THE INDIAN ACADEMY OF PEDIATRICS

genetic CLINICS

Newsletter of Genetics Chapter of Indian Academy of Pediatrics

Volume: 5
Issue: 1 (January-March 2012)

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Editorial

Medical Genetics in India: Catching up with the world!

Best wishes for the New Year!

The beginning of a new year always makes one look back. Though one may refrain from making ambitious new plans or resolutions, one can't help reflecting on one's achievements in the years that have gone by. The last few years have been quite satisfying for the growth of medical genetics in India. The number of medical genetics centers has increased during the last decade and so has the number of clinical geneticists. Clinical geneticists, though still a rare species in India, have gained a status and demand in the medical environment of India. All my medical genetics colleagues would agree with me that the referral has been improving over the last decade and is becoming specific and targeted, thanks to the increasing awareness about this medical specialty amongst medical practitioners and increasing availability of investigations for genetic disorders. The armamentarium of genetic tests is rapidly increasing and includes a wide spectrum of biochemical, molecular and molecular cytogenetic techniques. The availability of tandem mass spectrometry, gas chromatography mass spectrometry and various enzyme assays have enthused not only geneticists but also a number of neonatologists and paediatricians about inborn errors of metabolism. Appropriate and timely diagnosis of these rare metabolic disorders is a boon for affected families and helps in providing treatment, genetic counseling and prenatal diagnosis. There is another new activity in the area of genetic metabolic disorders and that is the beginning of pilot projects in newborn screening. These pilot projects funded by the Indian Council of Medical Research and the Department of Biotechnology will go a long way in not only establishing feasibility but also creating awareness about newborn screening amongst primary care physicians and laypersons alike. Our initial experience shows that newborn screening for preventable causes of mental retardation appeals even to families belonging to the lower socioeconomic strata and village folks. We are quite late entrants into the arena of newborn screening as compared to other developing countries. But the beginnings have been made and we hope that these initiatives gain further momentum and get the much needed government support, which is essential for such population- based screening programs.

The other welcome change I have noted in the area of medical genetics is the increasing demand for clinical geneticists especially in tertiary care hospitals. The number of clinical geneticists needed for this vast country is huge and there is a great need of many more centers providing formal training in genetics to medical doctors. New fellowships have been started and I hope that some genetics centers also start three year DM training programs in medical genetics. The increasing popularity of genetics workshops and symposia is a welcome trend and these programs fulfill a small but important role of delivering knowledge about the latest clinical applications of genetics to primary care physicians, different medical specialists and medical students. Awareness about genetic disorders is the most important step towards identifying patients with a possible genetic etiology and for doing the necessary work up or referral to a specialized medical genetics centre.

The genetic testing facilities also have greatly improved though the laboratories are limited in number. This is not much of a problem as the samples can easily be transported. More than 3000 monogenic phenotypes have a known genetic basis and DNA based tests for mutation detection play an important role in the confirmation of these disorders. DNA based tests also have an important role in carrier detection, prenatal diagnosis, presymptomatic diagnosis of late onset disorders,

determination of cancer susceptibility and pharmacogenetic studies to identify individuals at risk for adverse effects of drugs and are also increasingly finding a role in the evaluation of multifactorial diseases like venous thrombosis, diabetes, etc. This makes it essential for every medical practitioner to understand the principles of DNA diagnosis and the vocabulary of molecular genetics. The spectrum of tests for monogenic disorders is rapidly expanding and there are many technically sound molecular laboratories which can establish mutation detection tests for any new disorder in a week's time. It is a very exciting and welcome phenomenon for clinical geneticists and given the time and efforts India may establish itself, in the not so distant future, as a destination for quality genetic testing of samples from all over the world at reasonable and affordable costs. Similar progress has been made in various molecular cytogenetic techniques. Fluorescence in situ hybridization (FISH), Multiplex Ligation Probe Amplification (MLPA) and Quantitative Fluorescence PCR (QF PCR) are now well utilized techniques in India and have become popular within a short time. Similarly, cytogenetic microarray has also arrived in a big way and laboratories are getting experience with this fascinating test to study the whole genome in one go. It is only a year ago that the American Society of Medical Genetics had recommended microarray- based cytogenetic analysis as the first line of investigation for individuals with mental retardation and suggested replacing traditional cytogenetics with cytogenetic microarray. This may still not be cost- effective for our patients in India; but we are technically ready for the same. The latest exciting developments in genetics are next generation sequencing and the ability to sequence the whole genome or exome as required. Exome sequencing has proved to be an easy tool for mapping of genes for monogenic disorders. It is so powerful that even two- three cases of any rare or new genetic disorder are sufficient to identify the causative gene. It looks like the causative genes of all the many remaining monogenic disorders for which the genetic basis has not been established so far, will be identified soon. The power of exome sequencing is such that it has been used in identifying the molecular defect even in a single case and has been instrumental in changing the course of management. With such case based experiences, exome sequencing is likely to soon become available as a diagnostic test for patients. At present, exome sequencing is also being used quite extensively for research into the molecular pathology of cancers. At this juncture it is heartening to know that Indian laboratories have acquired next generation sequencing technology and research projects using exome sequencing to identify genes for new and rare syndromes are underway. India is a gold mine for rare and new syndromes due to the large size of our population and high rates of consanguinity. Hope we can make the best use of all these available opportunities and technology without any delay. Medical genetics in India appears to be catching up with the rest of the world; but we definitely have a long way to go!

Please do not miss the special article in this issue by Dr Judith Hall on Genetics in 2012 specially written for all of us!

Shubha Phadke
1st January, 2012

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http://www.iapindia.org/proforma/IAP_genetics_chapter_application_form.pdf

Medical Genetics in 2012

Dr Judith G Hall, OC, MD

Professor Emerita, Departments of Pediatrics and Medical Genetics, University of British Columbia

2012 seems to represent a point of change, that is, an exponential change in the influence and applicability of genetics to health care systems. Multiple factors seem to be coming together, including the internet, the availability of the sequence of the human genome, systems biology, developmental genetics, the recognition of developmental origins of health and disease, the availability of multiple genetic services for newborn screening, CGH array, exomics, and whole genome sequencing for individuals. We are indeed on the edge of a breakthrough into personalized medicine.

