Chronic Granulomatous Disease: detection of the defective proteins

Thesis

Submitted in Partial Fulfillment of MD Degree in Clinical and chemical pathology

Presented By

Mahitab Ahmed Abdelkawy

MSc. (Clinical and chemical pathology)

Faculty of medicine , cairo university

Supervised by

Prof. Dr.Taghrid Mohamed Gaafar

Professor of clinical and chemical pathology

Faculty of medicine , cairo university

Prof. Dr. Aisha Elmarsafy

Professor of Pediatrics

Faculty of medicine, Cairo university

Dr. Safa Sayed Meshaal

Lecturer of Clinical and Chemical pathology

Faculty of medicine, Cairo university

Dr. Rabab El Sayed El Hawary

Lecturer of Clinical and Chemical pathology

Faculty of medicine, Cairo university

Faculty of Medicine

Cairo University

2015

Acknowledgement

First and foremost, I would like to thank **GOD** for his infinite blessings till the completion of this work.

I would like to express my deepest gratitude and appreciation to **Prof. Dr. Tagrid Gaafar** Professor of Clinical and Chemical Pathology, Faculty of Medicine, Cairo University, for her ongoing constructive guidance, support and generous contribution to this work.

I would also want to thank **Prof. Aisha Mahmoud Khairy El Marsfy**, Professor of Pediatrics, Faculty of Medicine, Cairo University, for her great support, kind advice.

I am and will always be gratefull to **Dr. Safa Meshaal, & Dr. Rabab El-hawary,** Lecturers of Clinical and Chemical Pathology, Faculty of Medicine, Cairo University, for their endless ongoing support and encouragement, their generous time allocation as well as valuable suggestions and contributions to this work and above all their supportive words and kind advice that were always there whenever needed.

I would like to thank the staff members and my fellow colleagues at the Clinical and Chemical Pathology Department, Faculty of Medicine, Cairo University for all the support and help they offered me.

Also, I would like to express my gratitude to my fellow colleagues at the Pediatric Department, Faculty of Medicine, Cairo University for all their helpful advice and support.

Last but definitely not least, I could never be grateful enough to my beloved family and my supportive understanding husband for all their moral support and useful advice and guidance throughout this work.

List of Figures

List of Tables

List of Abbreviations

Abbreviation	Meaning
• ADA	Adenosine deaminase;
• AR	Autosomal recessive
• ATM	Ataxia telangiectasia mutated gene;
• BCG	BacilleCalmette-Guérin.
• BLM	Bloom syndrome gene;
• BMT	Bone marrow transplantation.
• Btk	Bruton tyrosine kinase.
• CBC	Complete blood picture.
• CGD	Chronic granulomatous disease.
• CID	Combined immunodeficiency.
• CRP	C-reactive protein.
• CT	Computed tomography.
• CVID	Common variable immunodeficiency.
• DCFH-DA	Dichlorofluoresceindiacetate.
• DGGE	Denaturing gradient gel electrophoresis.
• dHPLC	denaturing high-performance liquid chromatography.
• DHR	Dihydrorhodamine.

• EDTA	Ethylene diamine tetra-acetic acid.
• ESR	Erythrocyte sedimentation rate.
• FITC	Fluorescein isothiocyanate.
• GDP	Guanosinediphosphate.
• GTP	Guanosine-5'-triphosphate.
• H_2O_2	Hydrogen peroxide.
• HB	Hemoglobin
• HD	Heteroduplex
• HOCl	Hypochlorous acid.
• IFN-γ	Interferon-gamma.
• Ig	Immunoglobulin .
• IL	Interleukin
• LAD	Leukocyte adhesion deficiency;
• mAb	Monoclonal antibodies.
• MCH	Mean corpuscular Hemoglobin.
• MCV	Mean corpuscular volume.
• MHC	Major histocompatibility complex;
• NADPH	Nicotinamide adenine dinucleotide phosphate.
• NBS	Nijmegan breakage syndrome;
• NBT	NitroblueTetrazolium.
• NIH	National institute of health.

• NK	Natural killer
• O_2^-	Superoxide anion.
• OH	Hydroxyl radical.
• PBS	Phosphate buffer saline.
• PE	Phycoerythrin.
• Phox	Phagocyticoxidase.
• PID	Primary immunodeficiency.
• PMA	Phorbolmyristate acetate.
• PMNL	Polymorphoneuclear leucocytes.
• RAG	Recombination activating gene.
• Rap1	Ras-related protein 1.
• ROS	Reactive oxygen species.
• SAP	Signaling lymphocyte activation molecule
• SD	Standard deviation
• SCID	Severe combined immunodeficiency.
• SI	Stimulation index
• SLAM	SAP -associated adaptor protein;
• SSCP	Single-stranded conformation polymorphism.
• TCR	T-cell receptor.
• WASP	Wiskott-Aldrich syndrome protein;
• WBCs	White blood cells.

• XLA	X-linked agammaglobulinemia.

Abstract

Introduction

Chronic granulomatous disease (CGD) is an inherited disorder of the NADPH oxidase characterized by severe bacterial and fungal infections and excessive inflammation. CGD is caused by a defect in the burst of oxygen consumption that normally accompanies phagocytosis in myeloid cells. The enzyme that catalyzes the respiratory burst, the leukocyte NADPH oxidase, consists of subunits, four of which are important for CGD:gp91phox and p22phox, located in PMNL membranes, as well as two cytosolic oxidase components, p47phox and p67phox. CGD is caused by a defect in any of these four components.

