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ORIGINAL ARTICLE

Chronic granulomatous disease: Review of a cohort of Egyptian patients

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Abstract
Background: Chronic granulomatous disease (CGD) is an inherited disease that results from a defect in the phagocytic cells of the immune system. It is caused by defects in one of the major subunits of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase complex. The clinical presentations of CGD patients are heterogeneous.
Objectives: This is the first report from Egypt discussing clinical and laboratory data of twenty-nine patients (from 26 families) with CGD from a single tertiary referral centre.
Results: There were twenty male and nine female patients. The consanguinity rate was 76% (19/25). Their age of diagnosis ranged from 2 to 168 months with a mean of 52.8 months ± 49.6 SD. The most common manifestations were abscesses in 79.3% (deep organ abscesses in 37.9% of patients), followed by pneumonia in 75.8% and gastrointestinal symptoms in 27.5%. Rare but fatal complications were also reported among patients as one patient developed haemophagocytic lymphohistiocytosis (HLH) syndrome.

Although X linked-CGD universally constitutes the most common pattern of inheritance; only 6 of our patients 6/25 (24%) belonged to this group with a Stimulation Index (SI) of 1–5, and confirmed by carrier pattern of their mothers. Mothers were not available for testing in four male children. Nineteen patients (76%) had autosomal recessive patterns; ten males and nine females patients based on having abnormal SI, positive history of consanguinity and their mothers showing normal SI.
Conclusion: Increasing the awareness of physicians about symptoms of CGD may lead to earlier diagnosis of the disease, thus enhancing proper management and better quality of life.

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Introduction

Chronic granulomatous disease (CGD) is a primary immune deficiency disease that results from an inherited defect in the phagocytic cells of the immune system.[1] The disease occurs due to mutations of the genes that encode the components of nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase) which is responsible for transfer of electrons from NADPH in the cytosol across the phagosomal membrane to reduce oxygen to super oxide anion and deliver protons necessary for dismutation that produce hydrogen peroxide. The produced reactive oxygen intermediates (ROI) damage the phagocytosed microorganisms.[2]

The enzyme is formed of five subunits: gp*91*^{phox} (*CYBB*) and p*22*^{phox} (*CYBA*) which are integral membrane proteins that form the flavocytochrome b^{588} (the electron transport centre of the enzyme)[3] and p47$_{phox}$; neutrophil cytosolic factor 1(*NCF1*), p*67*^{phox}; neutrophil cytosolic factor 2(*NCF2*), p*40*^{phox}; neutrophil cytosolic factor4 (*NCF4*) which are cytosolic components.[4,5]

The gene encoding gp91 is found on the X chromosome, while other genes are located on autosomes.[6] Thus inheritance can be X linked (about 70% of cases) or autosomal recessive (about 30% of cases).[7]

Autosomal forms are more reported in certain areas such as Turkey and Iran due to the high rate of consanguineous marriages in these countries.[4]

The prevalence of CGD varies from one in 1,000,000 to one in 160,000 individuals depending on the populations investigated and clinicians' awareness.[8] The incidence to be reported is expected to be higher among the Arab population (1:111,000).[9]

The clinical presentations of the patients are heterogeneous, however most of them present with development of severe and recurrent bacterial and fungal infections. Most of the patients suffer from pneumonia, lymphadenitis, hepatosplenomegaly and abscesses.[1] However, diarrhoea and sepsis syndromes may also be a presenting feature and CGD may be misdiagnosed as Crohn's disease.[7] They may also develop granulomas and premature death.[1]

Laboratory diagnosis of CGD depends on the inability of phagocytes from affected individuals to produce a normal respiratory burst, this can be measured by direct measurement of superoxide production, super oxide dependent ferricytochrome c reduction, nitroblue tetrazolium test (NBT) and oxidative test dihydrorhodamine (DHR) by flow cytometry, which is progressively replacing other tests due to its higher reproducibility, objectiveness, rapidness and ability to detect X linked carriers.[1,5]

There is a paucity of data on CGD from developing countries especially the Middle East with the high consanguinity, and speculated higher incidence than reported. This study aimed at describing the characteristics of Egyptian children with CGD.

Patients and methods

Patients

The study was conducted in the Immunology Unit, Paediatric Department, Cairo University from 2012 through 2014. It was approved by the institutional review board and informed consents were obtained from children's guardians.

Among 117 patients screened for CGD, 29 patients were enrolled according to the following inclusion criteria suggestive of CGD (presentation with one or more of the following features): suppurative lymphadenitis, chronic diarrhoea, recurrent pneumonia, perirectal abscess and fistula, abscesses in the liver, spleen, lungs and/or brain, fungal infections, osteomyelitis, septicaemia and granulomas of the skin.

Patients underwent detailed clinical history taking and thorough examination with emphasis on their presenting symptoms, age at presentation, complications, outcome and detailed laboratory parameters.

Available family members were tested to determine the mode of inheritance whenever possible.

Diagnosis of CGD was based on flow cytometry Dihydrorhodmaine (DHR) 123 assay.[10,11]

One hundred age and sex matched healthy donors (attending for elective procedures e.g. tonsillectomy, circumcision) were included as a control group (mean age 60 months $\pm$ 15 SD), the mean fluorescence intensity (MFI) of the stimulated and non-stimulated granulocytes were determined to set the cut-off value for the stimulation index among the Egyptian population.

Dihydrorhodamine test

Peripheral blood was obtained from patients and healthy controls, one hundred microlitres of whole EDTA blood was diluted 1:10 with phosphate buffered saline (PBS) (Lonza, Belgium) in two test tubes, then 0.5 μl DHR 123(Sigma–Aldrich) at a concentration of 2.5 μg/ml was added to each tube.

After incubation for 15-min, an enzyme stimulant; Phorbol 12-myristate 13-acetate (PMA) (Sigma–Aldrich) was added to one tube marked stimulated at a concentration of 160 ng/ml and the tube was incubated for an additional 15 min at 37 °C in water bath.

At least 10,000 events were acquired on a CYTOMICS FC 500 Flow Cytometer (Beckman coulter, FL, USA).

The neutrophil stimulation index (SI) was calculated as the ratio of mean fluorescence intensity (MFI) of the stimulated cells to MFI in non-stimulated cells. The neutrophils were characterised by forward and side scatter properties.

In cases in which two distinct fluorescent populations were observed, MFI of each population was obtained and the SI for each population was calculated.

The flow cytometric analysis for 100 normal individuals was done (Fig. 1a) and the stimulation index of 70 was set as the cut-off value.

Immunophenotyping of peripheral blood lymphocytes and immunoglobulins level

Immunophenotyping of peripheral blood lymphocytes and immunoglobulins assay was performed for some patients to exclude other primary immunodeficiency disorders.

Serum immunoglobulin IgG, IgM, IgA were estimated by nephlometry. Complete blood count with the differential leucocytic count was done and lymphocyte subset were