As individual human genomes are sequenced, we begin to realize that every person, everyone of more than the 7 billion people on the earth, is totally unique genetically (even monozygotic twins). Not only are they unique in their nucleotide sequence, but also with regard to the epigenetic changes in the confirmation of their DNA. Each individual has had as many as 5,000 new mutations. In the past, we thought of new mutations as leading to selective disadvantage or advantage. However, in this modern day where we try to provide health and well being to all individuals, it may be possible to identify and interpret these changes in ways allowing one to avoid environments, medications, or psychological situations that could lead to poor health.

Observing the human response to information about health, up until this time however, it is not entirely clear that individuals will heed such warnings, or that their health care providers will provide appropriate testing. There has been great concern that there would be discrimination against those who carry genetic "abnormalities". However, now that we know that WE ALL carry them, potential stigma applied to carrying or having mutations will hopefully become a thing of the past. There is no "normal" human being.

Medical genetic services in developed countries are sometimes considered a luxury, but the public health data is clear that provision of diagnostic genetic services leads to markedly improved care, decreased costs (from other expensive testing) and more appropriate family planning.

The International Federation of Human Genetics Societies website serves as a resource of information about policies, standards, guidelines, etc., for genetic services and education. Experience from other countries hopefully will lead to streamlining the process of provision of clinical genetic services. Clinical genetic services often start with newborn screening. Then other appropriate services are adopted for the stage of development of a particular country. Almost all countries that provide newborn screening start with a limited number of tests (hypothyroidism, congenital virilizing adrenal hypoplasia, etc.) which are appropriate for that country. Creative approaches have been developed such as in the Philippines where grandparents were originally asked to provide the service as a birth gift. Generally, countries start with newborn screening for disorders that are treatable and will save enormous amounts of money in clinical services, such as hypothyroidism. Not only is treatment of hypothyroidism (1 in 3,000 newborns) simple and appropriate, but also produces a functional individual, and decreases the multiple ways in which that individual would cost society and the family.

The second phase of the development of genetics services usually includes organizing medical genetic clinics where expertise and diagnostic abilities are focussed. These clinics require information

databases, reliable laboratories able to interpret results, and well trained clinicians. Eventually, the educational systems for health care providers must educate and engage all members of the health care provision system about genetic services and genetic disorders in a way that leads to these problems being addressed in a timely and appropriate fashion.

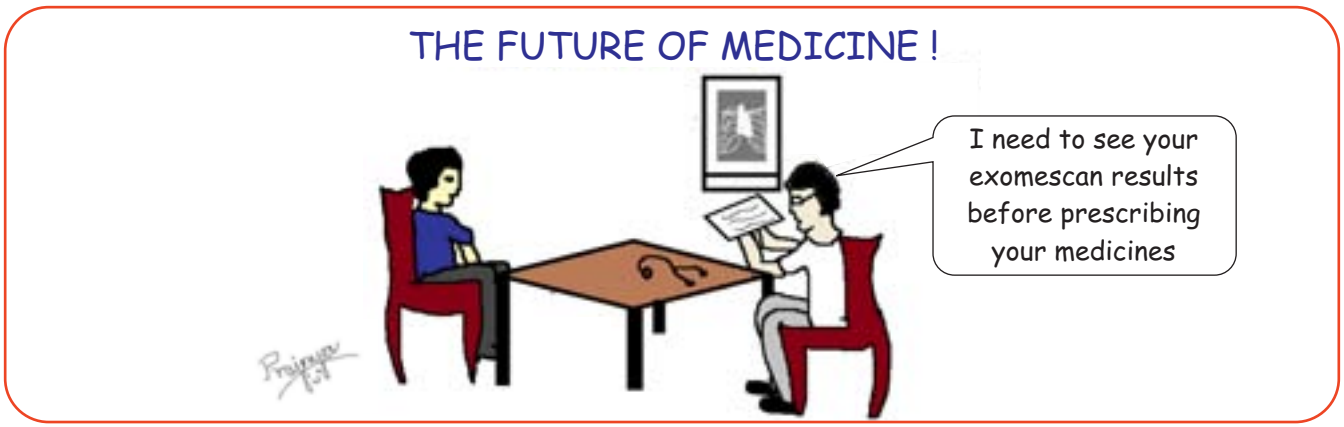
India is making real progress in moving from research genetics to practical applied clinical genetics. New specialists are being trained each year. New medical genetics centres are being established and the country quite clearly is moving toward providing a service appropriate for developed countries. Further development may require advocacy to government to demonstrate the value of clinical genetic services. Advocacy of clinical genetic services is best undertaken by the users of the service who often are the families and physicians. We can anticipate that human genetic knowledge will revolutionize medical care in the decades to come.

GenEvent

An Expert Group meeting on Prevention of Birth Defects in the WHO South-East Asian region from 13th-15th December 2011 was organized by the Division of Genetics, Department of Pediatrics, All India Institute of Medical Sciences, New Delhi, in collaboration with WHO/SEARO and CDC Atlanta . The objectives of this meeting were to review the current situation related to birth defects in the South East Asian Region, to identify the regional priorities in the area of Birth Defects, and to identify and discuss possible strategic directions for prevention and management of Birth defects in the Region. Country wise priorities and strategies were discussed. Experts recommended initiating a birth defect prevention program. Hope that this leads to beginning of population based programmes for neural tube defects, thalassemia and congenital hypothyroidism.

GeneToon

Contributed by: Prajnya Ranganath



Prenatal Diagnosis of Congenital Hyperinsulinism Caused by Mutations in ABCC8 (SUR1) Gene

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Abstract

Previous child with developmental delay is a common indication for prenatal diagnosis in the subsequent pregnancies. Clinical evaluation and investigations of the index case are necessary to confirm the cause of delay. Correct etiological diagnosis is a must for accurate prenatal diagnosis. Congenital hyperinsulinism is the most frequent cause of severe, persistent hypoglycemia in infancy and childhood. The hypoglycemia leads to brain injury. We report on prenatal diagnosis of congenital hyperinsulinism caused by mutations in the ABCC8 gene.

Introduction

Due to the huge population and a high birth rate there are a large number of patients with genetic diseases in India.[1] High rate of consanguinity among many communities further increases the prevalence of genetic disorders, while the lack of rehabilitative facilities escalates the burden of genetic disorders.[2] In a multi-centric study on the referral pattern for genetic counseling, the top four disorders were repeated abortions (12.4%), identifiable syndromes (12.1%), chromosomal disorders (11.3%) and mental retardation (11%). Verma et al reported that cases with mental retardation account for 13% cases referred for genetic counseling.[3] Many of these cases would seek genetic counseling so as to avoid the birth of another affected child. We present the case of prenatal diagnosis of congenital hyperinsulinism. Affected child had developmental delay and he was evaluated to determine the cause of delay.

