyle modification is easiest to achieve.

Insulin sensitizers: Association of overweight and insulin resistance with polycystic ovary syndrome (PCOS) has been clearly demonstrated.[15] Insulin sensitizers help the body to utilise insulin in a more efficient manner. Metformin is the drug, which has been most widely used in adolescents.[16,17] Metformin acts by decreasing glucose production by the liver. It produces improvement in insulin resistance, reduction in androgens and in many cases, spontaneous resumption of periods has been observed. The initial dose of 500 mg OD can be increased to 1000-1500 mg daily in divided doses. Side effects are nausea, vomiting and dyspepsia. Lactic acidosis is a rare side effect. It should be avoided in girls and women with altered renal or hepatic function. Long acting preparations are also available with fewer side effects. The use of metformin is best restricted to those with definite evidence of insulin resistance such as obesity, acanthosis nigricans or impaired

glucose tolerance. At the moment, lifestyle modification remains the best option in preventing longterm sequelae. Further research is awaited regarding long term use of metformin in preventing the metabolic syndrome.

Menstrual problems and hyperandrogenism

Combination pills: The low dose oral contraceptive pill containing ethinyl estradiol and the third generation progestin desogestrel is given to regularise menstrual cycle and will also combat hirsutism. 6-12 cycles are needed for a demonstrable effect on hirsutism. The best effects are seen in young girls and are found to have beneficial effects on future fertility as well, by normalising the hormonal milieu.[17] The drug prevents unopposed estrogenic stimulation of endometrium and future endometrial cancer.

Cyclical progesterone: Medroxyprogesterone acetate 10 mg twice daily for 5 days in a month will regularise periods if hirsutism is not a problem. It is important to remember that the young women to get endometrial cancer are those with PCOS or oestrogen secreting tumours. Hence, it is imperative that girls or women with PCOS should get withdrawal bleeds at least once in 2-3 months.

Antiandrogens: Antiandrogens are only recommended when the low dose combined pill has no effect on the hyperandrogenism. Spironolactone, an aldosterone antagonist, in the dose of 50-100mg daily twice daily is very effective in combating hirsutism. Electrolyte disturbances like hypokalemia should be monitored. Cyproterone acetate is associated with liver toxicity. Hence, assessment of liver function is advisable before commencing treatment, at 3 months and thereafter every 6 months. Other antiandrogens in clinical use are flutamide, finasteride and ketoconazole. Antiandrogens if used should be in combination with the combined pill.[18]

Acne therapy: Antibiotics such as tetracycline, erythromycin and minocycline are the mainstay of treatment for acne and can be used in conjunction with antiandrogen therapy. Retinoic acid is indicated in intractable acne with severe scarring but such drugs are best administered by a dermatologist, because of potential teratogenic effects.

Cosmetic procedures: Cosmetic procedures, primarily for improving body image, can be combined with the low dose combined pill or antiandrogens for tackling hirsutism to provide an immediate effect, while the impact of long-term hormonal treatment is awaited. Electrolysis and laser are acceptable if done in good centres and are especially useful in removing hair, which has been present for a long time.

Points to Remember

- ***Adolescent PCOS is diagnosed 2 years after menarche, if both irregular periods and hyperandrogenism (clinical and/or biochemical) are present.***
- ***Polycystic ovaries on ultrasound is not necessary for the diagnosis of adolescent PCOS.***
- ***Screening is recommended in adolescence to prevent long-term sequelae.***
- ***Management should include measures to prevent the metabolic syndrome.***

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CLIPPINGS

Screening for Iron Deficiency in Early Childhood Using Serum Ferritin in the Primary Care Setting.

