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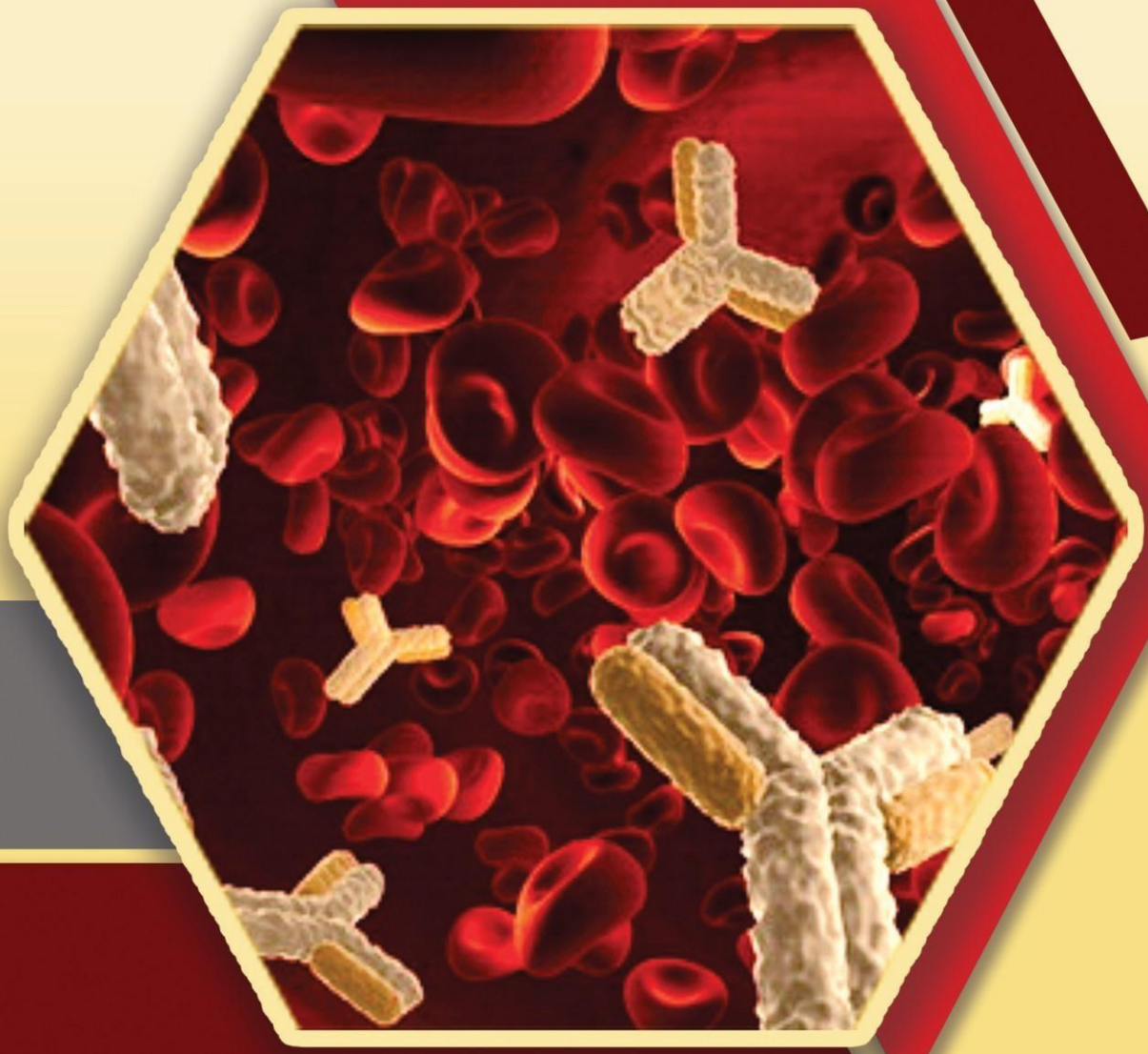

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Chronic Granulomatous Disease

Manasi Kulkarni

Introduction

Chronic Granulomatous Disease (CGD), is a rare (1: 200,000) inherited primary immune disorder (PID), caused due to a defect in any one of the five components of NADPH oxidase complex, characterized by the inability of phagocytes to form reactive oxygen species (ROS) upon interaction with bacterial or fungal pathogens (Winkelstein et al., 2000). The patients affected with CGD often show clinical manifestations in the first year of life including profound immunodeficiency to bacterial and fungal infections with unusual sites of infection involving unusual pathogens. The CGD may present any time from infancy to late adulthood; however, most of the affected individuals are diagnosed before age of five years.

Most commonly used assays for the diagnosis of CGD are nitrobluetetrazolium (NBT) slide test and oxidation of dihydrorhodamine (DHR) by flow cytometry. Molecular diagnosis is a confirmatory test for CGD, involving the identification of causative gene mutation. Molecular analysis of CGD is crucial due to its overlapping clinical manifestation and overall disease pattern. It is important for its therapeutic management, prenatal diagnosis, and genetic counselling. There is diversity in the pattern of underlying genetic defect with regards to the ethnicity and consanguinity. Overall, X- linked (XL)-CGD (due to mutations in *CYBB* gene) is the most prevalent (~70%) type of CGD. However, a higher rate of autosomal recessive (AR)-CGD (due to mutations in *CYBA*, *NCF1*, *NCF2*, *NCF4* genes) is reported in the regions where consanguineous marriage is more common, such as Turkey, Egypt, Oman, and Iran (Al-Zadjali et al., 2015; Fattahi et al., 2011; Meshaal et al., 2015). In India, due to ethnic diversity and high rate of consanguinity in some of the regions, the genetic pattern may be diverse and probably require a different diagnostic approach than that followed in other countries. The diagnostic approach may depend on the prevalence of affected gene, and designing the diagnostic algorithm reduces the turnaround time for the definitive diagnosis. The data generated could be utilized for prenatal diagnosis of affected families.

History

Chronic granulomatous disease was first described in 1957 and further characterized in 1959 and termed as a fatal granulomatous disease of childhood. It was associated with hypergammaglobulinemia and recurrent infections. Over the past six decades, CGD has evolved from a primary immunodeficiency associated (PID) with poor prognosis and severe infections to a disease with high survival using effective management.

