B D R News

The official newsletter of the Birth Defect Registry of India, (A unit of Fetal Care Research Foundation)

Volume 10 Issues 3 & 4 combined: July - Oct 2010

Proceedings of the third & fourth quarterly meetings of the Birth Defects Registry of India on 06.08.2010 & 17.11.2010 respectively.

The third & fourth quarterly BDR meetings for the year 2010 took place on 6th August & 17th November respectively at MediScan premises ,Chennai. Dr Indrani Suresh (Director, BDRI) welcomed Chennai registry members. She greeted the speakers & addressed the audience saying that BDRI has representative members reporting data from 26 states & 3 union territories making the total number of member hospitals to 600. She announced the most notable advancement of 100 hospitals reporting online which is a welcome trend among the members. She invited many more to join online data reporting mode which is easier, consumes lesser time, saves papers & thereby making us environment friendly! While discussing the preventive and supportive strategies of the parent foundation-FCRF, she went on to say that New Born Screening (NBS), Down syndrome Screening (11-13 weeks scan) and the Support Group for MPS were pioneering ventures of the foundation in the nation. It is now exploring another new area- NBS for Lysosomal storage disorders. She then introduced the speaker Dr Joan Keutzer, Vice President, Scientific Affairs, Genzyme Corpoaration USA who has extensive experience in NBS for Lysosomal Storage Disorders.

Following are the excepts of the presentations made at the third BDR meeting for 2010

Antenatal Diagnosis of Skin Disorders

Dr. Preeti Parekh Tomar, Fellow Fetal Medicine, MediScan

Dr. Preeti Tomar discussed the emerging role of molecular diagnosis in fetal skin disorders with the workup of two cases

Introduction

Genodermatoses are defined as a range of inheritable skin diseases that may be associated with significant mortality and long-term morbidity. In the past, options for prenatal diagnosis of these diseases were limited to fetal skin biopsy. The biopsy specimen was studied by light microscopy, electron microscopy and immunohistochemistry. As a result of recent advances in genetics and molecular biology, DNA-based prenatal diagnosis is now available for an increasing number of genodermatoses, and newer non-invasive methods are being developed that have the potential for tremendous future impact in dermatology.

USG & Feta Skin Disorders:

Ultrasound by itself has a limited role in diagnosis of fetal skin disorders, such as:

1)Assessment of gestational age

2)Looking for subtle features of Icthyosis (ectropion, eclabium, abnormal femur foot length) &

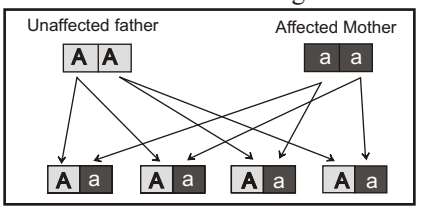
3)Ultrasound directed fetal skin biopsy

A 30 year old primigravida with albinism was referred for First Trimester Screening (FTS) and genetic workup. Maternal father and sister were also affected by albinism. Her marriage was non-consanguineous, but she was married within a small community ('Cholai Chettiyar' community, where consanguineous marriages are common). Her husband was 30 years old & was not affected. On workup, it was found that many other people from the community were similarly affected. No mutation analysis was available .A diagnosis of oculocutaneous albinism (OCA) was made. The work up was as follows:

Step 1: First trimester screen in the fetus was normal

Step 2: Mutation analysis in the mother revealed homozygous mutation in OCA2 (p.S641L:c. 1922C>T)

Step 3:Husband was tested and found negative for the mutation



Since this is an autosomal recessive disorder, all children would become obligate carriers, but none would be phenotypically affected. So, prenatal diagnosis was not required in this case.

Literature

Oculocutaneous albinism refers to a group of conditions that affect pigmentation of the skin, hair, and eyes. Cutaneous manifestations include very fair skin and white or light-coloured hair. Eyes show reduced pigmentation of the iris and the retina.

Lifespan in patients with OCA is not limited, and medical problems are generally not increased compared to those in the general population. Development and intelligence are normal. Persons with OCA have normal fertility. Skin cancers may occur and regular skin checks should be offered. Mutations in the genes involved in melanin production are:

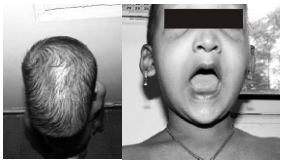
TYR	Type 1
OCA2	Type 2
TYRP2	Type 3
SLC45A2	Type 4

Carrier detection and prenatal diagnosis are possible when the disease causing mutations have been identified in the family. Testing can be done on DNA extracted from CVS at 10–12 weeks gestation or on DNA extracted from cultured amniocytes.

CASE 2- Anhydrotic Ectodermal Dysplasia

A couple with II degree consanguinity came for genetic counselling in their second conception. Their previous child was born by LSCS (2.6 kg) and had jaundice on second day of life. She had sparse hair, and by 3-4 months absent sweating,

no eyebrows were noted. The cognitive development was normal with normal milestones. Subsequently, poor dentition was noted.



A probable diagnosis of anhydrotic ectodermal dysplasia was made and index child's sample was sent to Germany for mutation analysis. EDAR Gene mutation – c.47T>C (p.Leu16Pro) homozygous was detected.

Since this has autosomal recessive mode of inheritance and the parents were unaffected carriers, the chance of subsequent child being affected would be 25%. Chorion villous sampling revealed that the fetus was affected by the disorder.

Literature:

Ectodermal dysplasia is a heterogeneous group of disorders characterized by developmental dystrophies of ectodermal structures, such as hypohidrosis, hypotrichosis, onychodysplasia and hypodontia or anodontia.