The American Academy of Pediatrics recommends universal screening for anemia using hemoglobin at 12 months.Authors objective was to assess a screening strategy for iron deficiency using serum ferritin. cross-sectional study of children 1 to 3 years old attending a health supervision visit was undertaken. Authors examined the relationship between child age and serum ferritin, age and hemoglobin, hemoglobin and serum ferritin and the prevalence of elevated C-reactive protein (CRP) results of the study was as follows. Analysis (n = 1735) revealed a nonlinear relationship between age and serum ferritin (P < .0001). From 12 to 15 months, for each 1-month increase in age, serum ferritin levels decreased by 9% (95% confidence interval [CI]: 5 to 13). From 15 to 24 months, the rate of change was nonsignificant. From 24 to 38 months, for each month increase in age, serum ferritin increased by 2% (95% CI: 1 to 2). For hemoglobin, from 12 to 24 months, the rate of change was nonsignificant. From 24 to 38 months, for each 1-month increase in age, hemoglobin increased by 20% (95% CI: 9 to 32). Compared with the serum ferritin cutoff of <12 ìg/L, the hemoglobin cutoff of <110 g/L had a sensitivity of 25% (95% CI: 19 to 32) and a specificity of 89% (95% CI: 87 to 91). Elevated CRP >10 mg/L occurred in 3.3% (95% CI: 2.5 to 4.2). Screening for iron deficiency using serum ferritin at 15 or 18 months may be a promising strategy. For children at low risk for acute inflammation, concurrent measurement of CRP may not be necessary.

Oatley H, Borkhoff CM, Chen S, Macarthur C, Persaud N, Birken CS, Maguire JL, Parkin PC, on behalf of the TARGet Kids! Collaboration. Screening for Iron Deficiency in Early Childhood Using Serum Ferritin in the Primary Care Setting. Pediatrics Dec 2018, 142 (6) e20182095; DOI: 10.1542/peds.2018-2095.

NEWS AND NOTES

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RADIOLOGY

HYDROCEPHALUS

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The embryonic brain is a hollow tube that undergoes constrictions and expansions in specific locations to give rise to the prosencephalon, mesencephalon and the rhombencephalon. Ventricles are the remnants of this central cavity in the neural tube. The parts of the brain related to the two lateral ventricles on either side of the midline are the cerebral hemispheres. The diencephalon structures which are the thalamus and hypothalamus are on either side of the centrally placed third ventricle. The aqueduct, a thin vertically linear cavity is not usually seen but runs through the midbrain which is derived from the embryonic mesencephalon. Anterior to the fourth ventricle is the pons while the cerebellum lies posteriorly. Therefore localisation of pathology can be done by identifying the ventricles.

Most of the CSF is produced by the choroid plexus in the floor of the lateral ventricles and the roof of the third and fourth ventricles. The CSF flows from the lateral ventricle through the interventricular foramina to the third ventricle and then to the fourth ventricle through the aqueduct. Finally it reaches the subarachnoid spaces through the foramina of Magendie and Luschka. Obstruction of this pathway causes proximal dilation which may be due to congenital or inflammatory stenosis or masses.

Fig.1 shows normal lateral ventricles as white triangular cavities in a T2 weighted film while the T1 film

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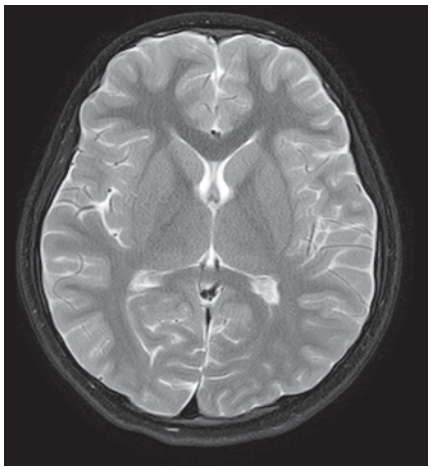

Fig.1. Normal hyperintense LV in T2 film.

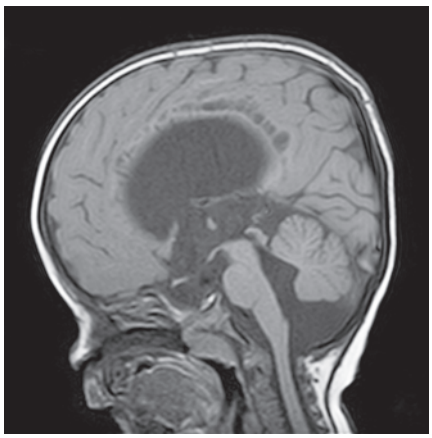

Fig.2. T1 midsagittal section showing dilated ventricles. Fluid is hypointense in T1 films.