Case Report

A 36-year-old third gravida was referred to our department for prenatal diagnosis at 13 weeks of gestation as the previous child had developmental delay. The index case was an 8-year-old boy, who had global developmental delay. He was born at 36 weeks of gestation by spontaneous vaginal delivery. His birth weight was 3.77 kg. There was no history of delayed cry. The mother had no history of gestational diabetes mellitus. He was referred to the neonatal intensive care unit at 12 hours of life with complaints of decreased activity and jitteriness. At the time of admission his blood sugar was 9 mg/dL. His blood sugar remained at a low level (less than 50mg/dL) with corresponding serum insulin levels of 62.5mIU/mL (3-35uIU/mL) for which he continued to have glucose requirements of 12-14 mg/kg/minute. He had normal serum lactate and ammonia levels. He was started on octreotide 1 microgram/kg/day which was increased gradually to 20 microgram/kg/day and diazoxide 15mg orally thrice daily along with growth hormone and hydrocortisone. He was put on oral feeds every 2 hourly supplemented with corn starch and added sugar. He was advised pancreatectomy in view of the high insulin levels and recurrent hypoglycemia and poor response to the medical treatment. The parents did not opt for pancreatectomy after understanding that there would be no cognitive improvement. The parents gradually decreased the medications prescribed and they started him on some traditional treatment. At one year of age parents noticed that he did not respond to sounds

and BERA revealed 85% hearing loss. His fundus examination was normal. His developmental milestones did not improve over the next year and he was investigated further. He was not dysmorphic and there was no visceromegaly. There was no family history of similar problem. Magnetic resonance imaging showed symmetric damage of both lobes of brain. His karyotype was 46,XY. His tandem mass spectrometry and organic acid levels revealed no abnormality. His parents were concerned about his developmental delay and did not want a recurrence of this problem in the next child. He was suspected to have congenital hyperinsulinism in view of hypoglycemia and high insulin levels and we did molecular tests for confirmation.

Molecular diagnosis: Genomic DNA was extracted from peripheral blood from the affected child and both parents. Mutation analysis of ABCC8 (encoding SUR1) gene was undertaken according to the protocol described previously.[4] The child was found to be homozygous for the G111R mutation of the ABCC8 gene. This result confirmed the diagnosis of autosomal recessive congenital hyperinsulinism. Sequencing analysis of ABCC8 gene showed that his father and mother were heterozygous for the G111R mutation. The risk that their next child would be affected by congenital hyperinsulinism was 25%. Prenatal testing was possible at 18 weeks of pregnancy. This showed the fetus to be a heterozygote for G111R mutation in the ABCC8 gene and so the parents chose to continue the pregnancy.

Discussion

Hyperinsulinism (HI) is the most common cause of persistent hypoglycemia in newborns and infants.[5] It has an incidence of 1 in 30,000–50,000 live births and results from a dysregulation of insulin secretion by the pancreatic islet cell.[5,6] Children often present within the first month of life with hypoglycemia, hypotonia, seizures, and loss of consciousness or are detected fortuitously. There is a marked heterogeneity in the clinical presentation, molecular mechanisms and histological basis of the disease.[7]

In the present family, the proband had repeated episodes of hypoglycemia in infancy and childhood. He had high serum insulin levels at the time of hypoglycemia. This strongly suggested the diagnosis. Congenital hyperinsulinism should be suspected clinically in every infant presenting with unexplained hypoglycemia. Other causes of developmental delay should be excluded before offering definitive prenatal diagnosis. In the Indian Council of Medical Research (ICMR) study on genetic causes of mental retardation, chromosomal disorders comprised 23.7%, identifiable syndromes 11.6%, and metabolic defects 5%. Of the chromosomal disorders 94.8% were Down syndrome.[2] Mutation analysis of hyperinsulinism was done in view of repeated episodes of hypoglycemia and high insulin. This confirmed the clinical diagnosis and prenatal diagnosis was done at 18 weeks of pregnancy.

Congenital hyperinsulinism is commonly caused by mutation in ABCC8 gene or KCNJ11 gene. These genes account for about 50% of cases with HI.[8] These genes regulate the ATP sensitive potassium channel (KATP), which is involved in insulin secretion in relation to meals. CHI can be inherited as autosomal recessive or dominant. Our patient had a missense mutation in exon 3 of the ABCC8 gene. The G>A mutation at nucleotide 331 (c.331G>A) results in the substitution of arginine for glycine at codon 111 (p.Gly111Arg) and has been reported previously.[9] This case highlights the fact that prenatal diagnosis is possible for even a rare genetic condition, but only after the diagnosis in the index case is confirmed.

Acknowledgements

The authors acknowledge Prof. Sian Ellard, Institute of Biomedical and Clinical Science, Peninsula Medical School, University of Exeter, UK for genetic testing.

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Fetal Ventriculomegaly

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Introduction

Central nervous system malformations are amongst the commonest malformations detected antenatally. Assessment of cerebral ventricles remains an important part of antenatal ultrasound. Ventriculomegaly is a term which defines the enlargement of ventricles in brain. It usually indicates the presence of excess cerebrospinal fluid (CSF) in the developing brain of the fetus. The term 'hydrocephalus' is used when ventriculomegaly is associated with an increase in intracranial pressure. As we can not measure the CSF pressure in utero, the term ventriculomegaly is preferred. Ventriculomegaly is among the most common abnormalities detected in prenatal ultrasound examination. As the prognosis varies greatly from normal outcome with or without surgery to stillbirth, it poses a great diagnostic and prognostic challenge to the genetic counselor and dilemma in decision making to the prospective parents.[1]

Diagnosis and classification of ventriculomegaly on prenatal ultrasonography

Prenatal diagnosis of ventriculomegaly is done by ultrasonographic measurement of the lateral ventricle atrial width. According to guidelines of the International Society of Ultrasound in Obstetrics and Gynecology formulated in 2007, measurement of lateral ventricle should be done in axial view of fetal head at the level of atrium in transventricular plane, where the glomus of choroid plexus lies.[2] Measurement should be done at perpendicular to ventricular cavity by keeping both the calipers at inner margin of lateral and medial wall of the lateral ventricle (Fig 1). If visibility in axial plane is not clear then coronal plane may be used at the