Anhidrotic (hypohidrotic) ectodermal dysplasia (EDA) is the most common ED (80%). It is characterized by hypoplasia of hair, teeth and sweat glands. Most patients with EDA have a normal life expectancy and normal intelligence. However, the lack of sweat glands may lead to hyperthermia, followed by brain damage or death in early infancy, if unrecognized.

Genetics of EDA: It has both X-linked & Autosomal recessive mode of inheritance. Mutations in the 1) autosomal recessive (EDARADD) gene & 2) ectodysplasin anhidrotic receptor (EDAR) gene are responsible for the autosomal recessive form of EDA. Families with EDA should therefore be offered genetic counseling. Early diagnosis is crucial for supportive care & prenatal diagnosis in subsequent pregnancies.

Advantages of Molecular Diagnosis in Genodermatoses

1) Facilitates early diagnosis & decision making in the first trimester of pregnancy 2) technically simpler 3) may present a lower risk to the pregnancy than fetoscopy and multiple skin biopsies

Conclusion :

Since most cases with these disorders have normal life expectancy and intelligence, prenatal diagnosis is probably not an option in most families as illustrated in the two cases above. Careful genetic counselling is thus indicated.

Increasing relevance of prenatal diagnosis in dermatology

(Dr. Murlidhar Rajagopalan, Consultant in Dermatology, Apollo Hospitals & Apollo Children's Hospital, Chennai)

Dr. Muralidhar Rajagopalan at the commencement of his presentation cherished his long term association with MediScan & the fetal dermatology team work he has been involved. He mentioned that molecular diagnosis for genodermatoses is a newly emerging concept & his presentation dealt with the inheritable disorders seen at birth. It would be a traumatic experience for the Gynecologists and Neonatologists as well as the parents when a child with genodermatosis is born. Though it cannot be treated, the aim of prenatal diagnosis is to prevent recurrence of the problem. Several tools are available for prenatal diagnosis in dermatology as mentioned below:

1) Fetal skin biopsies 2) Chorionic villus sampling
3) Amniocentesis 4) Periumbilical vein blood sampling
5) Preimplantation genetic diagnosis

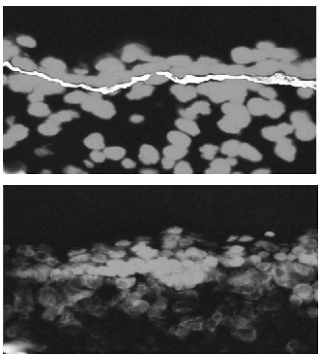
However one should remember that mild diseases and good postnatal treatment response are not an indication for prenatal diagnosis, even with a high degree of recurrence.

Fetal skin biopsy: Fetal skin biopsies taken prior to 20 weeks may not always be conclusive as the interfollicular keratinization of the skin takes place as late as 24 weeks. (which is beyond the legal limit of pregnancy termination).

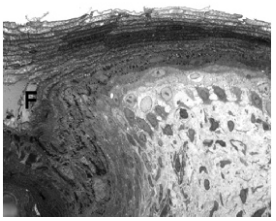
Skin biopsy can be used to diagnose conditions like epidermolysis bullosa, ichthyosis and ectodermal dysplasias.

Disorder	Methods
Epidermolysis bullosa	
Junctional EB (Herlitz)	LM, EM, IF
Junctional EB with PA	LM, EM, IF
Recessive dystrophic EB (Hallopeau - Siemens)	LM, EM, IF
Dominant dystrophic EB	LM, EM
EB simplex(Dowling - Meara)	LM, EM
Ichthyosis	
Epidermolytic hyperkeratosis*	LM, EM, AF
Harlequin ichthyosis	LM, EM, AF
Lamellar ichthiosis	LM, EM
Sjogren - Larsson syndrome	LM
Anhidrotic ectodermal dysplasia	LM, EM
Oculocutaneous albinism	LM, EM, EH

Most of the diagnoses require electron microscopy, immunofluorescence or even immunohistochemistry for confirmation. Index case workup is most important for reaching decisions on specific molecular tests to be performed.



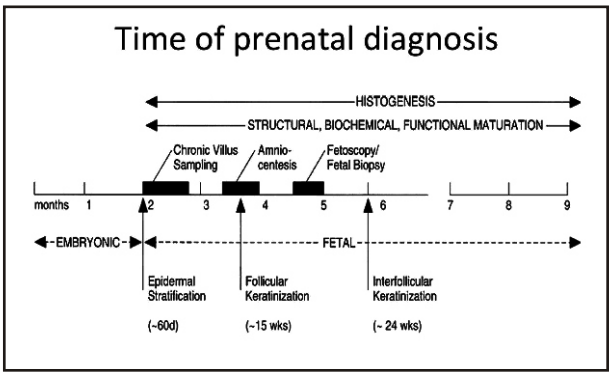
Immunofluorescence microscopy for Herlitz JEB—stain with gb3[laminin]



Biopsy samples from the scalp of affected fetuses of 22-23 weeks' gestation
Note abnormal stacking of newly keratinized cells around the upper part of a hair follicle (F).

Limitations of histopathology

1) Technically difficult 2) Requires excellent skin biopsy site selection 3) Good knowledge of fetal skin development 4) Inter follicular epidermis at 19 weeks EGA or earlier is not sufficiently developed to exhibit the characteristic morphologic changes of keratinization



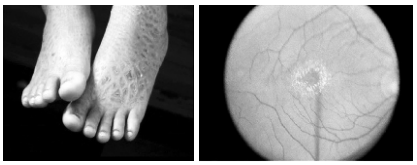
DNA based prenatal diagnosis

Most of the genodermatoses have got specific genetic markers. However DNA testing for these markers are not easily available in our country.