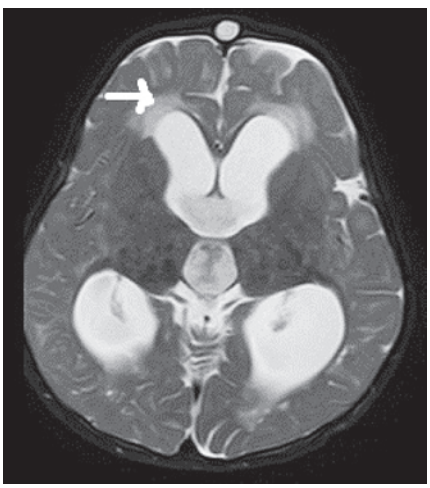

Fig.3. T2 film showing dilated LV and 3V. Note periventricular seepage(arrow)

shows the CSF as grey in color or hypointense (Fig.2). Fig.3 shows dilated lateral and third ventricles and hence the level of obstruction is at the aqueduct. In Fig.4 the fourth ventricle is also dilated and hence the block is at the exit points of the foramina of Magendie and Luschka. This happens often with meningitis or following intraventricular or subarachnoid hemorrhage.

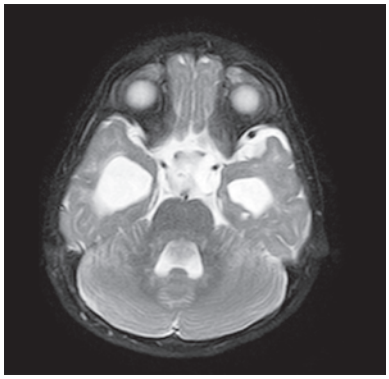

Fig.4. T2 film – dilated temporal horns and 4V.

The temporal horns have the greatest capacitance and its enlargement is an early sign of hydrocephalus. Rounding of the frontal horns and bulging of the third ventricle floor are other early signs. Fig.2 is a midsagittal section showing thinned and elevated corpus callosum and bulging third ventricle recesses. As pressure increases there is bulging of the lateral walls of the lateral ventricles with loss of its normal concave outer margins. Periventricular interstitial edema occurs due to seepage of fluid into the periventricular white matter (Fig.3). This is seen as finger like processes of water density extending peripherally (Fig.5). In MRI (Fig.3), periventricular seepage is appreciated when the normally dark white matter shows white areas in T2 films and the bright white matter in T1 shows grey areas.

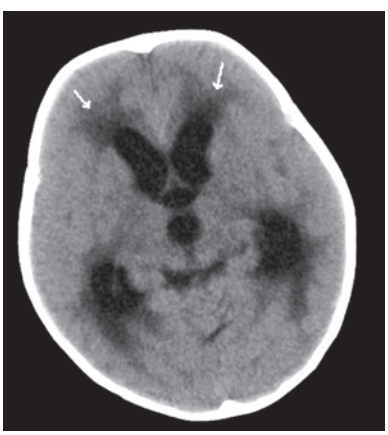

Fig.5. CT-dilated LV and 3V with periventricular seepage into white matter (arrows).

CSF flow is pulsatile corresponding to the systolic pulse wave in the choroid plexus arteries and subarachnoid portion of cerebral arteries. The normal flow velocity is about 5 to 10 cm/sec. Phase contrast study of CSF flow (Fig.6) is a sequence done to distinguish normal pressure hydrocephalus and late onset aqueduct stenosis in older patients, who may already have mild ventricular dilation

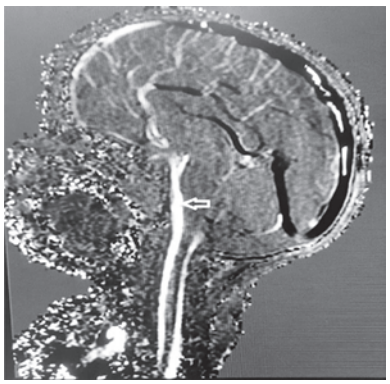

Fig.6. Phase contrast MRI in a normal child. Arrow points to flow in aqueduct.