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Molecular Pathology

CGD is a defect in phagocytic NADPH oxidase enzyme, resulting in failure of production of superoxides by phagocytes upon stimulation with foreign pathogen e.g. bacterial or fungal, and various other soluble inflammatory stimuli. Superoxides combine with water to produce hydrogen peroxide (H_2O_2) which has microbicidal activity. This further leads to production of other microbicidal components such as hypochlorous acid etc. The exact mechanism of pathogen killing within the phagosome is unknown. The complete reaction runs as below,

$$NADPH + 2O_2 \rightarrow NADP^+ + H^+ + \quad \text{(NADPH oxidase}$$

$$2O_2^- + 2H^+ \rightarrow O_2 + H_2O_2 \quad \text{(superoxide dismutase}$$

The functional NADPH oxidase complex comprises of six proteins (Figure 1). Each component of this enzyme complex is encoded by an individual gene: $gp91^{phox}$ by *CYBB* gene (cytochrome b-245 beta subunit) located on X chromosome, $p22^{phox}$ by *CYBA* gene (cytochrome b-245 alpha subunit) located on chromosome 16, $p47^{phox}$ by *NCF1* gene (neutrophil cytosolic factor 1) located on chromosome 7, $p67^{phox}$ by *NCF2* gene (neutrophil cytosolic factor 2) located on chromosome 1, and $p40^{phox}$ by *NCF4* gene (neutrophil cytosolic factor 4) located on chromosome 22. Mutation in any one of five genes leads to CGD. The 6th protein Rac2, is involved in cell migration and activation of NADPH oxidase. Similarly, in rare case defect in Rac2 and G6PD may show similarities to CGD including poor wound healing, decreased superoxide production and recurrent infections.

At a resting phagocyte, its inactive subunits reside in different compartments. Membrane bound components $gp91^{phox}$ and $p22^{phox}$ reside in transmembrane together form heterodimer called as flavocytochrome b558. They require each other's presence for stable and mature expression. That means loss of one protein result in absence of another component within phagocytes. Others are cytoplasmic components $p47^{phox}$, $p67^{phox}$ and $p40^{phox}$ forms a heterotrimer which is translocated to transmembrane upon cellular activation by ingested bacteria or fungi.

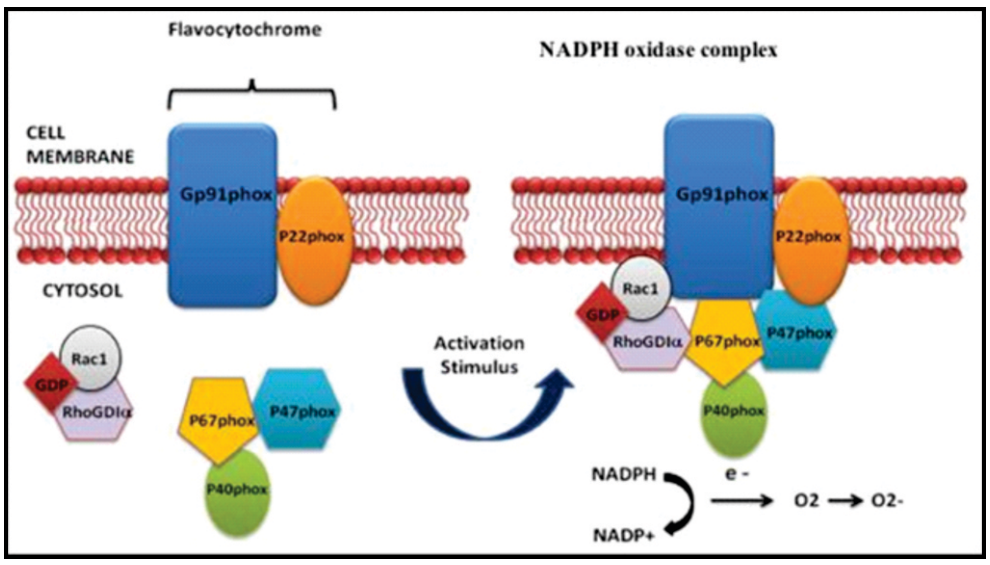

Figure 1:NADPH Oxidase complex

Mutation in gp91^{phox} (*CYBB*) are inherited in X-linked manner accounts for 65 to70% of the cases. AR-CGD is most commonly caused by p47^{phox} (*NCF1*) occurring in ~25% of the cases. The remaining 5% occur due to defect in p67^{phox} (*NCF2*) and p22^{phox} (*CYBA*). Very few cases are reported with p40^{phox} (*NCF4*) deficiency. The incidence of CGD is ~ 1: 200,000 to 1: 250,000 based on two large retrospective studies form USA and Europe (Gathmann et al., 2009; Winkelstein et al., 2000). Other counties have different frequencies that are depended on consanguineous marriages and ethnic practices: 1:300,000 in Japan (Ishibashi et al., 2000), 1: 111,000 in Israeli Arabs (Wolach et al., 2017) and 1: 450,000 in Sweden (Ahlin et al., 1995).

Diagnosis

The diagnosis of CGD is mainly dependent on measurement of superoxide activity. NBT is the most recognized and the oldest diagnostic test for CGD (Figure 2). It relies on microscopy to provide quantitative measurement of NADPH oxidase activity. It has been largely replaced by DHR assay for its sensitivity and rapid analysis (Roesler et al., 1991). The DHR assay uses flow cytometry to measure oxidation of non-fluorescent Dihydrorhodamine to highly fluorescent rhodamine by stimulated neutrophils. The presence of myeloperoxidase is also essential for neutrophils to produce superoxides, hence myeloperoxidase (MPO) deficiency can therefore lead to abnormal DHR assay. The DHR is preferable because of its ability to distinguish between XL-CGD and AR-CGD, its sensitivity to very low number of functional neutrophils and its capacity to accurately identify X-linked carriers (Figure 3).