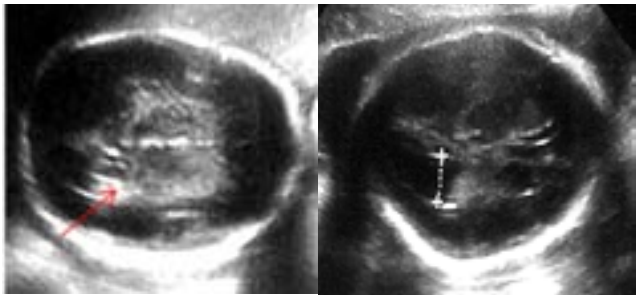

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Fig 1: (a) USG section of fetal brain to measure the atrial width of the lateral ventricle; arrow pointing towards the choroid plexus. (b) Fetal USG showing dilated ventricle.

level of atria, keeping the calipers at inner margin of ventricular walls. It is usually measured in the distal cerebral hemisphere due to technical difficulty in visualization of the proximal hemisphere. However an attempt should be made to visualize both the cerebral ventricles to differentiate between unilateral and bilateral ventriculomegaly as the prognosis may vary in both the cases. Along with the enlargement of lateral ventricles, the third and fourth ventricles may also be enlarged to varying degrees depending on the level of obstruction.[2]

Classification of ventriculomegaly is done on the basis of atrial width. Measurements up to 10 mm are considered normal during the period between 14 weeks of gestation and term. In newer ultrasound (USG) machines, it corresponds to 9.6 mm to 10.5 mm. Atrial width of 10 mm is taken as cut off for the upper limit of normal. Atrial width between 10-12 mm is said to be mild, between 12-15 mm moderate and › 15 mm is considered to be severe ventriculomegaly.[1] These categories differ in associated malformations, frequency of chromosomal abnormalities, perinatal mortality and neurocognitive abnormalities in surviving fetuses as

shown in table 2. Thus the classification helps in providing counseling regarding the prognosis in

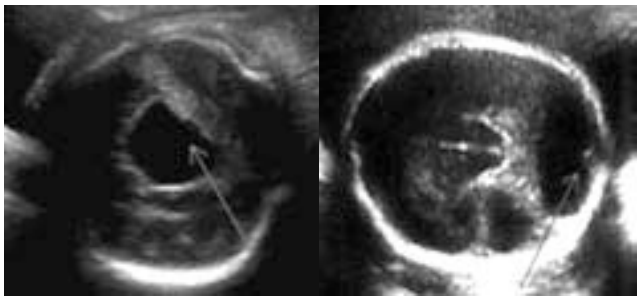
Table 1: Table showing commonly associated malformations and syndromes with prenatally diagnosed ventriculomegaly.

S.No.	Type of anomaly	Examples of conditions/ syndromes
1	Holoprosencephaly	Trisomy 13, Velocardiofacial syndrome, Diabetic embryopathy
2	Agenesis of corpus callosum	Acrocallosal syndrome, Aicardi syndrome
3	Abnormal shape of head, craniosynostosis	Apert syndrome, Antley Bixler syndrome
4	Cleft lip and/or palate	Orofaciodigital syndrome, Trisomy 13
5	Short long bones	Various skeletal dysplasias- Atelosteogenesis, Short rib polydactyly, Jeune thoracic dysplasia, Osteogenesis imperfecta
6	Hands	Adducted thumbs in X linked hydrocephalus, polydactyly in short rib polydactyly, syndactyly in Apert syndrome
7	Microphthalmia	Walker Waarburg syndrome
8	Cardiac malformations	Intrauterine infection, various genetic syndromes and chromosomal abnormalities
9	Renal abnormalities	Short rib polydactyly, VACTERL association

post natal period. However, there is a wide variation in reported incidence of neurodevelopmental delay in cases with prenatally diagnosed ventriculomegaly. This may be because of different populations and different study designs and lack of long term prospective studies.[1,3] Diagnosis and counseling of ventriculomegaly is a challenging clinical situation because prognosis may range from a completely benign outcome to considerable poor prognosis especially regarding neurological outcome even after surgery. Prognosis can not be predicted on the basis of the size of ventricles. The term 'isolated ventriculomegaly' is used when there is no other sonographically visible abnormality in the fetus besides ventriculomegaly and by definition it is a diagnosis of exclusion.[4]

Etiology and associated malformations

Causes of ventriculomegaly are heterogeneous ranging from increased CSF production, obstruction of CSF flow from lateral ventricles to subarachnoid space, CNS structural and migrational abnormalities and destructive processes by vascular or infective etiologies (Fig 2). Among the obstructive causes aqueductal stenosis is an important cause of fetal



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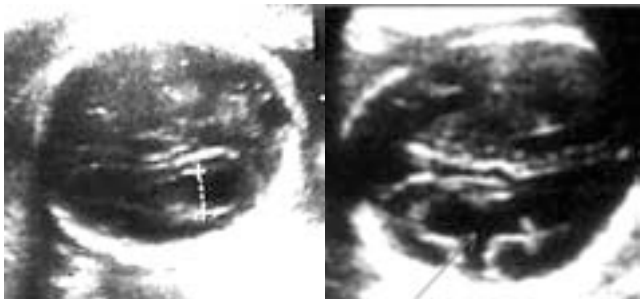

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Fig 2: Fetal ultrasonographic pictures showing (a) a single ventricle in holoprosencephaly, (b) Dandy-Walker malformation: posterior fossa cyst communicating with fourth ventricle and flattened cerebellar hemispheres (c) Distended posterior horns of lateral ventricles in a fetus with aplasia of corpus callosum and (d) Schizencephaly - Cleft in the cerebral hemisphere due to abnormality of neuronal migration.

ventriculomegaly. Some of these cases are associated with adducted thumbs as an important feature and suggest X-linked aqueductal stenosis caused due to mutation in L1CAM gene (Fig 3a & b). Non-obstructive causes of ventriculomegaly are many and present during intrauterine life.[1]

Fig 3: A neonate with X linked aqueductal stenosis

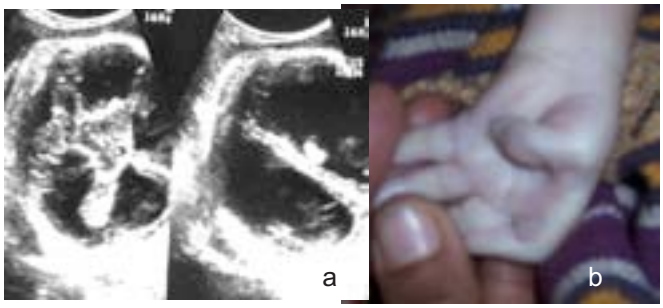

Fig 3 : (a) Enlarged lateral ventriclesand absent fourth ventricle suggestive of aqueductal stenosis.(b) Showing hypoplastic and adducted Thumb.