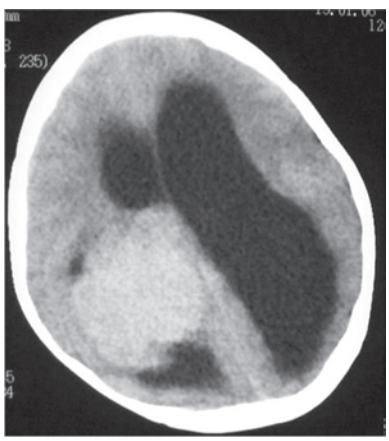

Fig.7. CT brain - Choroid plexus papilloma. Note effacement of sulci.

due to age or other causes. The study helps to rule out actual obstruction. In aqueductal stenosis, there is no flow in the aqueduct while normal pressure hydrocephalus shows high velocity flow.

Fig.7 is that of a four year old child with dilated lateral ventricles and a large hyperdense mass with a lobulated contour in the right lateral ventricle. This is a choroid plexus papilloma. There is increased secretion of CSF causing the dilation. The mass may also obstruct CSF pathways aggravating the dilation. As ventricles dilate further, other signs of pressure increase are seen, like effacement of the sulci (Fig.7) and cisterns and pressing of gyri against the calvarium.

CASE REPORT

DIABETES THAT IS DIFFICULT TO TREAT

*Sridevi A Naaraayan
*Prasanna Sudhakar
*Raghavan Velayudham Dhakshayani
**Rema Chandramohan

Abstract: *A five year old child who was diabetic from the age of one and half years presented with progressive loss of weight and abdominal distension. She had generalized loss of subcutaneous fat, dysmorphic facies, acanthosis nigricans and hepatomegaly. Her glycemic control was poor and she was negative for auto-antibodies. Genetic analysis clinched the diagnosis.*

Keywords: *Insulin resistance gene, Rabson-Mendenhall syndrome, Acanthosis nigricans.*

Genetic mutations causing diabetes are relatively rare conditions encountered in children. Genetic syndromes should be considered in children with diabetes in the presence of dysmorphic features, hearing impairment and defective vision.[1] This report is of a child with genetic mutation causing diabetes which is refractory to treatment.

Case

A five year old girl born of non-consanguineous marriage presented with history of poor weight gain and abdominal distension for three years. Antenatal and postnatal periods were uneventful. The child was born as a full term, low birth weight baby weighing 1.6 kg at birth. She was developmentally normal and was immunized up to age. Father had diabetes and was on oral hypoglycemic agent. At the age of one and half years, the child was diagnosed to have diabetes and was started on lantus insulin and metformin. Parents discontinued insulin on their own but continued metformin and were not on follow up in any hospital/pediatrician. On examination she was looking emaciated with generalized loss of subcutaneous fat (Fig.1),

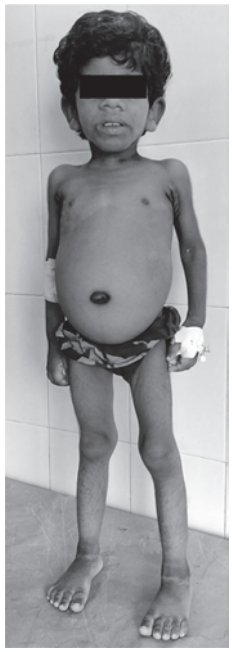

Fig.1. Short stature and umbilical hernia

had dysmorphic facies, hypertrichosis, acanthosis nigricans over neck and axillae, hyper pigmentation of periumbilical region, groin and perianal region and umbilical hernia with a protuberant abdomen. Her height and weight were less than 3rd centile, while her body mass index was between 25th and 50th centile of IAP standard. She had hepatomegaly with a liver span of 11 cm, but other systems examination were normal.

Complete blood count, renal function tests, liver function tests and serum lipids were normal. Ultrasound abdomen showed hepatomegaly with increased echogenecity and a normal pancreas. Her HbA1c was 15.5% with increased serum insulin of 404 mIU/L and 'c' peptide 4.11ng/ml. MRI brain was normal but her whole body MRI showed grossly reduced fat in trunk and limbs. Her thyroid function and bone age were normal. She was negative for auto antibodies for glutamic acid decarboxylase (GAD) and tyrosine phosphatase 2 (IA_2). Genetic analysis revealed homozygous missense mutation of insulin receptor (INSR) gene involving pHis236Tyr and a diagnosis of Rabson-Mendenhall syndrome was made. Both parents were heterozygous for the above mutation. The child is on oral metformin. After the initial visit and investigation she is on regular follow up for the past 12

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months. Though she is on metformin, her glycemic control continues to be poor.