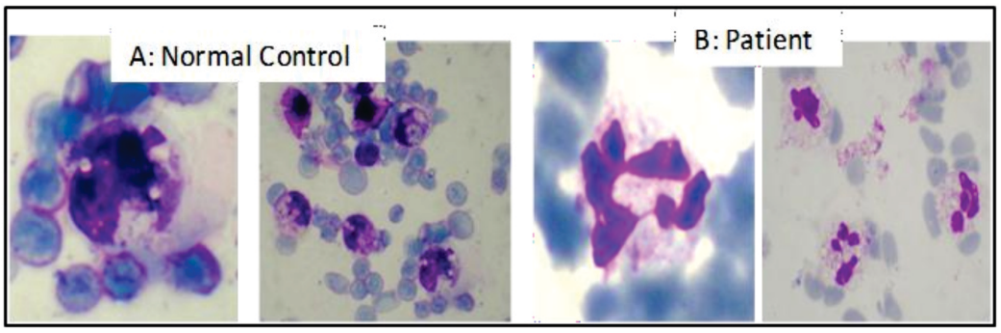

Figure 2:NBT Test

A. Nitro Blue Tetrazolium dye (NBT) reduction showing blue-black formazan particles deposited on neutrophils of healthy control on stimulation with phorbol myristate acetate (PMA).
B. No reduction of NBT dye after stimulation in CGD patient's neutrophils

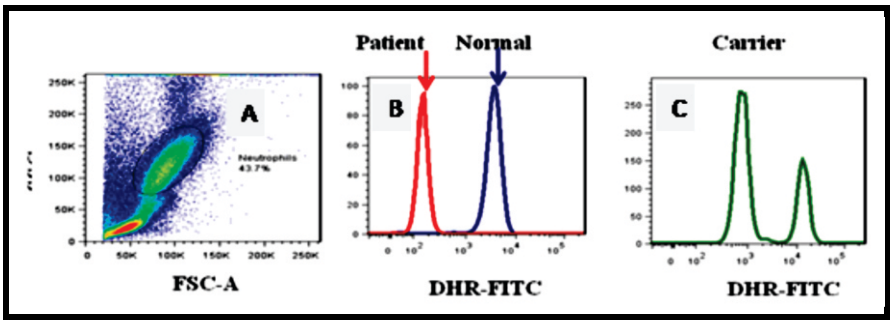

Figure 3:DHR Test

DHR assay in healthy control, CGD patient and in carrier parents.

A Gating of neutrophils

B Expression of Rhodamine in phorbol myristate acetate (PMA)-stimulated granulocytes obtained from CGD patient (Red line), healthy normal control (Blue line)

C From obligate carriers in XL-CGD.

Clinical Manifestations

Although the genetic basis for this disease is well known, the expected clinical course and outcome have extreme differences in terms of presentation between patients, varying from a relatively mild presentation late in life to fatal septicemia in infancy (Holland, 2010) (Jones et al., 2008). In most of the CGD patients, clinical manifestations are presented before the age of five years. Some of the first presenting symptoms are cough, fever, chest pain, and failure to thrive. Most commonly reported sites of infections are lung (pneumonia), lymph nodes (lymphadenitis), liver (abscess), bone (osteomyelitis), and skin (abscesses or cellulitis) (Holland, 2010). Possibility of CGD can be suspected microbiologically, since the spectrum of infection in CGD is distinct and narrow. A significant respiratory morbidity is reported in CGD patients (Holland, 2013).

The unusual organisms commonly observed in CGD patients include Staphylococcus aureus, Burkholderia cepacia complex, Serratia marcescens, Nocardia, Aspergillus, etc. Tuberculosis and fungal infections are the leading cause of mortality in CGD patients (Marciano et al., 2015). These are typically acquired through inhalation leading to pneumonia and can spread locally or metastatically. Fungal infections have been the leading cause of mortality in CGD (Winkelstein et al., 2000). However, the advent of itraconazole prophylaxis and the newer agents for treatment of filamentous fungal infections, such as voriconazole and posaconazole, have markedly reduced the frequency and mortality of fungal infections in CGD. (Table 1)

BCG vaccine, given to almost 90 % of newborns around the world, is usually the first important infectious challenge CGD patients will face. BCG complications range from none to self-limited localized BCGitis to fatal disseminated BCGosis (Zhou et al., 2018).

Table 1: Infection Pathogens Involved in CGD

Pathogen		Presentation
Bacterial infections	*Staphylococcus aureus*	Soft tissue infections Lymphadenitis, Liver abscess, Osteomyelitis, Pneumonia, Sepsis
	Burkholderia species (Greenberg et al., 2009) • *B cepacia* (Ong et al., 1993) • *B gladioli* • *B pseudomallei*	Pneumonia Sepsis
	Serratia marcescens (Friend et al., 2009; Galluzzo et al., 2008)	More common: • Osteomyelitis • Soft tissue infections Less common: • Pneumonia • Sepsis
	Nocardia species (Dorman et al., 2002) • *N asteroides* • *N nova* • *N otitidiscaviarum* • *N farcinica*	Pneumonia Osteomyelitis Brain abscess
	• *Chromobacterium violaceum* (Sirinavin et al., 2005)	Sepsis
Fungal infections	Aspergillus species (Beauté et al., 2011) • *A fumigatus* • *A nidulans* • *A viridinutans* • *A flavus* • *A terreus* • *A niger*	Pneumonia Osteomyelitis Brain abscess Lymphadenitis
Yeast infections	Candida • *C albicans* • *C glabrata* • *C lusitaniae*	Sepsis Soft tissue infection Liver abscess
Source: Chronic Granulomatous Disease, Gene Review, Leiding and Holland (De Ravin et al., 2008)		

Treatment and Management

The management of CGD is either supportive or curative. The supportive management is based on three principles:

1) lifelong antibacterial and antifungal prophylaxis
2) early diagnosis of infection and
3) aggressive management of infectious complications.

In CGD, it is important to start the treatment at an earliest for which determination of the exact complicating infections and selection of the most appropriate antibiotic or anti-fungal therapy is needed. For infections that fail to respond to therapy within 24 to 48 hours, additional diagnostic procedures should be used to identify the microorganism. Effective management of CGD relies on lifelong antibacterial and antifungal prophylaxis. It reduces frequency of major infections from one episode per year to one every 3.5 year (Margolis et al. 1990).

In CGD patients, steroids and aminosalicylates may improve colitis and other inflammatory complications, while a prophylactic treatment with antibacterial and antifungal prophylaxis for life with co-trimoxazole and itraconazole may have improved short and medium-term survival. However, these treatments do not correct the underlying genetic defect. Currently, HSCT is the only established curative treatment for CGD and a rapid diagnosis is important to identify if HSCT is possible. Overall survival rates for patients who underwent HSCT have improved as about >90% in children with <14 years of age. Still, prior infections and organ dysfunction may increase transplant-related complications and the patients with CGD are also found to be prone to graft failure. The optimal conditioning regimen

and management of adolescent and adult patients is still not clear. The decision for HSCT should be taken based on the final prognosis, donor availability, access to transplantation, and patient preference (Gennery, 2017).

