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Chronic Granulomatous Disease; Experience of a Rare Primary Immunodeficiency from a Tertiary Care Center in Pakistan

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ABSTRACT

Objective: To determine the clinical parameters, consanguinity of parents, previous family history of primary immunodeficiency (PID) and Neutrophil oxidative index (NOI) of Dihydrorhodamine (DHR) assay in chronic granulomatous disease (CGD).

Study Design: Cross-sectional study.

Place and Duration of Study: Immunology Department, Armed Forces Institute of Pathology, Rawalpindi, Pakistan, from Jan 2015 to Dec 2020.

Methodology: A total of 18 patients with chronic granulomatous disease were diagnosed over six years by Dihydrorhodamine assay on flow cytometry, in our institute.

Results: The mean age of patients at the time of symptoms was 4.0±3.01 months, while diagnosis was made at the mean age of 42.02±46.00 months. In 6 years, 13(72%) males and 5(28%) females were diagnosed with chronic granulomatous disease by DHR assay on flow cytometry. Parents of fifteen patients (83%) had consanguineous marriages, and 8(44%) patients had positive family history of PIDs. DHR assay showed two mothers had carrier states. The commonest clinical presentation noted was recurrent respiratory tract infections in 16 (89%) patients, repeated abscesses were seen in 13 (72%) and 5(28%) patients had a history of tuberculosis.

Conclusion: Chronic granulomatous disease should be suspected in patients with repeated chest infections (especially with catalase-positive organisms), abscesses, diarrhoea and death of siblings at an early age with undiagnosed PID.

Keywords: Chronic Abscess, Granulomatous Disease, Dihydrorhodamine, Neutrophil oxidative index

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INTRODUCTION

Chronic granulomatous disease (CGD) is a rare inherited primary immunodeficiency of the innate immune system caused by gene mutations encoding components to phagocyte oxidase enzyme complex.[1] Chronic granulomatous disease is a rare primary immunodeficiency, with males predominant, and the incidence of the disease varies in different populations.[2] In the USA (1 in 200,000), in Europe (1 in 250,000), in Iran and Morocco (1.5 in 100,000), the general population.[3] Prophylactic antibiotics and antifungals significantly reduce the frequency of bacterial and fungal infections. Gamma interferon is approved by the FDA for the prevention of infections. Bone marrow transplant is the curative management. Gene therapy requires the services of reference centers.[4] CGD is characterized by defective phagocytic activity of neutrophils, macrophages and monocytes, making patients more prone to bacterial and fungal infections (especially caused by catalase-positive organisms).[5]

Chronic granulomatous disease patients suffer from chronic, severe and persistent bacterial and fungal infections, specifically with catalase-positive organisms, which is due to inherited defects in the NADPH oxidase system, which leads to poor production of free radicals and decreased synthesis of HOCL, which results in defective killing of microorganisms by phagocytes.[6] This leads to repeated stimulation of T cells, which accumulate to form granulomas, hence the name Granulomatous Disease.[7] Most of the CGD patients present before one year of age. The most common presenting features are pneumonia, abscesses (skin, tissue, organs), lymphadenitis, osteomyelitis, superficial skin infections (cellulitis/impetigo) and persistent diarrhoea.[8]

Different diagnostic investigations are available for chronic granulomatous disease. NBT assay is a simple screening test for CGD. Dihydrorhodamine assay is the gold standard diagnostic test for chronic

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granulomatous disease. However, few neutrophils possess normal oxidative burst activity in X-linked CGD carriers, so we get two peaks. A specific gene mutation is useful for establishing the genetic inheritance pattern and aids in family counselling. The chorionic villous biopsy can be performed to obtain DNA to diagnose the affected fetus.[9] The tests used for diagnosing the carrier of XL-CGD are the same as those used for diagnosing CGD and are performed using whole blood samples.[10]

Pakistan does not have a CGD or PID registry like most developing countries. However, it is expected to have a high incidence of CGD in Pakistan because of very common consanguineous marriages. Lack of awareness of CGD and non-availability of diagnostic facilities at tertiary care centres are key reasons for the delay in diagnosis and diagnostic delay leading to complications, mortality and morbidity in CGD patients. The rationale of this study was to document the 6-year-long experience with a subset of CGD patients from Pakistan, as only a few case reports have been published to date. Data available on common clinical presentations, time of onset of symptoms, average delay in diagnosis, mode of inheritance, positive family history of PID and consanguinity of parents is scanty.