Genodermatosis	Gene locus
Junctional epidermolysis bullosa(Herlitz)	18q11.2 1q32 1q25-31
Junctional epidermolysis bullosa (with pyrolic atresia)	17q11-qter
Dystrophic epidermloysis bullosa(recessive)	3p21.3
Epidermolysis bullosa simplex(Dowling Meara)	17q21-22
Bullous congenita ichthyisiform erythroderma (Epidermolytic hyperkeratosis)	17q21-22
Netherton syndrome	5q32
Ocultaneous albinism (tyrosinase - negative, OCA1A)	11q14-21
Lamellar ichthyosis	14q11
Sjogren-Larsson syndrome	17p11
Smith-Lemli-Opitz syndrome	11q12-q13
Mucopolysaccharidosis: (Hunter, type II)	Xq27.3-q28
Ectrodactyly, ectodermal dysplasia, clefting (EEC) syndrome	3q27

Many cases with various types of genedermatosis were discussed in detail.

1). Sjogren-Larssen syndrome: Characterized by thick adherent scaling and associated with neurological menifestations. The fundal examination shows perifoveal glistening dots of the retina. An index case of Sjogren Larssen was confirmed on clinical, MRI and ophthalmological grounds. However electron microscopic (EM) confirmation was not done. Therefore fetal skin biopsy was planned in subsequent pregnancy for histopathology to prove the presence of icthyosis and cord blood sampling for FADH enzyme levels in UK.



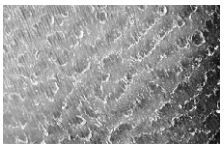
2). Epidermolytic hyperkeratosis: There is generalized skin involvement with thick, dark brown, verrucous hyperkeratosis.

Generalized skin involvement with thick, dark brown, verrucous

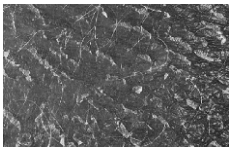
Note the ridged pattern in the knee folds. Epidermolytic hyperkeratosis

Generalized Erythroderma, scaling and peeling

Severe hyperkeratosis with cobblestone surface pattern over the extensor surface of the knee joint and linear arrangement in the knee fold.

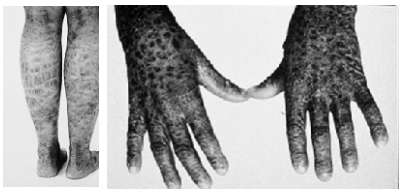


Lamellar ichthyosis with large, dark, plate-like scales, ectropion and without discernible erythroderma



The intermediate phenotypes fall anywhere between with variable degrees of erythema and scaling

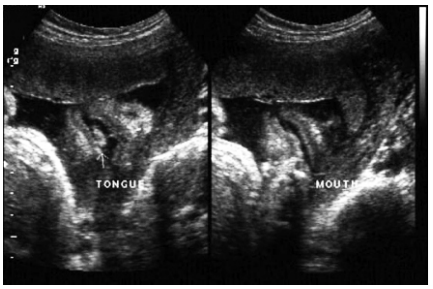
Ectropion and madarosis

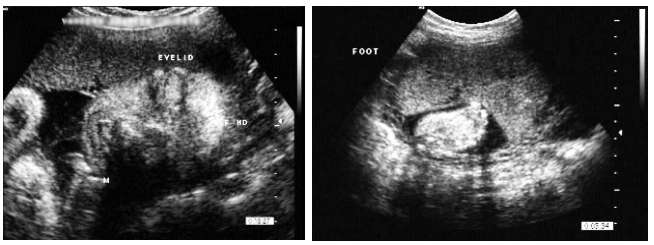


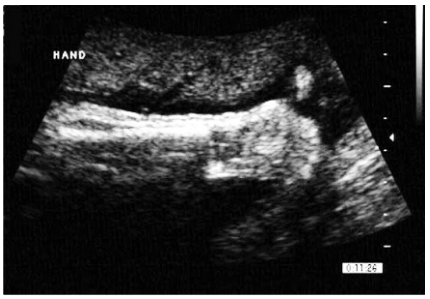
Large, brown, plate-like scale

Many lamellar icthyoses have no discernible EM changes by adulthood, as the icthyosis starts disappearing. Hence histopatholgy still seems a practical diagnostic technique. Molecular markers will be more useful in future.

3).Collodion Baby: Most of these ichthyoses are encased by the "collodion membrane" which is a thick sheet like covering and can be made out even on ultrasound.





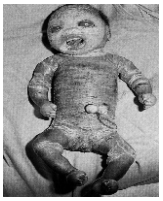


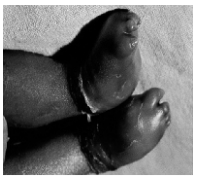
Occurrence of collodion membrane at birth.

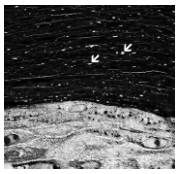
Disorder	Frequency
Lamellar ichthyosis	Common
Congenital ichthyosiform erythroderma	Common
CIE/LI intermediate phenotypes	Common
Autosomal dominant lamellar ichthyosis/congenital ichthyosiform erythroderma	Rare
Self-healing collodion baby	Always
Sjogren-Larsson syndrome	Rare
Trichothiodystrophy	Rare
Infantile Gaucher disease	Rare
Hay-Wells syndrome	Rare

4)Harlequin

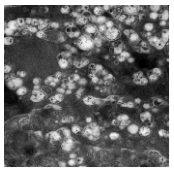
This condition is characterized by abnormal lamellar bodies seen under electron microscopy. Typical inclusions are also seen between the layers of the newly keratinized follicular cells.