New Treatment Strategies

With recent advances, gene therapy is seen as an alternative to HSCT for patients without an HLA-matched donor (Siler et al., 2015). Research on gene therapy trials in CGD is very active globally with several researchers involved in clinical development of gene therapy. Development of gene therapy in CGD patients could be logical and feasible as genes involved in NADPH oxidase are metabolic genes and are not involved in cellular proliferation. Use of zinc-finger nucleases (ZFN), transcription activator-like effector nucleases, or the clustered regularly interspaced short palindromic repeat (CRISPR)-Cas9 system for targeted genome editing approaches are proposed to allow gene correction in situ such that gene expression would remain under the control of the gene's own cell-specific promoters. The rapid advances in gene therapy have been extremely exciting, and clinical trials using gene-editing techniques are likely to be developed within the next five years (Brendel et al., 2018). In future, gene therapy may provide viable alternative cure for CGD patients. (Arnold and Heimall, 2017)

NIIH Experience

In our study at NIIH, 100 CGD patients from 94 families were evaluated. Of which, four families had two or more siblings affected with CGD; 53% of patients were male and 47% of patients were female (Ratio: 1.12:1); 24% of patients had family history suggestive of CGD. Consanguinity was noted in 43% of the patients, all of them had AR-CGD. On the basis of religion, 75% of patients were Hindu, 21% of patients were Muslim, and 4% of patients were Christian, suggestive of the ethnic diversity. In our cohort, AR-CGD was more common (70%) than X-linked CGD (30%). Among AR-CGD patients, *NCF1* gene defect was the most common (56%) followed by *CYBA* (7%) and *NCF2* (7%). Molecular characterization of 90 CGD patients revealed 41 different mutations (15 novel, 26 reported): 25 mutations in *CYBB* gene, five mutations each in *CYBA* and *NCF2* genes, and six mutations in *CYBA* gene. Mutation spectrum seen in our cohort: 47% DelGT, 21% missense, 16% nonsense, 11% small deletion, 3% splice variant, 1% duplication and 1% insertion mutation. The common mode of presentation was pneumonia, tuberculosis, skin and subcutaneous abscess, lymphadenitis, and osteomyelitis. Most common organisms isolated in our series were *Mycobacterium sp., Aspergillus sp., Staphylococcus aureus sp., Klebsiella sp., Candida sp.,* and Gram-negative *bacilli*. An aggressive culture policy needs to be followed to isolate organisms to initiate specific therapy as early as possible. This may help in reduction of morbidity and mortality seen in these especially due to fungal infections. There is a wide clinical heterogeneity observed among Indian CGD patients even with the common mutation (42 cases of Del-GT mutation in *NCF1* gene) in terms of age of presentation, severity, and overall disease prognosis. This suggests that, not only the genetic defect but epigenetic and environmental factors also play an important role in phenotypic variation (Kulkarni et al., 2016). The comprehensive approach with detection of mosaic pattern in NBT and DHR assay in mother's sample along with component expression analysis in patient's sample is recommended before planning the molecular diagnostic strategy (Figure 4). Based on the flow cytometric analysis of 100 patients, following diagnostic strategy was designed for the molecular diagnosis of Indian CGD patients. (Figure 4)

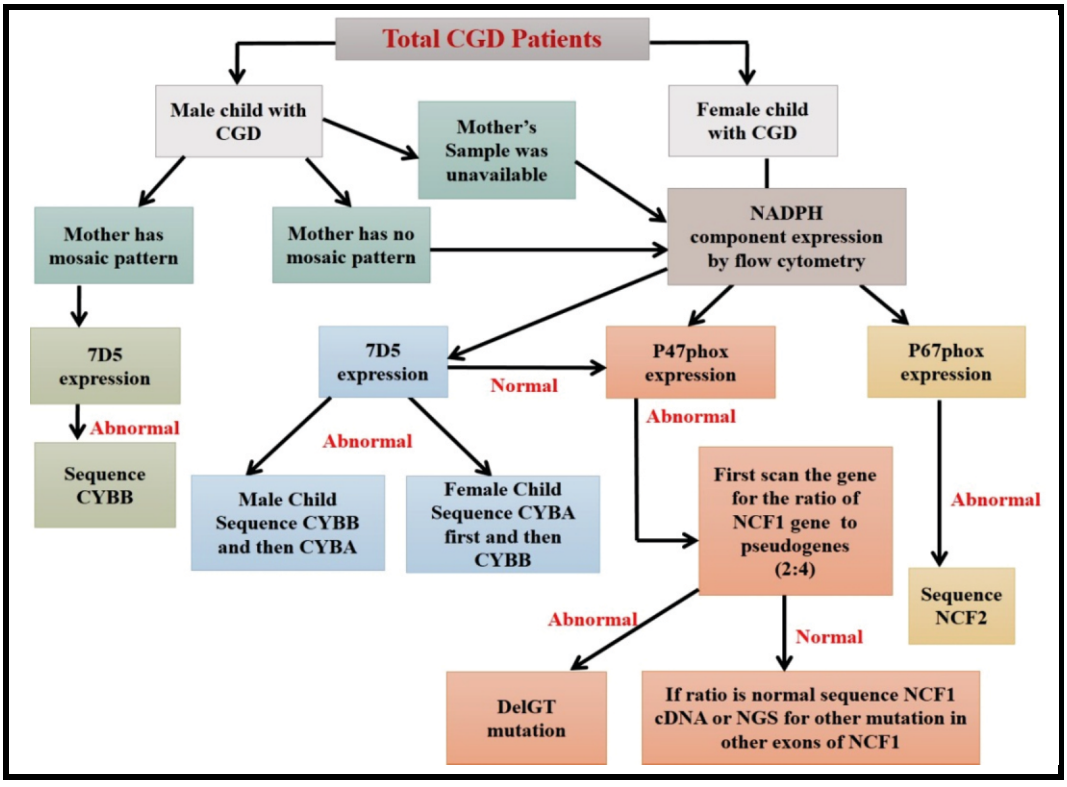

Figure 4: Diagnostic Strategy for Indian CGD Patients at the Time of Initial Diagnosis

Genotype-Phenotype Correlation

A marked phenotypic heterogeneity is seen in patients with CGD. Underlying genetic defect is one of the strong predictors of severity of disease and survival in CGD patients. The difference in survival was found to be statistically significant by Log Rank (Mantel-Cox) test based on their underlying affected genotype (p=0.018). Autosomal recessive p47^{phox} CGD has a significantly better prognosis than XL-CGD (Roos et al., 2010). Factors like sex (male), early onset, and increased severity of disease often suggests X-linked disease. Similar findings were observed in our series with early age of presentation in patients with XL-CGD compared to AR-CGD. However, severe disease may also be seen in patients with AR-CGD. Notably, the XL-CGD patients with missense mutations have better overall survival comparable to p47^{phox} CGD. The amount of residual NADPH oxidase activity is one of the factors responsible for such diversity. Nonsense mutation with nearly absent residual superoxide production are likely to be associated with more severe disease compared to missense mutations with partially retained superoxide production (Kuhns et al., 2010).