Once the ventriculomegaly has been diagnosed and classified according to the size of the atrial width the next step is thorough examination for other structural central nervous system (CNS) and non CNS abnormalities. These might range from 10% in cases with mild ventriculomegaly to 60%-80% in moderate and severe ventriculomegaly.[1,3] The most common CNS structural abnormalities associated with ventriculomegaly are neural tube defects and agenesis of the corpus callosum. Other associated abnormalities may be Dandy Walker malformation, holoprosencephaly and various neuronal migration abnormalities like lissencepahly (Fig 4). Presence of other non CNS structural abnormalities may indicate possibility of chromosomal (Trisomy 13, 18, 21) and non-chromosomal syndromes (Apert syndrome, Aicardi syndrome, Acrocallosal syndrome, Smith Lemli Opitz syndrome, Walker Warburg syndrome etc.) (Fig 4a & b). Table 2 lists some syndromes associated with ventriculomegaly and associated malformations. Presence of associated malformations and prenatal diagnosis of a

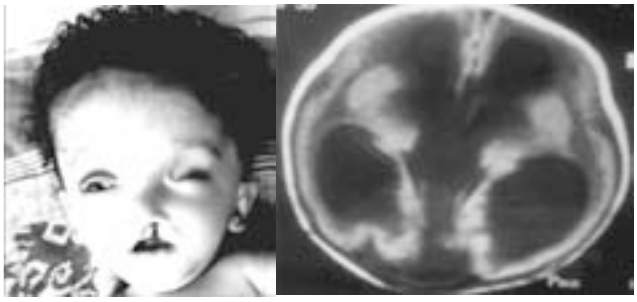

Fig 4: Walker Warburg syndrome (a) Microphthalmia and cleft lip (b) Ventriculomegaly and flattened surface of cerebral hemispheres

syndrome helps in giving definitive information regarding outcome. Therefore, a pregnant woman with a diagnosis of fetal ventriculomegaly should be referred to centers specialized in evaluation of fetal structural malformations as presence of these may change the prognosis and counseling considerably.[1,3] In these cases postnatal prognosis is guided by presence of other structural abnormalities and syndromic association. Some studies suggest that even after targeted USG as many as 10% of associated abnormalities are detected after birth in cases of isolated ventriculomegaly. Especially, neuronal migrational disorder may not be detected before the third trimester. In a study done by Lee et al as many as 50% of the cases with prenatally detected ventriculomegaly who were sent for prenatal neurosurgical consultation were found to have additional abnormalities which were not detected on prenatal USG.[5] The presence of associated anomalies has implications in planning management and overall prognosis of the baby. Examples of the some of these abnormalities are given in table 2.

Table 2: Associated abnormalities and outcome in cases of mild, moderate and severe ventriculomegaly

Condition	Chromosomal abnormality (%)	Associated structural malformation (%)	Perinatal mortality (%)	Neurocognitive abnormality in surviving fetuses (%)
Mild	3-4%	6%	Comparable to general population	4% - 10 %
Moderate	3-15%	10-70%	10%	25 %
Severe	Low	60%	25%	70 %

In the recent studies fetal MRI has been shown to be marginally superior to USG for detection of CNS structural abnormalities. In one of the largest studies done on 185 antenatally diagnosed cases of isolated mild ventriculomegaly fetal MRI detected additional abnormalities in 5% of the cases. In other studies the percentage of additional abnormalities detected by fetal MRI was found to be up to 50%. Overall gain in detection rate depends upon operator skill, indication of fetal MRI and gestational age. Fetal MRI especially provides useful clues for diagnosis of migrational abnormalities of the fetal brain. [6,7]

The risk of chromosomal abnormalities in cases of ventriculomegaly ranges from 3-15% and is high especially in cases associated with other structural malformations. In cases with isolated ventriculomegaly it is around 3.5% and therefore, fetal karyotyping should be offered to all cases with a prenatal diagnosis of ventriculomegaly even if the ventricular atrial width is between 10-12 mm. Intrauterine fetal infection is another important etiological factor for ventriculomegaly and is found in 10-20% of isolated severe ventriculomegaly and 1-5% of mild to moderate ventriculomegaly.[1] Possible organisms include Rubella, Toxoplasma and CMV and evaluation for these infections is indicated. USG signs like intracerebral or liver calcification provide further clues for an infective etiology. Definitive diagnosis of fetal infection can be made by testing for IgM positivity in the fetal cord blood sample or doing amniotic fluid PCR for nucleic acid of the specific organism. A four- fold rise in IgG or positivity of IgM in the maternal serum, which was negative before pregnancy may also point towards the possibility of an intrauterine infection. However none of these techniques give an idea about the severity and extent of fetal effects. Intracranial calcification similar to fetal toxoplasmosis is seen in an autosomal recessive condition known as pseudoTORCH syndrome (Fig 5a &b).

Intraventricular haemorrhage due to alloimmune thrombocytopenia or maternal therapy with warfarin is a rare cause of fetal ventriculomegaly. Songraphic findings of intraventricular haemorrhage are subtle and difficult to detect and MRI may be helpful in these cases. Work up for this should only be done in those cases where all other causes are ruled out and intraventricular hemorrhage is suspected.[1]

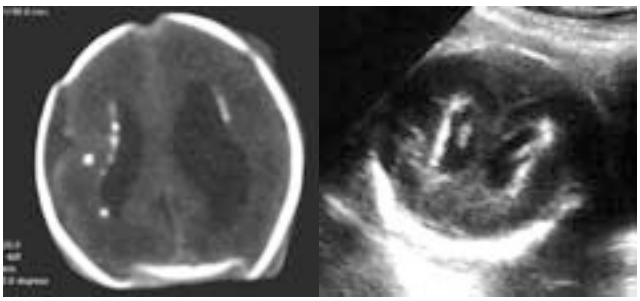

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Fig 5: Pseudo TORCH syndrome in a consanguineous couple: (a) Post termination CT scan of a fetus showing ventriculomegaly with periventricular calcification (b) Prenatal USG during next pregnancy showed mild ventricular prominence and periventricular calcification

Another important consideration in prenatal detection of ventriculomegaly is timing of diagnosis. In many cases it is diagnosed after 20 weeks of gestation and unless severe it poses difficulty in counseling and providing prognosis and sometimes serial USG has to be done at 2 weekly intervals to look for the progression of ventriculomegaly and to provide definite counseling.