Discussion

Rabson-Mendenhall syndrome, a rare syndrome of insulin resistance was described by Rabson and Mendenhall in 1956 in three siblings. The insulin receptor (INSR) gene which is located in the short arm of chromosome 19 codes for insulin receptor that is found in many types of cells. Insulin receptor is heterotetrameric with two alpha and two beta subunits.[2] Binding of insulin to this receptor activates insulin signalling pathway regulating glucose uptake and release into cells. Mutations in INSR gene result in a spectrum of insulin resistance syndromes like Donohue syndrome (Leprechaunism), Rabson-Mendenhall syndrome and type A insulin resistance syndrome.[3] Donohue syndrome is the most severe of the three syndromes; affected children do not survive beyond two years of age. Type A insulin resistance syndrome is the mildest and commonly presents during pubertal age.

Rabson – Mendenhall syndrome is of intermediate severity like the index case. The affected individuals have features similar to those of Donohue syndrome (Leprechaunism), but usually survive into adolescence.[4] Rabson- Mendenhall syndrome is estimated to affect less than one per million people worldwide. Acanthosis nigricans which is a marker of insulin resistance is characteristically seen. Hyperpigmentation, hypertrichosis and emaciation are common features. Other features like multiple cysts in ovaries and pineal hyperplasia that are usually present, were not seen in this child. Distinctive facies with prominent, widely spaced eyes, broad nose and large, low set ears are seen.[5]

Glycemic control in these patients remains poor and long term complications are frequent. They can be treated with insulin sensitizers like biguanides and thiazolidinediones.[6] Insulin use is restricted to ketoacidosis. Other therapies under trial are human recombinant insulin-like growth factor 1 (IGF-1) and leptin.[7] Confirmation by genetic analysis is essential in counseling the parents on the risk of recurrence in subsequent pregnancies.

Acknowledgement

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NEWS AND NOTES

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CASE REPORT

IMMUNE DEFICIENCY, AUTO IMMUNITY AND MALIGNANCY – AN INTERACTIVE RELATIONSHIP

*Sarala Rajajee
**Ezhilarasi Subbiah
***Nikhil Lohiya

Abstract: *Interaction between auto immunity, immune deficiency and malignancy is well recognized but often missed in clinical practice. There is a fine balance between recognition of self and foreign antigens. When this is breached, it results in autoimmunity or failure to effective recognition of malignant cells. Underlying immune deficiency perpetuates this process. This is a case series of such children from our unit.*

Keywords: *Interaction, Auto immunity, Immune deficiency, Malignancy*

Immune system plays a central role in malignancy, immunodeficiency and autoimmunity. Failure of immune system regulation is responsible for cancer and autoimmunity.[1] Immune system finely balances foreign and self-antigens. Lymphocytes are under check normally by peripheral tolerance but in the presence of stimuli like recurrent infections, as in primary immunodeficiency, this self-tolerance is breached and lead to autoimmune disease.[1,2] Similarly self-reactive cells when depleted will lead to failure of effective recognition of growing cancers that express altered self-antigens. Hence, a complex relationship between autoimmunity, immunodeficiency and cancer exists. A case series of six children with this basis of their presentation and progression have been reported here.

In the hemato-oncology unit of Mehta Children's Hospital, Chennai during January 2003 to January 2015, children with immunodeficiency, malignancy and autoimmunity are followed up regularly. Children who had overlapping features of the above or who went on to have a switch over of disease spectrum of autoimmunity, immunodeficiency and malignancy were studied. Detailed history and work-up were done to find out the possible link between the overlap of the three entities and inference made.