Treatment and outcome

Allogenic hematopoietic stem cell transplant (HSCT) is the only known curative therapy for CGD. Though, HSCT is an attractive alternative to cure patients with CGD. The overwhelming majority of CGD patients survive without HSCT. Survival of CGD has improved overall since last decades. Out of 100 cases in our study, 46 patients were lost to follow-up after the diagnosis, 22 patients succumbed to illness, and 32 patients are alive and doing well with the use of antimicrobial and antifungal prophylaxis. Residual superoxide activity usually correlates well with the survival. AR- CGD patients typically have higher levels of residual superoxide activity than XL-CGD patients. Mutations that abolish the protein expression are associated with severe and overall worse outcome, whereas patients with positive protein expression tend to have better outcome. Therefore, knowing the genetic cause of the mutation can help to predict superoxide production, overall risk of mortality, and providing information that can help to decide about HSCT.

Prenatal Diagnosis

The data obtained in the present study was successfully utilized for genotypic (1) or phenotypic (9) PND in ten families at risk of getting CGD affected child in next pregnancy. PND is challenging in families approaching late in pregnancy with an uncharacterized molecular defect. Analysis of fetal blood sample by cordocentesis at 18 to 20 weeks of gestation helped to predict whether the fetus is to be affected or unaffected with CGD in seven families. However, its application is restricted to a condition where a family with a known history of CGD approaches late in pregnancy, genetic diagnosis is not available, and time for diagnosis is very limited (Kulkarni et al., 2017).

Summary

The large cohort study on clinical, immunological and molecular characterization of CGD patients from India, emphasizes the influence of ethnic and cultural practices on the spectrum of molecular pathology of CGD. Early diagnosis and prompt treatment may help CGD patient for the better survival with reduced morbidity and mortality. In our cohort, AR-CGD was more common (70%) than X-linked CGD (30%). Among AR-CGD patients, *NCF1* gene defect was the most common (56%) followed by *CYBA* (7%) and *NCF2* (7%). This study emphasizes that, substantial number of the patient's had defect in *NCF1* gene (41%) of which 73% patients lack *NCF1* gene on both the alleles. The same mutation has been found to be described in different cohort studies; however, the molecular characterization of this pathology done for the first time in India is important for diagnostic classification, patient prognosis, and adequate genetic advice and a possible future therapy. This mutation is often missed by advanced molecular techniques like Sanger sequencing and NGS due to the presence of pseudogenes and requires a simple Genescan method for confirmation. It can be concluded from this study that, in Indian population, the Genescan method should be preferred as the primary molecular test to rule out *NCF1* gene mutations prior to Sanger sequencing and NGS (Kulkarni et al., 2018). Thus, the diagnostic approach may depend on the prevalence of affected genes in respective population. In this study, flow cytometry-based classifications of CGD subtypes, facilitated rapid identification of affected gene for the molecular confirmation.

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चिरकालीन ग्रॅन्युलोमॅटस डिजिज

मनिषा पटवर्धन

चिरकालीन ग्रॅन्युलोमॅटस डिजिज (सी.जी.डी.) दुर्लभ बिमारी है। आनुवांशिक प्राथमिक प्रतिरक्षित (Primary Immune Disorder) की यह बिमारी से दो लाख जनसंख्या में से एक बच्चा ग्रस्त होता है। शरीर में फॅगोसाईट कोशिका प्रतिकियाशिल (reactive) ऑक्सिजन (ROS) बनाती है। एन.ए.डी.पी.एच ऑक्सिडेज (NADPH oxidise) के पॉच भागों में से किसी एक भाग में दोष होने सें, फॅगोसाईट कोशिका प्रतिकियाशिल ऑक्सिजन बनाने में नाकाम रहती है। सी.जी.डी. से बाधित रूग्ण में आयु के पहले वर्ष में ही रोग के लक्षण दिखने लगते हैं। जीवाणू (bacteria) और फफूंद (fungus) के हमलों को रोकने में, यह रूग्ण नाकामयाव रहते हैं। यद्यपि, सी.जी.डी की शुरूवात वचपन से लेकर प्रौढ होने पर हो सकती हैं, किंतु ज्यादातर रूग्णो में आयु के पॉच या उससे पहले वर्षो में सी.जी.डी.के लक्षण प्रकट होते हैं।

प्रयोगशाला में सी.जी.डी.की पहचान के लिए नायट्रोब्लू टेट्राओलियम (NBT) और डाय हायड्रोरोडामीन (DHR) परीक्षा की जाती हैं। हाल ही में मोलेक्युलर परीक्षा निदान पध्दती विकसित हुई हैं। भविष्य में चिकित्सा पध्दती आंखने में यह परीक्षा लाभदायक होती हैं। सजाति अर्थात सगोत्र शादी–व्याह की रिती चलाने वाले जन जातियों में सी.जी.डी.की मात्रा अधिक होती हैं। तुर्की (इजिप्त), ओमान और ईराण के बाशिंदो में सी.जी.डी. में जीन म्युटेशन (बदलाव) का प्रतिशत काफी हैं।

सी.जी.डी. का इतिहास

सन १९५७ में सी.जी.डी.का पहला रूग्ण पहचाना गया। इस रूग्ण में बार बार जंतु प्रादुर्भाव को देखा गया था। पिछले छह दशकों में सी.जी.डी.में प्रशंसनीय अनुसंधान हुआ। प्रभावी दवा बनने से रूग्ण में आयुमर्यादा बढीं हैं।