Prognosis and counseling:

The outcome of fetal ventriculomegaly is greatly variable and depends upon the severity of ventriculomegaly, associated structural or chromosomal abnormalities and progression of ventriculomegaly during intrauterine life. The outcome of ventriculomegaly associated with other structural and/or chromosomal abnormalities depends upon the type of associated anomaly. Presence of an unbalanced chromosomal abnormality suggests poor outcome. Presence of central nervous system malformations like holoprosencephaly suggests universal poor outcome while satisfactory neurological outcome is seen in

50% of cases with Dandy Walker malformation and more than 50% cases with isolated aplasia of corpus callosum. There is wide variation in reported incidence of neurodevelopmental delay in cases with prenatally diagnosed ventriculomegaly. This may be because of differences in the cases studied, differences in study designs and lack of long term prospective studies.

Among the isolated cases of ventriculomegaly prognosis is poor in cases with severe ventriculomegaly with perinatal mortality of about 25% and some neurocognitive abnormality in as many as 70% of surviving fetuses (Table 2). In cases with moderate ventriculomegaly, perinatal mortality is in the range of 10% with normal neurodevelopmental outcome in around 75% of cases. In cases with isolated mild ventriculomegaly (atrial width in range of 10-12 mm with no other structural abnormalities) normal neurocognitive outcome is seen in as many as 96% of cases which is almost similar to the normal population. Some studies have quoted risk up to 10% of learning disabilities and cognitive dysfunction in cases with isolated mild ventriculomegaly. However all cases need to be offered chromosomal analysis and long term follow up.[1,8] Recent studies do not support any association between ventriculomegaly and neuropsychiatric conditions like autism and schizophrenia but post natal follow up of these children with a neurodevelopmental pediatrician and brain MRI is recommended. Regarding the post-delivery shunt placement there is not much difference in final outcome between isolated ventriculomegaly and those with associated malformations.[9]

Besides the size of ventricles, other factors affecting the prognosis of antenatally diagnosed ventriculomegaly include a progressive increase in size of ventricles in fetal life which always indicates poor prognosis. Some studies suggest as many as 40% of isolated bilateral mild ventriculomegaly may resolve in utero. Whether ventriculomegaly is unilateral or bilateral does not affect the outcome.

There are some reports which suggest better outcome in male fetuses with ventriculomegaly in comparison to female fetuses. In one study prevalence of neurodevelopmental delay in female fetuses was 10.6% in comparison to 5.6% in male fetuses. These results were not statistically significant and there are no gender specific guidelines for defining ventriculomegaly; however, it may suggest that male fetuses have a slightly higher atrial width diameter than female fetuses.[1] Some recent studies suggest that bilateral and symmetrical ventriculomegaly has a better prognosis than asymmetrical ventriculomegaly (difference in atrial width >2mm) as risk of neurodevelopmental delay is lower i.e. 4% as compared to 50% in the latter group.[10]

Surgical treatment

If the family continues the pregnancy, the delivery should be conducted at a centre with good neonatal facilities and the neonate needs to be investigated appropriately. Surgical intervention will be needed for aqueductal stenosis, Dandy Walker malformation and meningocele / encephalocele. Surgical interventions are not indicated for holoprosencephaly and neuronal migrational abnormalities like lissencepahly where the outcome is universally poor due to inherently abnormal brain. Fetal shunt placement is technically possible but has not been shown to change the outcome significantly.[11]

Counseling regarding recurrence in future pregnancies

Whatever may be the outcome of the fetus, affected the family needs to be provided counseling regarding risk of recurrence in the next pregnancy. The risk of recurrence depends on the etiology. Chromosomal analysis, syndromic diagnosis by examination of the baby after delivery or autopsy in case of stillbirth or termination of pregnancy is necessary to arrive at the etiological diagnosis which is the prerequisite for genetic counseling.

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MESSAGES

- Ventriculomegaly is one of the commonest abnormalities detected on antenatal ultrasonography.
- Measurement of atrial width of the lateral ventricles should be done in the proper plane.
- Ventriculomegaly is diagnosed when the atrial width diameter is › 10 mm. Atrial width is 10-12 mm in mild ventriculomegaly, 13-15 mm in moderate ventriculomegaly and more than 15 mm in severe cases.
- A thorough search for other associated structural CNS and non CNS malformations is necessary for giving prognosis.
- Work up for fetal ventriculomegaly includes karyotype and investigations for congenital infections and is indicated even in cases with mild ventriculomegaly.
- Poor prognosis regarding the neurodevelopmental outcome is likely in cases with associated malformations and/or chromosomal abnormality.
- Overall outcome in cases with mild isolated ventriculomegaly seems to be good with a normal neurodevelopmental outcome; however, prenatal follow up with serial USGs is indicated as some of the cases may show progression.
- All cases need postnatal follow up by a pediatrician.
- Cases with moderate and severe ventriculomegaly will need postnatal surgery. Outcome depends upon associated abnormalities and to some extent on the severity of ventriculomegaly and is highly variable.
- The prospective parents must be told that some of the associated malformations may not be diagnosable by antenatal USG.
- Fetal MRI may be useful in some selected cases and is only marginally superior in providing useful information.
- The presence of unilateral or bilateral ventriculomegaly does not affect the outcome and there are no gender specific differences.
- In cases of isolated ventriculomegaly the figures of outcome, which are already available from published data, should be used for genetic counseling.
- Around 40% of cases of mild ventriculomegaly do not progress or resolve in-utero.
- Severe ventriculomegaly is associated with poor outcome in a significant number of cases but most of these cases present after 20 weeks of pregnancy which poses difficulty in counseling and decision making in view of the MTP act in India.
- Post delivery or after termination of pregnancy, examination of the baby / fetus is important as 20-30% of associated malformations may not be detected on antenatal USG.
- Prenatal shunt placement has not been found to change the postnatal outcome significantly.