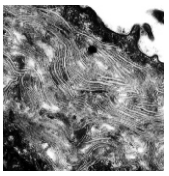




At higher magnification, this section from another fetus shows the abnormal inclusions (arrows) in different layers of newly keratinized follicular epithelial cells.



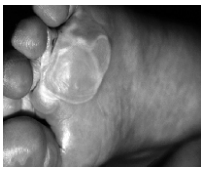
Electron micrographs showing the same typical inclusions in greater detail.



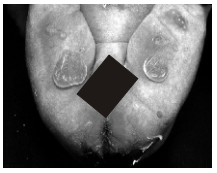
An amniotic fluid cell from an affected fetus of 20 weeks' gestation.

5) Hypohydroitic Ectodermal Dysplasia:
This is characterized by alopecia,hypo dentition, febrile fits & other complications due to the absence of sweat glands.

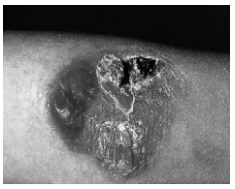
6) Epidermolysis Bullosa (EB) :
There are various types of this condition namely
1) EB simplex type 2) Junctional epidermolysis bullosa &
3) Dystrophic epidermolysis bullosa depending on their severity



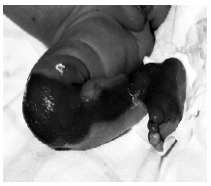
EB simplex-Weber-Cockayne bullae on forefoot



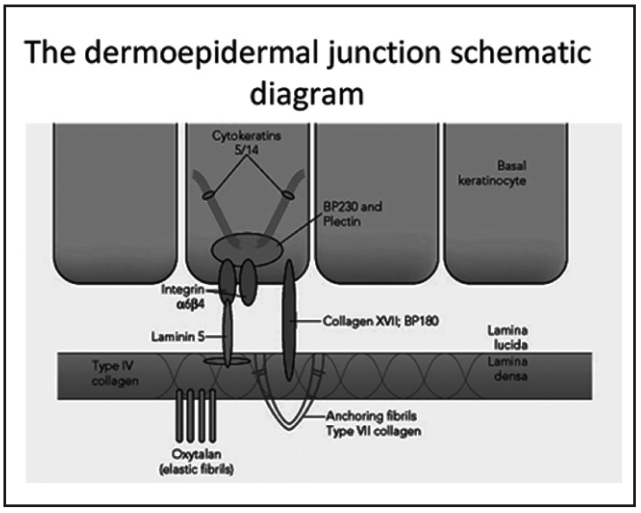
JEB-Herlitz bullae on the buttocks and thighs



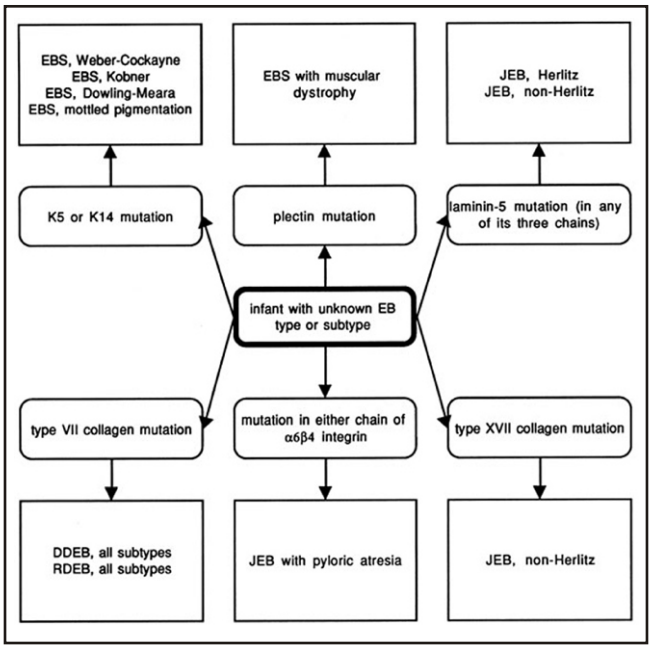
Dominant DEB bullae and scarring on the knee



Recessive DEB congenital absence of skin (Bart syndrome)



As seen from the picture, laminen is the protein that joins the lamina densa of the dermis to the epidermal cells. By staining laminin, diagnosis of EB can be made with Immunofluorescence microscopy & HPE. Any malformation of the keratin proteins can give rise to various types of EB. The genomic interpretation of EB for arriving at a diagnosis of mild to severe forms of EB was discussed & the details are shown in the flow charts as follows.

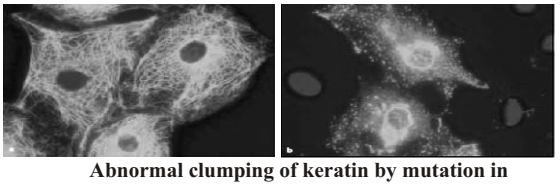


Future Progress

In less than 2 decades, prenatal testing has progressed from mid-trimester fetal skin biopsies or protein analysis in a limited Number of conditions to first trimester chorionic villus sampling. Advances in in-vitro fertilization protocols and embryo manipulation technology have further led to the feasibility of even earlier prenatal diagnosis through preimplantation genetic diagnosis.