सी.जी.डी. की कारणमिमांसा शरीर में जिवाणू दाखिल होने पर कई प्रकार की कोशिका (फॅगोस) उत्तेजित होती हैं। इन में एन.ए.डी.पी.एच ऑक्सिडेज (NADPH oxidise) की मात्रा बढ़ जाती हैं। जिसके फलस्वरूप 'सुपरॉक्साईड' निर्माण होता है। सुपर आक्साईड का पानी से संयोग होने पर हायड्रोजन पेरोक्साइड (H_2O_2) बनती हैं। जीवाणू को नष्ट करने का महत्त्वपूर्ण काम इस 'द्रव्य' द्वारा होता हैं। कियाशिल (functional) एन.ए.डी.पी.एच.ऑक्सिडेज छह (6) प्रोटीन से बनता हैं। हर एक प्रोटीन के लिए एक जीन (gene) निर्धारीत होता हैं। इन में से किसी भी जीन में बदलाव के कारण एन.ए.डी.पी.एच.ऑक्सिडेज के कार्य में बाधा उत्पन्न होती है।

सी.जी.डी. का रोजनिदान

सुपरऑक्साइड की मात्रा का मुल्यमापान सी.जी.डी. के होने की पुष्टी करता हैं। नायट्रोब्लू टेट्राझोलियम (NBT) चिकित्सा से सी.जी.डी. का निदान होता हैं। किंतु यह अर्धमात्रांकित (semi quantitative) प्रकार की चिकित्सा हैं। लिहाजा निश्चित तौर पर चिकित्सा के लिए डायहायड्रोरोडामाईन (DHR) चिकित्सा का उपयोग किया जाता हैं। आधुनिक 'फ्लोसायटोमीटर' उपकरण से की जाने वाली यह चिकित्सा ज्यादा 'विश्वसनीय' हैं।

सी.जी.डी. के लक्षण

सी.जी.डी.के रूग्णों में लक्ष्णों का प्रकटीकरण, उतार - चढाव में समानता नहीं होती हैं। रूग्णों में पॉच साल से कम उम्र में लक्षण दिखायी देते हैं। खांसी, बुखार, सीनें में दर्द आदि लक्षण शुरूआती दौर में दिखते हैं। यथा अवकाश फेफड़े, लसिका ग्रंथी (lymph node), यकृत (liver) हडडी और त्वचा में प्रादुर्भाव के लक्षण देखनें में आते हैं। फफुंदीय प्रादुर्भाव (fungal infection) सी.जी.डी.के रूग्ण में तीव्र स्वरूप धारण कर सकती हैं। इन्ट्राकोनझाल, वोरीकोनाझाल, पोसाकोनाझोल जैसे प्रभावी रोगनिरोध दवा के कारण, प्रादुर्भाव की तीव्रता और पुनरावृती कम हो जाती हैं। टी.वी. (TB) के प्रतिरोध के लिए नवजात शिंशु को बी.सी.जी. (BCG) के टीके लगाये जाते हैं। सी.जी.डी.के रूग्ण में यह टिका भी घातक हो सकता हैं। सी.जी.डी.में प्रादुर्भाव पैदा करनेवालों में जीवाणू (bacteria) फफुंदीय जीव (Fungus- Asperigillus species) और किण्व (Yeast- Candida) शामील हैं।

सी.जी.डी.की अभिव्यक्ति (Manifestation)

सी.जी.डी.के रूग्णों में प्रादुर्भाव के कारण व्रण (granuloma) होता हैं। कुछ रूग्णों में फोडे या गॉठ बनती हैं। इन के रूग्णों में मसुडों का प्रदाह (gingivitis), प्रकाश संवेदनशीलता (photo sensitivity), रक्तवाहीका शोथ (vasculitis), गुर्दा और हृदय संबंधी विकृती हो सकती हैं। इन रूग्णों में आम तौर पर बड़ी आंत का प्रदाह (collitis) की शिकायत होती हैं। रूग्ण में वजन की कमी, दस्त होते हैं। आयु बढ़ने के साथ साथ सी.जी.डी.के रूग्ण तीव्र लक्षण से झुंझने लगते है।

सी.जी.डी.की उपचारपद्ती तथा प्रबंधन (Management)

सी.जी.डी.का प्रबंधन दो प्रकार से किया जाता हैं, सहायक पद्धती (supportive) और रोगनिवारक (curative) पद्धती।

सहायक प्रबंधन के तीन तरीके -

१. आजीवन जीवाणू, फफुंद के प्रादुर्भाव को रोखने के लिए दवा का उपयोग

२. प्रादुर्भाव का जल्द निदान

३. प्रादुर्भाव के जटील होने से पहले ही रोकना

हालांकि आनुवंशिक सी.जी.डी.को ठीक करने में दवा नाकाफी हैं किंतु आयु बढ़ाने में परिणामकारक जरूर साबित हुई हैं।

नई उपचारप्रणाली

जीन थैरपी (gene therapy) उभरती उपचारपद्धती में सबसे आशादायी नजर आती हैं। किंतु अभी तक के नतीजे मिश्र स्वरूप हैं। दुनियाभर के वैज्ञानिक जीन थेरपी को यशस्वी बनाने में जुटे हुए हैं।

एन.आय़.आय.एच. में अनुभव

एन.आय़.आय.एच. में ९४ परिवार अनुसंधान में लिए गये। इऩमें से चार परिवारों में दो से ज्यादा बच्चे सी.जी.डी. से पिडीत थे। कुल सी.जी.डी.रूग्णों में ५३(%) प्रतिशत पुरूष और ४७ प्रतिशत महिलायें थी। सगोत्र शादियों का औसत ४३ प्रतिशत था। धार्मिक आधार पर सी.जी.डी. रूग्णों में ७५ प्रतिशत हिंदू, २१ प्रतिशत मुसलमान और चार प्रतिशत खिश्चन थे। ज्यादातर रूग्णों में न्युमोनिया, टी.बी., त्वचा पे व्रण, लसिकाग्रंथी शोध देखा गया।