Case 1

A 2½ year old boy, with history of recurrent respiratory tract infections (RRTI) and gastrointestinal (GI) infections from 6 months of age hospitalized twice for acute gastroenteritis, presented with fever, rash, joint pain and swelling of large joints (knee, ankle and elbow). Child had elevated total leucocyte count (TLC), ESR and was diagnosed as systemic onset juvenile idiopathic arthritis (SOJIA). After ruling out tuberculosis, child was started on oral steroids and oral methotrexate weekly. Fever and joint pain subsided but RRTI persisted. Child was admitted in PICU with fever, seizures and was diagnosed to have meningoencephalitis. He recovered with residual intellectual disability and spasticity. Immunoglobulin profile showed very low IgG, M and A levels. Flow cytometry had low CD19 and normal CD3 and CD7. Hence, child was started on IVIG every 3 weekly. He received 12 cycles of IVIG and is doing well with no recurrent infections but residual mental retardation and spasticity persisting. In this child, underlying immunodeficiency presented as an autoimmune disorder.

Case 2

Eight year old girl presented with mild yellowish discolouration of eyes, progressive pallor and hepatosplenomegaly and generalized lymphadenopathy. Evaluation revealed low hemoglobin (Hb) with high reticulocyte count, ESR and LDH (lactate dehydrogenase). Peripheral smear showed hypochromic, microcytic red cells, polychromasia, normoblast, microspherocytes with lymphocytic response with many atypical lymphocytes and mild thrombocytopenia. Direct Coomb's test was negative and Epstein-Barr virus (EBV) IgM positive. Bone marrow aspiration (BMA) and immunoglobulin profile were normal. Child was given pulse methylprednisolone for 3

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days and was tapered on oral prednisolone. Hemoglobin and reticulocyte count improved. One month later child again had anemia and on evaluation showed low Hb with raised reticulocyte count. This time intravenous immunoglobulin (IVIG) was given followed by low dose prednisolone and child responded well. Two months later child again had low hemoglobin with low total leucocyte counts and thrombocytopenia. Peripheral smear showed presence of blast cells and further workup with bone marrow aspiration diagnosed as acute lymphoblastic leukemia (L1 + L2), flow cytometry proved to be T cell type. Autoimmune hemolytic anemia post EBV infection leading to malignancy is a possibility in this child.

Case 3

Fourteen years old adolescent boy presented with fever of 10 days with a history of RRTI and GI infections with recurrent abdominal distension and vomiting in past 1 year, diagnosed to have GERD (gastro oesophageal reflux disease) and treated with PPI (Proton pump inhibitors). Examination showed generalized lymphadenopathy and hepatosplenomegaly. Blood counts showed lymphocytic response with atypical lymphocytes, low hemoglobin, thrombocytopenia and EBV IgM was positive. Chest Xray and USG abdomen were normal. Lymphnode biopsy showed T cell non Hodgkin lymphoma on histology. BMA was normal. Child had low immunoglobulin (IgG, IgM and IgA). Flow cytometry showed low CD19 and CD20 with normal CD7 cells. On echocardiography found to have right atrial intracardiac mass. Child was started on chemotherapy and IVIG every 3 weekly. He responded well initially but later developed febrile neutropenia and refractory septic shock. Child died due to cardiac dysfunction. This child presented initially as immunodeficiency but later progressed to T-cell Non Hodgkin lymphoma post Epstein-Barr virus infection.

Case 4

A ten years old boy presented with fever, swellings in the posterior cervical region and suprasternal area. Lymphnode biopsy showed features of anaplastic large cell lymphoma. He was started on chemotherapy with BFM NHL protocol. Chemotherapy was successful and child attained remission. After 5 years child presented with petechiae and oral bleeds for one week with no fever and axillary lymphadenopathy. CBC showed thrombocytopenia and normal Hb and WBC. Pet scan showed multiple foci in axillary nodes. Axillary lymphnode biopsy showed no malignancy. BMA was consistent with immune thrombocytopenia. ESR, LDH, HIV serology, immunoglobulin profile were normal. Child was started on oral prednisolone 2 mg/kg/day. He showed good response to steroids and medication was tapered. For 1 year he was off any therapy when he came with recurrence of petechiae and oral bleeds. PET scan, BMA and flow cytometry were normal but showed severe thrombocytopenia. Prednisolone was started and child improved but had adverse effects in the form of cushingoid features and hence steroids were stopped. Child was started on danazol, improved and is well till date. Hence, this is a child with malignancy who progressed to develop autoimmune disorder after successful treatment of malignancy.