METHODOLOGY

The cross-sectional study was conducted at the Department of Immunology, Armed Forces Institute of Pathology, Rawalpindi, Pakistan, from January 2015 to December 2020. Institutional Review Board approved the study (IERB ltr No: 43)

Inclusion Criteria: Patients of either gender with chronic granulomatous disease were diagnosed over six years by Dihydrorhodamine assay on flow cytometry were excluded.

Exclusion Criteria: Patients with normal DHR were excluded.

A total of 18 patients with chronic granulomatous disease were diagnosed over six years in our institute by DHR assay on flow cytometry by measuring the respiratory burst activity of neutrophils. After taking the complete history of patients from parents, including common symptoms (recurrent respiratory tract infections, recurrent abscesses, diarrhoea and tuberculosis), the onset of symptoms, family history of PID, consanguinity and general physical examination was undertaken. Informed consent was obtained from the patient's guardians. DHR assay was performed on suspected patients of CGD. Three ml blood samples were collected in an EDTA tube. The flow cytometry instrument FACS Canto II was used to assess the neutrophil function after stimulation with phorbol myristate acetate (PMA) and mixing with DHR. Patients with CGD were only included in the study, and patients with normal DHR assay were excluded. This study has the limitation of genetic confirmation through NGS, which is unavailable in Pakistan.

The data was analyzed using the Statistical Package for Social Sciences (SPSS) version 25.0. Quantitative variables were expressed as Mean±SD and qualitative variables were expressed as frequency and percentages.

RESULTS

Eighteen patients were diagnosed with CGD over six years by DHR assay on flow cytometry, including 13 (72%) males and 5 (28%) females. Fifteen (83%) parents had consanguineous marriages, and 8 (44%) patients had a positive family history of PIDs. The mean age of patients at the time of symptoms was 4.0±3.01 months, while diagnosis was made at the mean age of 42.02±46.00 months. CGD presented with different clinical symptoms, including repeated respiratory tract infections in 16(89%), repeated abscesses in 13(72%), frequent diarrhoea in 9(50%), failure to thrive in 6(33%), history of tuberculosis in 5(28%), mouth ulcers in (22%) and lymphadenitis in 2(11%) while no patient presented with history of liver abscess or osteomyelitis (Figure-1).

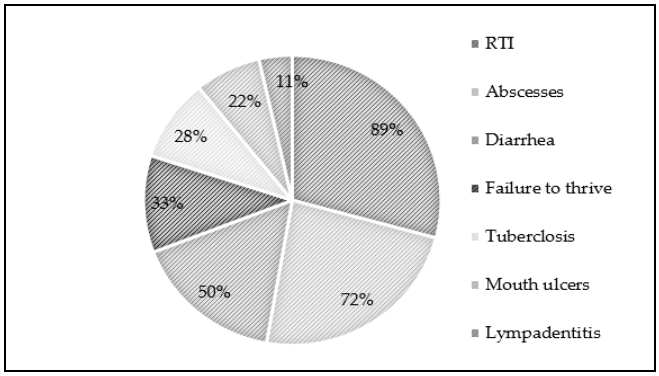

Figure-1: Predominant Clinical Manifestations in Children with CGD (n=18)

One patient was diagnosed on the second day of life because of the previous history of deaths of two male siblings within the first year of life.

The Neutrophil Oxidative Index (NOI) of the DHR assay was also analyzed for channel shift, and 17

cases had a NOI of <5 where, whereas one patient had a NOI of 20. DHR assay of mothers was also analyzed for carrier state, and we found that two mothers had two peaks of neutrophils (stimulated and unstimulated) in the histogram (Figure-2). After analysis of mothers' DHR assays, we concluded that most cases were of autosomal recessive inheritance patterns in our community.

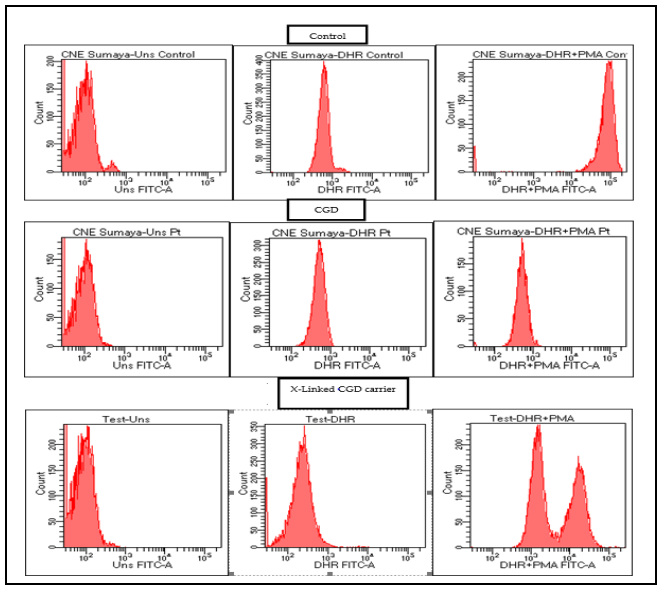

Figure-2: Control Shows Shift of Neutrophil Channel to the right upon Stimulation with PMA, Suggestive of good Respiratory Burst Activity (n=18)