Genetic analysis of fetal DNA is now routinely performed from chorionic villus samples or by amniocentesis from week 15 onwards. In the future,non-invasive tests would be available through the identification, characterization and isolation of fetal cells or free fetal DNA from the maternal circulation.

The future simpler techniques



Abnormal clumping of keratin by mutation in keratin14- marker of EBS

Gene therapy in Genodermatoses

Gene therapy has been truly successful only in a handful of patients worldwide. In vivo gene therapy uses a gene gun or liposomal coated DNA repair proteins like in Xenodermatosis Pigmentosa.

Discussion: It was discussed that to create facilities in our country for genodermatoses & fetal dermatology work up, one needs to know the baseline prevalence of such conditions

In India. Dr. Muralidhar suggested that BDRI develops a link with the Genodermatoses work group in India so that the incidence of skin anomalies enters the common database for further work up.

Value of Pathology in Genodermatoses

(Dr. S. Lata, Senior Consultant, Perinatal Pathology , MediScan)

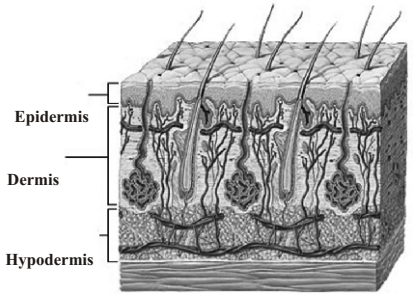
Dr. Lata commenced her talk by saying although the relevance of pathology in the era of molecular study for Genodermatoses is quite obsolete, a few specific conditions can be antenatally detected with LM which is easily available when compared to EM & other facilities.There are more than 700 inherited skin disorders identified from ABCD syndrome to Zimmermann Laband syndrome.

Genodermatoses of interest that were discussed were

1) Congenital icthyosis group (Harlequin,Collodion)
2)Bullous disorders (Epidermolysis bullosa)
3) Restrictive dermopathy 4) Ectodermal dysplasia &
5) Oculocutaneous albinism. These can be diagnosed with the help of LM. Fetal skin biopsy is useful in diagnosing these conditions.

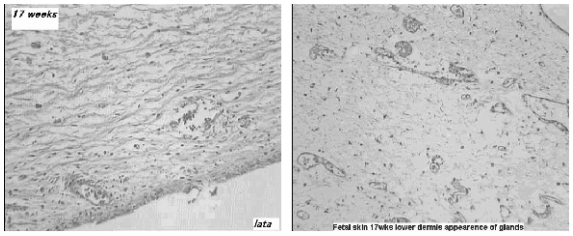
To diagnose these conditions the pathologist should have a clear understanding of the fetal skin development corresponding to gestational weeks. Fetal skin development was explained with beautiful illustrations as shown.

Normal adult skin

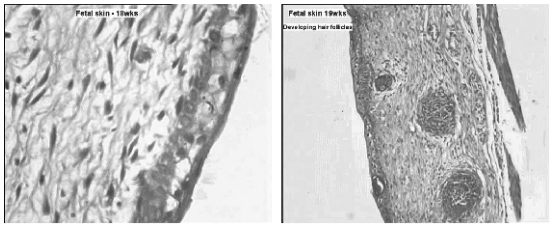


Fetal skin histology

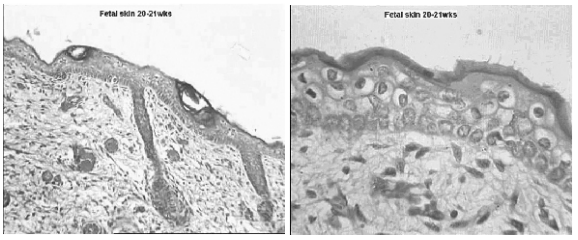
By 12 weeks the epidermis & dermis is not differentiated, At 17weeks, mesenchyme is more defined with no appendages & primordium of hair follicles are seen.The epidermis is about 3 cell layers thick with a clear basal layer. At 20-22 weeks the epidermis & dermis are well defined with keratinised hair follicles. This is the ideal time for fetal skin biopsy to rule out ichthyosis As weeks progress, the flat epidermis becomes more keratinsed (6 cell layer thickness) with retepegs & by 32 weeks the fetal skin almost resembles the neonatal skin with well defined dermis with clear undulations & appendages.



17 weeks



18 weeks **19 weeks**



20 - 21 weeks

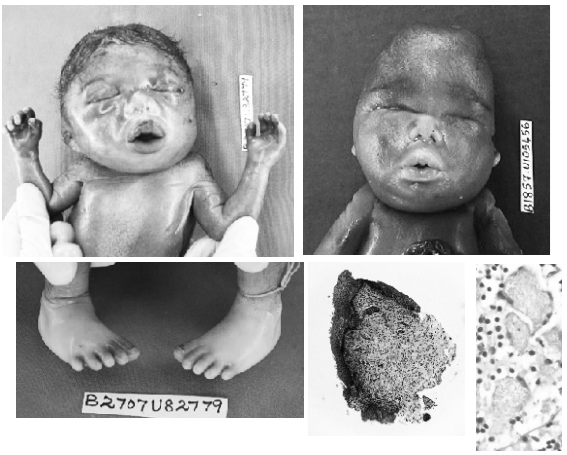
Criteria for Fetal Skin Biopsy

Certain criteria should be met before deciding to perform fetal skin biopsy for diagnosing genodermatosis

1) Disorder should be sufficiently severe to warrant procedure. 2) Diagnosis of index case or affected parent should be firmly established. 3) Abnormality in fetus should be expressed at the time of the biopsy. 4) Biopsy site should be carefully selected.