Late termination of pregnancies for severe fetal abnormalities: To do or not to do?

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A thirty-two-year-old second gravida, a resident of Bihar married non-consanguineously, visited our genetics clinic at 31 weeks of gestation for prenatal counseling for antenatally detected short fetal limbs. On taking a detailed family history, it was found that there was history of a previous malformed female stillbirth. In the previous affected pregnancy the couple was told that the baby had tetraphocomelia. However, neither autopsy nor infantogram was performed in that pregnancy. In the present pregnancy, a level II scan showed presence of short four limbs (corresponding to 23 weeks of gestation against the menstrual age of 31 weeks).There was no cleft lip and palate, polydactyly or cardiac defect. Possibility of a lethal skeletal dysplasia was kept and the couple was counseled. The couple opted for termination of pregnancy. But..... according to the legislation for medical termination of pregnancy, termination of pregnancy for fetal conditions is permissible only up to 20 weeks of gestation and thereafter only on grounds of maternal health (The Medical Termination of Pregnancy Act, No. 34 of 1971).[1] Sadly, even after finding out about this catastrophe, the couple was forced to carry on with the pregnancy and finally delivered a female baby at 37 weeks. The baby had dysmorphic facies, corneal opacity, upper limb hypomelia (Figure 1) with oligodactyl and, bilateral severe shortening of femora and leg bones (hypomelia) with club foot. Overall, the features in this baby were suggestive of Roberts syndrome which is an autosomal recessive disorder and is characterized by mild to severe prenatal growth retardation and limb malformations (including bilateral symmetric tetraphocomelia or hypomelia caused by mesomelic shortening). The prognosis depends on the malformations present: the severity of manifestations correlates with survival. Mortality is high among most of the severely affected pregnancies and newborns. Patient's blood samples were taken for cytogenetic studies to look for premature centromere separation (PCS) and separation of the heterochromatic regions as well as for molecular studies.

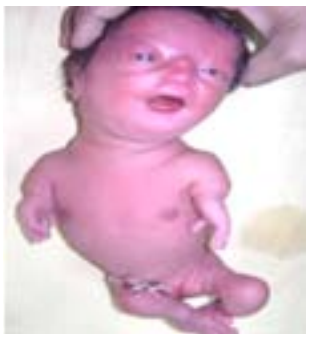

Fig 1. Baby shows features of Roberts phocomelia

This is not an uncommon situation especially at a tertiary care centre. Nowadays, with increasing availability of expertise, modern techniques such as 3D, 4D ultrasound, and genetic testing for various genetic disorders, prenatal diagnosis for various malformations and other single gene disorders has become a reality in various states of India. However, in the existing health system, the referral for high risk pregnancies with suspected fetal malformation or genetic disorder is sometimes delayed till late second trimester or third trimester. Moreover, many conditions such as cardiac defects, ventriculomegaly, microcephaly with or without neuronal migration defects or skeletal dysplasias may not be obvious until 20 weeks of gestation and often the diagnosis of a few fetal conditions may be delayed. Diagnosis of any fetal abnormality creates tremendous stress, anxiety, depression, and worry to the woman as well as to the family at any stage. In accordance with the Indian MTP act, late

termination of pregnancies is not permissible even if the fetal condition is associated with high morbidity and mortality. Despite many ethical and legal controversies associated with the late termination of pregnancies, in most countries there have been differences in the gestational age for termination of pregnancy depending upon the fetal abnormality and its severity. In India, this law is still being followed, totally ignoring the parents' perspective and non-availability of resources (support groups, financial) to deal with such complex situations. In the recent article published by Phadke et al, the authors have tried to ascertain the opinion of lay persons and medical practitioners in India regarding late termination of pregnancies for fetal abnormalities.[2] In their survey they have found that majority of the clinicians and more than two thirds of the lay persons opted for late termination for fetal conditions with poor prognosis.

Hence, in the current health scenario, there is a felt need for the policy-makers to revise the MTP act to extend the gestational age for termination, especially for fetal abnormalities with a poor prognosis.

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Exome sequencing: translating genetic research into patient care

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Exome sequencing – from bench to bedside [1,2]

Over the past few years exome sequencing has emerged as a powerful tool for genetic research and its utility in mapping genes for Mendelian disorders has been well documented. However, so far its applicability in a clinical setting has been limited. Worthey et al have now demonstrated the utility of exome sequencing as a clinical diagnostic modality, using the illustrative example of a case where exome sequencing helped to establish a definitive diagnosis in a patient with a rare clinical condition and thereby enabled appropriate therapeutic intervention, after all other standard investigative modalities had failed to identify the etiology. They have reported the case of a 15 month old male child who presented with a severe and unusual form of inflammatory bowel disease in whom, despite a thorough clinical and laboratory evaluation, a definitive diagnosis could not be made. Whole exome sequencing was done and after filtering and analysis of the identified 16,124 variants, a novel, hemizygous missense mutation in the X-linked inhibitor of the apoptosis gene was found, on the basis of which X-linked inhibitor of apoptosis deficiency was diagnosed, which was consistent with the clinical picture. Based on this diagnosis, an allogeneic hematopoietic progenitor cell transplant was performed and post-transplant the child showed a significant amelioration of his symptoms. Given the rapid advancements in this technology, the day may not be far when requisitions for a whole-exome scan become as common place as for a CT or an MRI scan!

FBN1-the long and short of it [3]

Geleophysic dysplasia (GD) and acromicric dysplasia (AD) are both acromelic skeletal dysplasias characterized by severe short stature, short extremities and stiff joints. ADAMTSL2 mutations have previously been identified in a subset of patients with geleophysic dysplasia, but the molecular basis of acromicric dysplasia was hitherto unknown. Le Goff et al selected 19 GD patients without ADAMTSL2 mutations and 10 AD patients; of these, whole exome sequencing was initially performed for two GD and three AD patients. Because of the phenotypic overlap between AD and GD, a shared mutated gene was searched for among the five cases; of the three likely genes (MUC17, HYDIN and FBN1) thus identified, FBN1 was considered the best candidate gene because of the known link between tall stature and Marfan syndrome caused by FBN1 mutations. Subsequently, Sanger sequencing of the FBN1 gene in all the 29 GD and AD cases showed mutations in all the cases and a total of 16 distinct heterozygous FBN1 mutations were identified in the 29 patients, all of which were clustered in the same region (exons 41 and 42) encoding the TGFb-binding protein-like 5 (TB5) domain of FBN1. Fibroblast studies in the GD and AD patients with FBN1 mutations revealed disorganization of the microfibrillar network, enhanced TGFβ signalling and disruption of interactions between ADAMTSL2 and FBN1. Thus, while enhanced TGFb signaling due to FBN1 mutations leads to the Marfan syndrome phenotype, the same effect due to mutations in the TB5 domain of FBN1 and disturbance in interactions with ADAMTSL2 leads to short stature phenotypes. These findings highlight the phenotypic diversity that can occur in different allelic disorders arising from different mutations in the same gene.