DISCUSSION

Over six years, our institute has diagnosed 18 cases of CGD, one of the rare defects of PIDs. Most patients who visited our immunodeficiency clinic were from Punjab, Khyber Pakhtunkhwa and Gilgit-Baltistan province because of the location of this institute. This study provided information about differences in this rare disease in Pakistan, Asia, and other parts of the world, as this study has yet to be conducted on a large scale in the Pakistani population.

In CGD patients, minimum or no channel shift to the right after PMA stimulation confirms the absence of respiratory burst activity in the neutrophils. Half of the neutrophils in X-linked CGD carriers possess normal oxidative burst activity, so two peaks are observed on the histogram.[11] A USA study by Marciano *et al*. showed lung infection in 87%, abscesses in 32% and lymphadenitis in 25% of CGD patients.[12] A study in Iran by Mortaz *et al*. showed symptoms of lymphadenitis (68%), abscesses (59%), pneumonia (50%), osteomyelitis (27%) and mouth ulcers as prominent features.[13] Two previous studies on CGD patients described lymph-adenopathy, chest infections, abscesses, diarrhoea and failure to thrive.[14,15] A study conducted by Rawat *et al*. in India showed that chest infections in (72%), lymphadenitis in (32%), abscesses in (37%) and osteo-myelitis in (9%) of patients who presented with CGD.[16] In contrast, our study showed respiratory infections (89%), abscesses (72%), diarrhoea (50%), failure to thrive (33%), history of tuberculosis (28%), mouth ulcers (22%), lymphadenitis (11%) and no patient presented with a history of liver abscess or osteomyelitis.

A large single-centre experience in the USA showed male predominance with 70% cases of X-linked inheritance pattern.[17] In contrast, an Iranian study showed only 16% of cases of X-linked inheritance pattern while most cases were also male but of autosomal recessive inheritance pattern.[13] Another study by Koker *et al*. in Turkey showed 38% of X-linked inheritance patterns.[14] Our study showed 72% of male patients, but most cases were of auto-somal recessive inheritance patterns, which is in agreement with the Iranian population. Pakistani and Iranian populations exhibit more autosomal recessive patterns because consanguineous marriages are common in Pakistan and Iran.

A study conducted by Bortoletto *et al*. in the USA mentioned three years of mean age of diagnosis of CGD patients.[17] An Iranian study observed that the mean diagnosis was 5.5 years, whereas our study showed a mean age of diagnosis of 3.5 years. The delay in diagnosis in the Pakistani and Irani populations may be due to a need for more awareness and diagnostic facilities in all parts of the country.

A study conducted in the USA mentioned that 44% of CGD patients had a previous history of tuberculosis.[18] A study in India showed that about 19% of CGD patients had mycobacterial infections,[16] whereas our study showed 28% of patients who had tuberculosis in the past. Tuberculosis is endemic in our country, and children with tuberculosis, along with warning signs of primary immunodeficiency, must be ruled out for CGD as mycobacterium tuberculosis is a catalase-positive organism. BCG is contraindicated in patients suspected of CGD. There is a need to focus on awareness of the disease and genetic counselling of the family with a CGD child. Prenatal testing must be conducted in female carriers by PCR after getting a sample through chronic villous sampling. DHR can be

performed on peripheral blood collected from the fetus after 18 weeks of gestation.

CONCLUSION

Chronic Granulomatous Disease (CGD is the third most common primary immunodeficiency in Pakistan after severe combined immunodeficiency and antibody deficiency, respectively. Children with fever, repeated chest infections, abscesses and a history of tuberculosis must be ruled out for chronic granulomatous disease. DHR is the gold standard test for the diagnosis of CGD. A blood culture may also be helpful for diagnosis if it shows the growth of catalase-positive organisms.

Conflict of Interest: None.

Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

MH & HNT: Data acquisition, data analysis, drafting the manuscript, critical review, approval of the final version to be published.

DA & MA: Study design, data interpretation, drafting the manuscript, critical review, approval of the final version to be published.

YI & EQ: Conception, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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