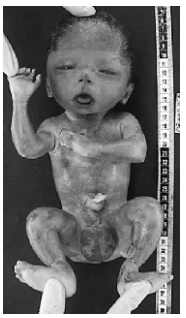
Characteristic features of fetuses presenting with congenital Harlequin,Lamellar ichthyosis were discussed. A few fetuses with ichthyosis were diagnosed to have Gaucher-Lysosomal Storage disorder. They were not compatible with life & biopsy revealed classical storage cells in crumpled tissue like cytoplasm all over the body.

Gaucher Disease



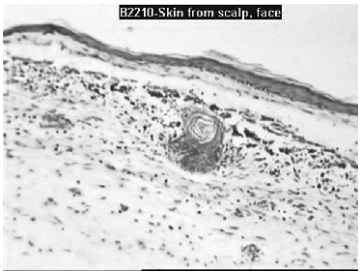
A few cases diagnosed with a condition called Restrictive dermopathy was discussed. It is characterized by :

1) Abnormally tight noncompliant skin, flexion contractures of the extremities
2) abnormalities of hair and nails, linear skin splits, and ectropion variably present
3) low set malformed ears
4) micrognathia and hypertelorism
5) small fixed open mouth with a pinched nose



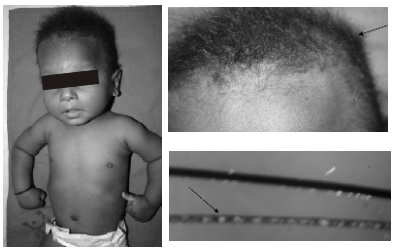
Histopathology of the skin shows:

Thin dermis, poorly developed skin appendages, flattened epidermal rete ridges& straight dermal-epidermal border.



Tay syndrome is another form of Icthyosis with Trichothiodystrophy

Affected babies show abnormal brittle hair & it is associated with multiple developmental defects.When individual hair is examined microscopically between polarizing filters, they show alternating light and dark banding.



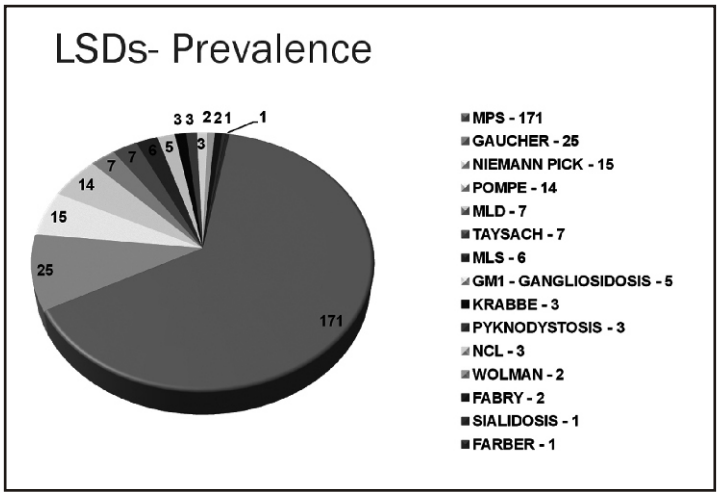
The lecture ended with a comparative note on fetal skin biopsy Vs molecular diagnosis.

Fetal Skin Biopsy	Molecular diagnosis
1) Allows direct examination of affected tissue	1) Mutation analysis of index case essential
2) Can be done only after 20 wks	2) Early diagnosis possible
3) Unreliability due to regional variation of fetal skin	3) Regional variability does not affect diagnosis
4) Procedure related risk involved	4) Procedure related risk involved

Proceedings of the 4th BDR meeting held on 17th November 2010

Dr. Sujatha Jagadeesh, Clinical Geneticist, MediScan opened the session by giving an introduction to Lysosomal storage disorders(LSD),its incidence in random & also the support services provided by the organization to children with LSDs in particular.

Lysosomal storage disorders result from the deficiency of lysosomal enzymes. Lysosomes are cellular organelles which contain hydrolase enzymes to breakdown waste material and cellular debris. There are different groups of LSDs depending upon the material or substrate that is accumulated due to deficiency of the required enzyme. The most commonly known LSDs - Mucopolysaccaridoses (MPS) is caused due to the accumulation of glycosaminoglycans, Gaucher's disease due to accumulation of sphingolipids and Pompe's disease due to accumulation of glycogen. These substrates accumulate in cells of different systems impairing their function and LSD is therefore a multisystem disorder.



MPS is the most prevalent LSD in India. There are 171 cases enrolled at present, of which 144 cases have been confirmed. Following MPS, Gaucher's disease, Niemann Pick disease and Pompe's disease are also the more prevalent LSDs in India. The maximum number of referrals from Tamil Nadu as well as other states, is for MPS, which may be attributed to the MPS support group. The Support Group for MPS, a unit of FCRF, helps in the diagnosis, treatment, prenatal diagnosis and supportive therapy for the families with MPS children. Genzyme is an indispensable colleague for the MPS support group by providing free diagnostic services as well as Enzyme Replacement Therapy (ERT) for patients with MPS I, Gaucher and Pompe diseases. Currently, there are 4 MPS I, 15 Gaucher and 5 Pompe patients benefiting from ERT.