Chemotherapy prescription based on exome profile! [4]

Choosing the appropriate drug therapy for cancer patients is often a difficult task, because the anti-cancer effects of drugs can vary greatly from one individual to another. Multiple different genetic mutations in human cancers as well as the wide variety of single nucleotide polymorphisms (SNPs) and other pharmacogenetic variations in different individuals significantly influence the response to cancer therapy. He et al hypothesized that targeted exome sequencing technology can be used to study the multiple genome-wide variations in individual cancer patients and as proof-of-concept they performed whole exome sequencing in a unique patient with high cancer susceptibility who had developed three different cancers in her lifetime (breast cancer, gall bladder adenocarcinoma and lung cancer at 41, 63 and 66 years of age respectively). Amongst the variants identified in this patient were homozygous mutations in 18 genes of the RAS-MAPK pathway and in genes encoding 15 growth factors/cytokines and their receptors, 9 transcription factors, 6 proteins on WNT signaling pathway, and 16 cell surface and extracellular proteins. These variants are likely to confer cancer susceptibility and therefore could be potential therapeutic targets. Having demonstrated its applicability in this one patient, the authors believe that targeted exome sequencing technology could be used to develop a cost effective strategy for studying genome-wide variations and important carcinogenic mutations in each cancer patient. This in turn would help in designing genome-based individualized cancer therapy and care.

After bone scan it is exome scan for osteoporosis! [5]

Osteoporosis is a common metabolic bone disorder world-wide, but the molecular mechanisms underlying the causation remain largely unknown. In an attempt to uncover the pathways involved in the development of osteoporosis and thereby to discover potential therapeutic targets for this condition, Simpson et al studied the molecular basis underlying Hajdu–Cheney syndrome (HCS), an autosomal dominant multisystem disorder also characterized by severe and progressive bone loss. The scarcity of large families with multiple affected members has until now hampered studies into the genetic basis of HCS. However, exome sequencing being such a powerful tool requires only a few cases for evaluation and Simpson et al made use of this technology. They performed whole exome sequencing in three unrelated individuals with HCS and identified NOTCH2 to be the candidate gene. Thereafter, NOTCH2 sequence analysis was performed in an additional 12 unrelated HCS patients and revealed presence of heterozygous sequence variants within the gene in all of them. NOTCH2-mediated modulation of RANKL-induced osteoclastogenesis may be the pathway responsible in part for the pathogenesis of HCS. This study provides compelling evidence that NOTCH2 and its associated pathways are involved in the causation of HCS and potentially of osteoporosis, which in turn suggests that these pathways may be potential therapeutic targets for the treatment of osteoporosis.

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Suggested Reading

1. Bamshad MJ, et al. Nat Rev Genet 2011; 12: 745-55.

2. Clin Genet 2011; 80 (2): 101 – 201. Special issue on exome sequencing.

genetic CLINICS

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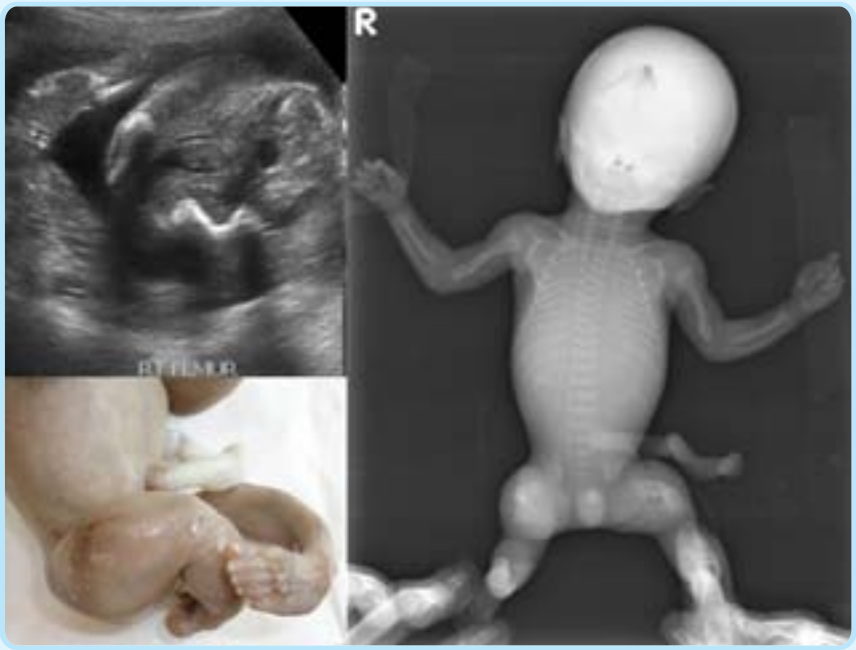
The pregnancy was terminated following antenatal diagnosis of short limbs in the fetus. See the ultrasound image, clinical photograph and radiographs and identify the condition.



Answer to the PhotoQuiz 14 of the previous issue

Roberts (pseudothalidomide) syndrome (OMIM 268300)

Roberts syndrome is an autosomal recessive condition characterized by prenatal growth retardation and limb malformations. The limb defects can include severe shortening of all limbs (bilateral symmetric tetraphocomelia), mesomelic shortening of limbs, radial ray defects, oligodactyly or syndactyly. Associated craniofacial abnormalities include hypertelorism, cleft lip, a prominent premaxilla, mid-face capillary hemangioma, cloudy corneae or cataracts and dysplastic or small ears. Other defects that may occur with this condition are large genitalia, congenital heart defects and cystic kidneys. The chromosomes show premature separation of centromeres. The disorder is caused by mutations in the ESCO2 gene on chromosome 8p.

Correct response to PhotoQuiz No. 14 was given by

Prochi Madon, Mumbai

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