Case 5

Two and half years old boy with history of recurrent diarrhea presented with ear infections and recurrent respiratory tract infections. History revealed death of elder sibling at 11 months of age due to acute gastroenteritis. Child was evaluated for immunodeficiency. HIV screening was negative and had low immunoglobulin levels (IgG, IgM and IgA). Child was treated with IVIG 400 mg/kg every 3 weeks; on follow-up had skin lesions which on biopsy showed dermal fibrosis and perivascular round cell infiltration suggestive of scleroderma. Dermatologist opined it to be scleroderma secondary to immunodeficiency. Thus an autoimmune disorder occurred due to immunodeficient state.

Case 6

Twelve years old adolescent boy presented with fever, jaundice, anemia and hepatosplenomegaly. On evaluation his hemoglobin was 3 g% and reticulocyte count was 15%. Peripheral smear showed lymphocytic response with many atypical lymphocytes and normal platelets. Indirect bilirubin and LDH were elevated. Direct Coomb's test was positive. EBV IgM was positive and bone marrow aspirate was normal. He was given pulse methylprednisolone for 3 days, followed by oral prednisolone. Child was doing well for 6 months, when he had recurrence of fever, enlarged nodes with hepato-splenomegaly and anemia. Peripheral smear at this stage revealed features suggestive of acute myeloid leukemia (M1). Flow-cytometry confirmed the diagnosis of acute myeloid leukemia (AML). Child was subsequently started on chemotherapy and is currently doing well. This child presented as autoimmune hemolytic anemia (AIHA) which progressed to AML.

Table I and II gives a brief synopsis of the initial presentation, underlying diseases and management of all the six children.

Table I. Clinical presentation and underlying diseases

Case	Age	Sex	Initial presentation	Progression / Underlying diseases	EBV
1	2 ½	Male	SOJIA	Agammaglobulinemia	Negative
2	8	Female	AIHA	T Cell ALL	Positive
3	14	Male	Recurrent infections. Humoral immune deficiency (Not diagnosed)	T Cell NHL Intracardiac right atrial tumour	Positive
4	10	Male	Anaplastic NHL	ITP	Negative
5	12	Male	AIHA	AML	Positive
6	2 ½	Male	Agammaglobulinemia	Scleroderma	Negative

Table II. Management in this case series

Case	Initial treatment	Later treatment	Spectrum
1	Steroids, methotrexate	IVIG once in 3 weeks	SOJIA with Immune Deficiency
2	Steroids, IV immunoglobulin	Chemotherapy for ALL	AIHA EBV ALL
3	Treatment of infections	Chemotherapy for NHL plus	NHL, intracardiac right atrial tumor IVIG once in 3 weeks EBV, Cell NHL Immune Deficiency
4	Chemotherapy for NHL	IVIG and steroids	NHL, ITP
5	Steroids	Chemotherapy	AIHA, EBV, AML
6	IVIG once in 3 weeks	Tacrolimus	Immune deficiency Scleroderma

Discussion

Immune system has a complex relationship with malignancy. It generates anticancer response but also may promote tumor genesis. Anticancer response is the reason why tumor infiltration by lymphocytes, correlates with improved prognosis and patient survival and also occurrence of paraneoplastic syndrome, suggesting the possibility of antitumor response of immune system.[3-5] Successful anti-tumor response cross reacts with the normal self-tissue, lose self-tolerance and with increasing magnitude of immune response, may lead to chronic inflammation and autoimmune disorders.[6,7]

The reason for the above problem is said to be that chemotherapy given in malignancy non-specific immunosuppression and directed against the cell surface molecules, receptors and function involving common activation and effector pathways of immune system. These pathways get hyperactivated and causes enhanced release of pro-inflammatory mediators leading to autoimmunity.[6,7] Similarly, inhibition of this common activation and effector pathway to counteract autoimmunity could negatively affect desired responses that are involved in host defense.