Dr. Joan Keutzer while addressing the audience on NBS for LSDs said that LSDs are not clinically obvious at birth which progresses to become a debilitating and often a life-threatening disease. It may often take years to arrive at the correct diagnosis solely based on clinical presentation, by which time the disease burden may not be reversed satisfactorily if treatment is given. There is ERT available for few of the LSDs while some are in clinical trials and some more in the development stage. Early treatment may make a significant difference in the patient outcome. Hence, early diagnosis and intervention through newborn screening (NBS) for LSD would be very beneficial, the magnitude of which can only be determined by employing NBS. Hence, LSDs as candidates for NBS cannot be more justified. Through NBS, all patients could be diagnosed at birth and routinely monitored for clinical and biochemical changes in biomarkers. Appropriate patients are transplanted early or ERT is initiated when a change is detected. This facilitates early intervention and treatment which could be modified as per requirement based on the patient's response and progress. This would include changing the dosage of ERT, using adjunctive therapies and symptom-based intervention.

A five year follow up on two siblings with MPS I has elucidated the clear benefits of NBS and early treatment. The first born female child was diagnosed as MPS I at the age of 4.5 years based on clinical presentation.She had skeletal and cardiac abnormalities with hepatosplenomegaly, corneal clouding and dysostosis multiplex, but normal intelligence. She was started on ERT at the age of 5. Five years later, she has normal liver and spleen volume, shoulder flexion and extension showed mild improvement, had only mild corneal clouding and was growing on the 90th centile. However, her skeletal and cardiac abnormalities did not improve, but were stable. When her younger brother was born, a geneticist confirmed that he also had MPS !. He had NBS on postnatal day 3 & confirmed later. He was started on ERT on the same day as his sister when he was 5 months old. His only symptom was elevated levels of urinary GAGs. Five years later, there is no evidence of valvular, bone, joint, or cardiac disease. He has only mild corneal clouding but normal visual acuity. He is growing at the 90th centile, with normal intelligence and appearance.

NBS does however face a few **challenges**. After having diagnosed an asymptomatic patient, it would be difficult to predict the age of onset. This in turn makes it difficult to decide the best patient management plan to avoid treating earlier than necessary. It would also be difficult to predict the extent of disease burden and neurological involvement. In addition, one must be weary that NBS may also identify individuals who may remain asymptomatic always. Nonetheless, NBS would help diagnose all patients early, brings awareness to the parents earlier and provides potential for earliest intervention.

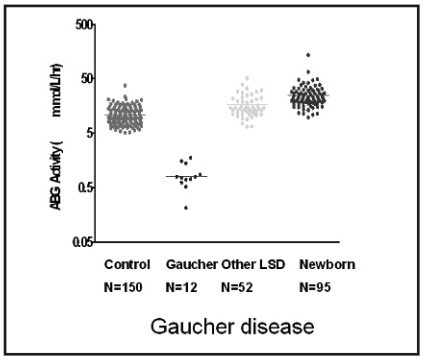
Genzyme plays a pivotal role in the development of NBS for LSD by creating awareness, identification and validation of biomarkers, assay development, supporting NBS pilot programs and providing reagents for LSD NBS worldwide. Several methods have been developed for testing in collaboration with Genzyme which include enzyme activity assays for many LSDs and genotyping assays. Samples could include dried blood spots (DBS), lymphocytes, leukocytes, serum, plasma, urine, fibroblasts, cerebral spinal fluid, bile and tissues (for pre-natal tests). Dried blood spots are however most commonly used for NBS. It is advantageous as it involves a less invasive sample collection, eliminates sample preparation and associated error, more stable and allows for easy shipment protocols.

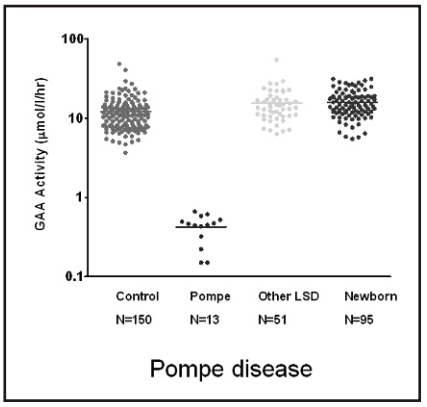
Enzyme activity assays can be performed by **using one of two equally reliable methods. One method uses 4-MU substrates** which generates fluorescent products. This method is used to measure the activity of a single enzyme. The other method uses MS/MS substrates which generates products with unique masses and helps measure the activity of several enzymes at once. The choice of the method obviously depends upon the number of enzymes to estimate and the availability of the specialized equipment in the lab.

The methodology for the **first method** involves extraction from a 3.2mm DBS, followed by addition of substrate. This mixture is then incubated for 20-22 quenched and analyzed using a flourometer. The enzyme activity from the patient sample is measured against normal controls. If the activity is below the lower limit of the control group, the patient is screened positive for the tested LSD. This has proven to be sensitive, cost-effective and high throughput for Gaucher's Disease by the measurement of glucocerebrosidase activity. However, the applicability of this method for diagnosis of Gaucher patients remains to be determined.

The **second method** involves the use of substrate-internal standard pairs for analysis with mass spectrometer. The internal standard is a synthetic molecule which can be acted upon by the enzyme as it has a structure similar to the substrate. However, the molecular mass of the internal standard and the substrate as well as their respective products are different to allow detection by the mass spectrometer. On the mass spectrometer read, the ratio of the area under the substrate product peak by the area under the internal standard peak gives the amount of product formed, which is turn quantifies the specific activity for the enzyme. For an affected patient, there would be negligible product formed indicating deficient enzyme activity. This assay has been developed in a way to allow for multiplex assays by designing unique internal standard-substrate pairs and products with unique masses for different LSDs. If an assay for an LSD not previously tested for is to be added, a unique internal standard-substrate pair needs to be designed. Hence, a newborn can be screened for different LSD using one DBS by this multiplex assay.