मोलेक्युलर म्युटेशन (बदलाव) की दृष्टी से सी.जी.डी. दो प्रकारों में बटती हैं। ऑटोसोमल म्युटेशन AR-CGD और एक्स लिंक्ड म्युटेशन XR-CGD. ऐन.आय.आय. एच. के अध्ययन में कुल सी.जी.डी.रूग्णों में, AR-CGD का औसत ७0 प्रतिशत तथा XR-CGD का औसत ३0 प्रतिशत मिला। अब तक, सी.जी.डी.में उपयुक्त और सबसे परिणामकारक पद्धती अलोजेनिक हिमोपोयटिक स्टेम सेल ट्रान्सप्लांट (HSCT) रही हैं। इस प्रणाली से रूग्णों में आयुमर्यादा बढ़ी।
ऐन.आय.आय. एच.में अनुसंधान में संमलित रूग्णों (१00) में से ४६ रूग्ण दोबारा जॉच (Followup) में हिस्सा नहीं लिया २२ रूग्ण किसी ना किसी विमारी से चल बसे। जिवित ३२ रूग्णों में जीवाणु विरोधी (anti bacteria) फंगस विरोधी (anti fungal) दवाएँ निरंतर चलती रही।

प्रसव-पूर्व चिकित्सा

जिन परिवारों में पहले से सी.जी.डी.का इतिहास था, ऐसे १0 परिवारों का मोलेक्युलर जॉच किया गया। भ्रून के रक्त में (fetal blood) प्रसुतीपूर्व १८ से २0 हफ्ते में, जॉच करने पर बच्चा सी.जी.डी. बाधित होने की संम्भावना का पता भी ऐन.आय.आय. एच.के अनुसंधान में लगाया जा सका।

सी.जी.डी.को पहली बार समजने से आज तक इस विमारी में दुनियाभर में अनुसंधान हो रहा हैं। नये तकनीक, प्रभावशाली दवा कारगर शावित हो रहीं हैं। रूग्ण का आयुमान बढ़ाना, प्रादुर्भाव की आवृत्ति (frequency) घटाना सी.जी.डी.की तत्पर जॉच भी अनुसंधान काफी उपयुक्त शावित हुए हैं। मोलेक्युलर तकनीक में दुनिया के साथ भारत में लगातार नये आविष्कार हो रहे हैं सी.जी.डी. रूग्णों को दिलासा देने में वैज्ञानिक निश्चित दृष्टीकोन से काम कर रहे हैं।

AWARD WINNING ABSTRACTS

1. First prize for an oral presentation at 7th Annual conference of Indian Society of Transfusion Medicine held at Kochi on 23rd-25th Nov, 2018.

Title: Molecular characterization of rare D--/D-- variants in individuals of Indian origin

Swati Kulkarni, Garima Mishra, K. Vasantha, Harita Gogri, Disha Parchure, Manisha Madkaikar

Introduction: The Rh system is the most polymorphic and immunogenic protein based blood group system with five main antigens: D, C, c, E, e. Rh antigens are important in haemolytic disease of the foetus and newborn. Unusual Rh phenotypes such as Rhnull and D-- are rarely encountered in routine blood bank testing. D-- phenotype is a rare blood group characterized by the lack of expression of C, c, E and e on the red cells because of mutations in both alleles of the *RHCE* gene. The D antigen expression is exalted (up to 2,00,000 D antigenic sites per RBC) to the extent that IgG anti-D can agglutinate the RBCs in saline. Such individuals show presence of anti-Rh17 or anti-Hro.

Aim: To determine the molecular basis of D-- individuals (n=5) of Indian origin.

Material & methods: Five RhD positive postnatal women who had produced antibodies against all Rh antigens except D, leading to HDFN and fetal loss were referred to ICMR -NIIH for further evaluation. Extensive serological and molecular analysis was carried out.

Results: Serological testing with anti-C, anti-c, anti-E, and anti-e showed absence of C, c, E and e antigen, thus identifying the rare Rh variant as D--/D--. Flow cytometry confirmed absence of these antigens with exhalted expression of D antigen. Antibody screening and identification showed presence of anti-Rh 17. Molecular analysis by QMPSF showed gene conversion event between RHCE and RHD causing D-- phenotype. Most common hybrid was found to be RHCE-D(3-9)-CE followed by RHCE-D(3-8)-CE and RHCE-D(2-6)-CE.

Conclusion: This is the first study reporting molecular mechanism of D-- phenotypes in Indian population. Identification of RHCE-null variants facilitates confirmation of D-- phenotypes in patients and donors, helping improve transfusion safety.

2. Third prize for an Oral presentation at 59th National Annual Conference- Haematocon 2018, organized by Indian Society of Haematology and Blood Tansfusion, held at Kochi during October 25th-28th, 2018

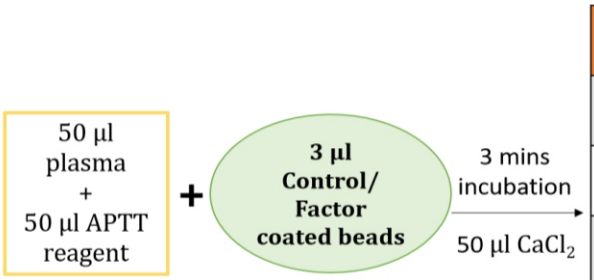
Title: Simple APTT based tests for differential diagnosis of haemophilia A and B - Preliminary results

Rucha Patil, Shrimati Shetty

Introduction: Haemophilia is an X-linked genetic disorder in which the patient tends to bleed excessively. There is a shortage of diagnostic laboratories for this disorder. Many laboratories perform APTT and PT assays but for factor assays they need to be referred elsewhere. This non-availability of factor assays is thus a major limitation.

Aims & Objectives: The main aim of this study is to standardize and establish a rapid, simple and cost-effective screening APTT assay which will differentially diagnose FVIII and FIX deficient patients, with no additional requirement of expensive instruments or reagents.

Materials & Methods: Different factor VIII(FVIII) and factor IX(FIX) concentrates (450U) were coupled to 2µm polystyrene beads by passive adsorption using different protocols and stored at 4°C. These were checked for binding efficacy and stability by flow cytometry using FITC-labelled anti-human FVIII/ FIX antibody. These beads were then used for correction in APTT test in 60 haemophiliacs and 30 controls.