It has been proposed by Chen, et al[8] that chronic systemic inflammation and concurrent B cell activation in Rheumatoid arthritis (RA) patient leads to hematological malignancy. AIHA may develop prior to malignancy which has been reported earlier.[9] EBV is notoriously known to cause both AIHA and hematological malignancy. Hence, the relationship between autoimmune disease and cancer is due to the complexity of immune system. Specific immunotherapy may help to reduce the incidence of malignancy in autoimmune disorders but it may not be possible with all autoimmune disorders if antibodies have not been identified.

It is well known that with immunodeficiency, there is a high risk of cancer and lymphoma.[10] The histological manifestation could vary from normal to grossly abnormal marrow response. It has been proven that there is an increased risk of lymphoma in immunodeficient cases.[9,11] Proposed reason is in patients with CVID, there is an increased proliferation of T-cells leading to lymphoma. There is an increased risk of malignancy even in T-cell immunodeficiency. In such cases the diagnosis and management is extremely difficult and there is an increased mortality due to the immunodeficiency state.[11]

One of the consequences following a successful anti cancer therapy is autoimmunity. It has been established previously that few conditions like uveitis, vitiligo, psoriasis and colitis are the autimmune side effects following a successful chemotherapy of cancer.[12] Vitiligo, for example, is a result of autoimmune destruction of melanocytes.

Conclusion

Interaction between autoimmunity, immune deficiency and malignancy needs to be remembered during management and follow up. High index of suspicion is needed for diagnosing immunodeficiency and malignancy in children with autoimmune disorders before initiating immunosuppressive therapy. In children with lymphoreticular malignancy, autoimmune diseases may occur and not related to relapse. In immunodeficient children, malignancy at unusual location should be kept in mind while treating them.

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SCIENTIFIC PROGRAM

TIME	SESSIONS	SPEAKERS
08.00-08.30 am	**REGISTRATION**	
08.30-09.30 am	**SESSION I**	
08.30-08.50 am	Follow up of preterms – Growth charts, feeding advise, vaccination	Dr.Anitha
08.50-09.05 am	Kawasaki disease – What is new?	Dr.Sathish Kumar
09.05-09.20 am	Break through seizures	Dr.V.Viswanathan
09.20-09.30 am	**Discussion**	
09.30-10.30 am	**SESSION II**	
09.30-09.50 am	Vesico ureteric reflux - Issues in management	Dr.Priya Pais
09.50-10.05 am	Neurorehabilitation in developmental disability	Dr.Vijayalakshmi
10.05-10.20 am	NAC in liver disease	Dr.Malathy Sathiyasekaran
10.20-10.30 am	**Discussion**	
10.30-11.00 am	**INAUGURATION**	
11.00-12.00 noon	**SESSION III** **Panel discussion I: Fever panel (<7days, 7-14 days, >14 days)**	**Moderator:** Dr.Digant D Shastri **Panelists:** Dr.P.Ramachandran Dr.A.Vijayaraghavan Dr.Vidhya Krishna
12.00-01.00 pm	**SESSION IV**	
12.00-12.20pm	Infant and young child feeding	Dr.S.Srinivasan
12.20-12.35pm	X-ray- clues to diagnosis	Dr.G.Vijayalakshmi
12.35-12.50pm	Vitamin D Deficiency myths and reality	Dr.S.Balasubramanian
12.50-01.00 pm	**Discussion**	
01.00-02.00 pm	**LUNCH**	
02.00-03.00 pm	**SESSION V** **Panel discussion II: Tuberculosis ¬ Issues in management**	**Moderator:** Dr.N.C.Gowrishankar **Panelists:** Dr.S.Elilarasi Dr.So.Sivabalan Dr.S.Lakshmi
03.00-04.00 pm	**SESSION VI**	
03.00-03.20 pm	Acute abdominal pain – Medical / Surgical	Dr.R.Senthilnathan
03.20-03.35 pm		Dr.V.Poovazhagi
03.35-03.50 pm	Transfusion safety	Dr.Aruna Rajendran
03.50-04.00 pm	**Discussion**	
04.00-05.00 pm	**SESION VII**	
04.00-04.20 pm	Septic shock – Recent advances	Dr.Deepika Gandhi
04.20-04.35 pm	Cardiac failure – Recent advances	Dr.Sai Leela
04.35-04.50 pm	Cleansers	Dr.C.Vijayabhaskar
04.50-05.00 pm	**Discussion**	

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