Multiplex Assay





So far, the multiplex assay has incorporated screening for Niemann Pick disease, Gaucher disease, Fabry disease, Pompe disease, Krabbe disease and MPS I recently. Genzyme manufactures vials containing the optimized ratio of substrate-internal standard for 1200 tests for these diseases. Reagents for each disorder as packed separately allows the end-user to choose menu-style. Assays for MPS II, IV and VI are currently under development at the University of Washington.

Genzyme, in collaboration with University of Washington, has helped launch a 2 year NBS pilot program in Washington for screening Fabry, Pompe and MPS I by a **triplex assay**. This was started in Spring 2010 and has a turn-over of approximately 30, 000 samples so far. The assay has been found to be very sensitive and specific with a virtually no false positives.

To support the quality of NBS, Genzyme collaborated with CDC to develop quality control by developing mock patient samples which could be used in routine analysis and assay method development. Firstly, a base pool is created which has negligible lysosomal enzyme activity. This is done by preparing leukocyte- reduced blood. This blood would then

contain only RBCs which do not have any lysozymes and can hence not produce any enzyme. The serum is heat-inactivated to get rid off residual enzyme activity. The leukocyte reduced blood is mixed with heat-inactivated serum to achieve a mean (SD) 55% ± 5% hematocrit to match the hematocrit for the newborns. Then, 5%, 50% or 100% cord blood samples adjusted to 55% ± 5% hematocrit are mixed with the base pool. This achieves a linear range of enzyme activity levels serving as low activity, medium activity and high activity controls. These QC samples are spotted onto DBS for use by laboratories performing screening. Pompe disease is reported to be more prevalent in Netherlands and Taiwan.

Pompe disease is caused due deficiency of alpla-glucosidase (GAA) leading to accumulation of glycogen in muscle fibres, which in turn causes inflammation, fibrosis and disruption of contractile elements of muscle. The disease mainly affects muscle function and hence affects the cardiovascular, respiratory and skeletal systems. Confirmation requires muscle biopsy to view glycogen accumulation in the muscle fibres.

Pompe disease is of two types – infant onset and late onset. The infantile-onset form of Pompe disease is characterized by massive deposition of glycogen in the heart, liver, and skeletal muscle resulting in rapidly progressive cardiomyopathy, generalized muscle weakness, hypotonia, motor delay, and hepatomegaly. Cardiomegaly is a prominent feature of infantile-onset disease. Death from cardiac and/or respiratory failure generally occurs before the end of the first year. A subset of patients with infantile-onset Pompe disease has been described in which there is a slower progression of cardiomyopathy and longer survival, with respiratory failure generally developing between 1 and 2 years of age. Diagnosis is based on clinical manifestation and is usually not before 6 months of age by which time the disease is advanced. In late-onset disease, first manifestation of symptoms occurs at a later age and disease progression is slower and more variable than in the infantile patients. Death usually results from respiratory failure.

The rapid disease progression and fatality of Infant-onset disease makes it an important candidate for NBS. Pompe disease NBS pilot program was launched in 2006 in collaboration with National Taiwan University Hosipital, wherein 45% of the newborns in Taiwan were screened 3 days after birth. Assay used 4MU substrates and estimation of fluorescent product by flourometer. The rest of the newborn from other hospitals in Taiwan served as controls. Posters were put up in participating hospitals to increase awareness. Informed consent was obtained from the parents before sample collection.

Growth charts and information brochures were given to the consented families.

Six newborns were identified in the NBS pilot program, all of whom were diagnosed before 45 days of life. They were all asymptomatic except for feeding or crying cyanosis in three of the neonates. They were given their first infusion of myozyme before 35 days of life except for one. This early intervention was found to be very beneficial in terms of survival and preserving muscle function. Taiwan University Hosipital, wherein 45% of the newborns in Taiwan were screened 3 days after birth. Assay used 4MU substrates and estimation of fluorescent product by flourometer. The rest of the newborn from other hospitals in Taiwan served as controls. Posters were put up in participating hospitals to increase awareness. Informed consent was obtained from the parents before sample collection. Growth charts and information brochures were given to the consented families.

Untreated patients never usually survive a year and are not able to sit or walk. On the other hand, almost on patients started on ERT have good survival rate and are able to walk by the age of 20 months.

One of the 6 newborns identified by NBS on day 22 of life can be used to elucidate the benefit of early treatment. When he was examined for confirmation, he had a normal physical exam with normal muscle tone and strength. His chest X-ray revealed massive cardiomegaly and his EKG was abnormal. Disappointingly, his muscle biopsy revealed massive glycogen accumulation with H&E staining and PAS staining. It was feared that the disease had already progressed and the course could not be reversed very satisfactorily. He was started on myozyme infusion on day 29 of life. Follow up X-ray three months later revealed regression of cardiomegaly. His muscle biopsy showed significant improvement with atleast 95% of normal muscle fibres and only occasional fibres with glycogen accumulation. The child has thereafter followed up to have normal motor milestones. This case clearly illustrates the benefit of NBS facilitating early intervention and better outcome.

In conclusion, NBS decreases the time to diagnose LSDs and really has the potential to improve patient outcome, as illustrated by NBS for Pompe disease. New methods are available for NBS for LSDs and enzymes are stable in DBS enabling their use in NBS and high risk screening for LSDs.

For BDRI membership please contact

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