Beads	Sample 1	Sample 2	Sample 3
Control beads	32.0	72.1	68.9
FVIII coated beads	29.0	36.5	66.5
FIX coated beads	31.3	70.1	35.7
Conclusion	Normal Pooled Plasma	HA Patient or vWD	HB Patient

Results: Out of the 60 haemophiliacs, 48 were haemophilia A(HA) (16 inhibitor positive) and 12 were haemophilia B(HB). Complete correction was seen in HA patients with no correction in HB patients using FVIII beads, which was confirmed using FIX beads which showed opposite results. Bead stability: pdFVIII coated beads was found to be stable till 6 months; those coated with rFVIII till 1-2 months; Fc-fusion FVIII binding was completely unsuccessful. The best results with maximum correction (CB: 71.1 secs; FVIIIB: 27.1 secs) and stability has been found using pd FVIII. FIX beads showed partial correction (CB: 68 secs; FIXB: 45.5 secs), having a stability of only 1 month. The cost of this test for 1 patient with all 3 beads- CB, F8B & F9B will be approx.72.6 Rs.

Discussion & Conclusion: It is a dilemma for both the treating physicians as well as patient when diagnosis for hemophilia cannot be given especially in emergency situations. This APTT based screening test established in our laboratory is rapid, simple and cost effective which will differentially diagnose FVIII and FIX deficient patients, wherein the reagent can be stored at 4^0C itself.

In countries like India, where many patients are still left undiagnosed, such simple inexpensive test which could be performed in basic laboratories will help diagnose majority of our haemophilia patients all over the country which in turn would reduce the morbidity and mortality during major haemostatic challenge in such new patients.

3. Best oral presentation award at Quarterly meeting of Mumbai Haematology Group held on 9th September 2018

Title: Triple jeopardy: A case of Glanzmanns thrombasthenia with inhibitors and HPA incompatibility resulting in still birth

Puja Soni, Chandrakala S, Aniket Prabhudesai, Shrimati Shetty

Introduction: Glanzmann thrombasthenia (GT) is a rare, autosomal recessive platelet disorder characterized by failure of platelet aggregation due to qualitative or quantitative abnormalities of a platelet glycoprotein complex (GPIIb-IIIa) leading to excessive bleeding. Pregnancy in such GT patients may present with complications of severe bleeding in the mother during delivery or post-partum and the risk of development of isoantibodies against the GPIIb–IIIa receptor due to frequent platelet transfusions and subsequent transplacental passage of these maternal antibodies in the foetus resulting in foetal immune thrombocytopenia. Thus, early attention and intensive management are essential for improving the maternal and fetal outcomes.

Case Summary: A 30 year old female with Type I Glanzmann Thrombasthenia (GT) underwent preterm vaginal delivery (PTVD) during her 1st pregnancy complicated by perinatal morbidity. This patient has had severe bleeding manifestation including epistaxis, ecchymosis on trauma, gastrointestinal bleeding, severe menorrhagia and bleeding from minor wounds during childhood till adolescent years and received multiple platelet and blood transfusions. Antenatal scan during the third trimester showed foetus had developed macrocaphaly along with intraventricular haemorrhage. Prior to delivery, patient was given platelet transfusions and NovoSeven however, pregnancy outcome was stillbirth. Due to serious obstetric complications, both parents were tested for complete coagulation profile and for presence of any Human platelet antigen (HPA) incompatibility. Mother (patient) showed lack of platelet aggregation with ADP, Epinephrine and Collagen and absence of platelet GPIIb-IIIa receptors on flow cytometry. Father showed normal platelet function tests and platelet receptor study. Addition of mother's plasma to father's platelet showed no aggregation with all agonists suggesting presence of isoantibodies against the platelet GPIIb–IIIa receptor. HPA genotyping of both parents revealed incompatibity of HPA-15 (CD109) as father had b/b and mother had a/a genotype.

Discussion: GT Type I patients are at greater risk of developing isoantibodies due to multiple platelet transfusions. Similarly in this patient, due to multiple platelet transfusions, isoantibodies against the platelet GP IIb-IIIa receptor might have been produced. This has been checked *in-vitro* by incubating father's platelets with mother's plasma and performing platelet function tests. Transplacental passage of these maternal isoantibodies in the foetus may have resulted in intracranial hemorrhage (ICH). Also, in addition due to HPA-15 incompatibility it may result in production of isoantibodies against HPA-15. Alloimmunization due to HPA-15 antibodies is rare but has been reported to result in ICH and intrauterine death during third trimester.

Conclusion: In GT patients, mixing platelet aggregation studies should be carried out after frequent platelet transfusion to know the presence of anti-platelet antibody. Also HPA genotyping must be carried out in GT patients as it might help proper management by anti-IgG along with plasmapharesis to decrease the frequency of intra and postpartum bleeding and thus may also decrease the risk of stillbirth child.

References:

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Dr. Rucha Patil, Department of Haemostasis and Thrombosis, was awarded SERB Women Excellence Award for the project entitled "A flow cytometry assay and rapid agglutination test for the detection of anti-FVIII antibodies"

Dr. Manisha Madkaikar, Director, ICMR-NIIH presented Oral paper entitled — Molecular Diagnostic Strategy for Chronic Granulomatous Disease (CGD) In Indian Population: A Large Cohort Study of 90 Indian Patients at the 18th Biennial Meeting of the European Society for Immunodeficiencies (ESID 2018), held in Lisbon, Portugal|24-27, October 2018.

Ms. Puja Soni Ph.D. student at Department of Haemostasis and Thrombosis, was awarded Best oral presentation award at Quarterly meeting of MHG-2018, held on 9th September 2018

Dr. Rucha Patil, Department of Haemostasis and Thrombosis, was awarded Best Oral presentation award- third prize at the 59th National Annual Conference- Haematocon 2018, Indian Society of Haematology and Blood Tansfusion, Kochi held from October 25-28, 2018

Dr. Swati Kulkarni, Department of Transfusion Medicine was awarded Best Oral presentation award - First prize at 7th Annual conference of Indian Society of Transfusion Medicine Kochi on 23rd-25th November, 2018

Dr. Swati Kulkarni received **"Award of Excellence" for extraordinary contribution in the field of Transfusion Medicine** at 43rd Annual Conference of Indian Society of Blood Transfusion and Immunohaematology- Transcon 2018 held at Vishakhapatnam from 26th to 28th October, 2018.

Two day symposium and one day flowcytometry workshop on diagnosis of Primary immunodeficiency disorders (PID) was organized by Pediatric Immunology and leukocyte biology department at National Institute of Immunohaematology from 5-7th September. Symposium was organized for creating awareness about PID disorders, their diagnosis and management and a total 60 clinicians, pediatricians, and residents participated. The workshop focused on understanding the application of flow cytometry in diagnosis of PID and hands on training was provided to 15 participants including technicians, pediatricians, clinicians, flow cytometer users and researchers.

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