Aim of work

The aim is to identify the defective proteins implicated in the pathogenesis of the CGD in Egyptian families, to study the mode of inheritance in such families.

Subjects and methods

Our study included 28 CGD patients (15 males and 13 females) with ages ranging from 2 months to 14years. Patients with CGD diagnosed by DHR test (dihydrorhodamine) together with the clinical picture of the disease.

Anticoagulated (EDTA) samples were stained and analyzed by flow cytometry.

Results

The present study revealed deficiency in p22phox in 13 patients (46.4%) followed by p47phox in 9 patients (32.1%), gp91phox in 4 patients (14.3%) and p67phox in 2 patients (7.1%).

Conclusion

The analysis of the defective proteins by flow cytometry will be the optimum solution for confirming the diagnosis, especially in laboratories that carry the DHR test and are already equipped with flow cytometry.

Keywords: Chronic granulomatous disease (CGD), Flow cytometry.

Introduction

Chronic granulomatous disease (CGD) is an inherited disorder of the NADPH oxidase characterized by severe bacterial and fungal infections and excessive inflammation. CGD was first described in the 1950s as a fatal granulomatous disease of childhood ***(Segal et al, 2012)***.

CGD is caused by a defect in the burst of oxygen consumption that normally accompanies phagocytosis in myeloid cells (i.e. neutrophils, eosinophils, monocytes, and macrophages). The ‘‘respiratory burst’’ involves the catalytic conversion of molecular oxygen to the oxygen free-radical superoxide (O_2^-), which in turn gives rise to hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), and hydroxyl radical (OH). These oxygen derivatives play a critical role in the killing of certain pathogenic bacteria and fungi. As a result of the failure to mount a respiratory burst in their phagocytes, the majority of CGD patients suffer from severe recurrent infections and also from dysregulated Th-17-lymphocyte-controlled inflammation.

Therefore, CGD patients can develop diffuse granulomas that can become sufficiently large to cause obstructive or painful symptoms in the esophagus, stomach, ureters or urinary bladder, or dysfunctional disorders secondary to extensive fibrosis of the different systems (pulmonary, gastrointestinal, genitourinary, central nervous system) ***(Van der Berg et al., 2009)***.

The enzyme that catalyzes the respiratory burst, the leukocyte NADPH oxidase, consists of subunits, four of which are important for CGD (designated phox for phagocyte oxidase): gp91phox (or Nox2) and p22phox, located in membranes, as well as two cytosolic oxidase components, p47phox and p67phox. CGD is caused by a defect in any of these four components. Mutations in the

gp91phox gene (CYBB on chromosome Xp21.1) cause the X-linked recessive form of the disease that affects the majority of CGD patients (70%). The remaining 30% of cases has inherited the disease in an autosomal recessive (AR) manner, in which males and females are equally affected. These patients have mutations in the genes encoding p47phox (NCF1 on chromosome 7q11.23), p67phox (NCF2 on chromosome 1q25), or p22phox (CYBA on chromosome 16q24) ***(Roesler et al, 2000).***

Due to the high rate of consanguinity in North Africa and Egypt, the occurrence of the autosomal recessive form of the disease is more likely. Unfortunately, records for the incidence of the disease and its genetic basis in Egypt or North Africa are not available. A study conducted in Tunisia 2006 would support this assumption. Elkares et al. conducted their study on 15 Tunisian patients from 14 unrelated families. Haplotype analyses and homozygosity mapping with microsatellite markers around known CGD genes assigned the genetic defect to p47phox in four patients, to p67phox in four patients and to p22 phox in two patients **(Elkares et al., 2006).**

Aim of work

The aim of the study is to identify the defective proteins implicated in the pathogenesis of the CGD in Egyptian families, to study the mode of inheritance in such families.

Primary immunodeficiency disorder

Primary immunodeficiency disorder (PID) refers to a heterogeneous group of disorders characterized by poor or absent function in one or more components of the immune system. Over 130 different disorders have been identified to date, with new disorders continually being recognized ***(Bousfiha et al., 2010)***. Most PIDs result from inherited defects in immune system development and/or function; however, acquired forms have also been described ***(Notarangelo ,2010)***.

It is important to note that PIDs are distinct from secondary immunodeficiencies that may result from other causes, such as viral or bacterial infections, malnutrition, or treatment with drugs that induce immunosuppression. With the exception of immunoglobulin A (IgA) deficiency, PIDs are rare; the estimated prevalence of these disorders in the United States is approximately 1 in 1200 live births. IgA deficiency is the most common PID, occurring in approximately 1 in 300 to 1 in 500 persons ***(Boyle and Buckley, 2007)***.

The clinical presentation of PIDs is highly variable; however, most disorders involve increased susceptibility to infection. In fact, many PIDs present as "routine" infections (often of the sinuses, ears and lungs) and, therefore, may go undetected in the primary-care setting. The accurate and timely diagnosis of these disorders requires a high index of suspicion and specialized testing. Therefore, consultation with a clinical immunologist who is experienced in the evaluation and management of immunodeficiencies is essential, since early diagnosis and treatment are critical for preventing significant disease-associated morbidity and improving patient outcomes ***(Bonilla et al., 2005; Shehata et al., 2010)***.

- **Classification:**

PIDs are broadly classified according to the component of the immune system that is primarily disrupted: adaptive or innate immunity

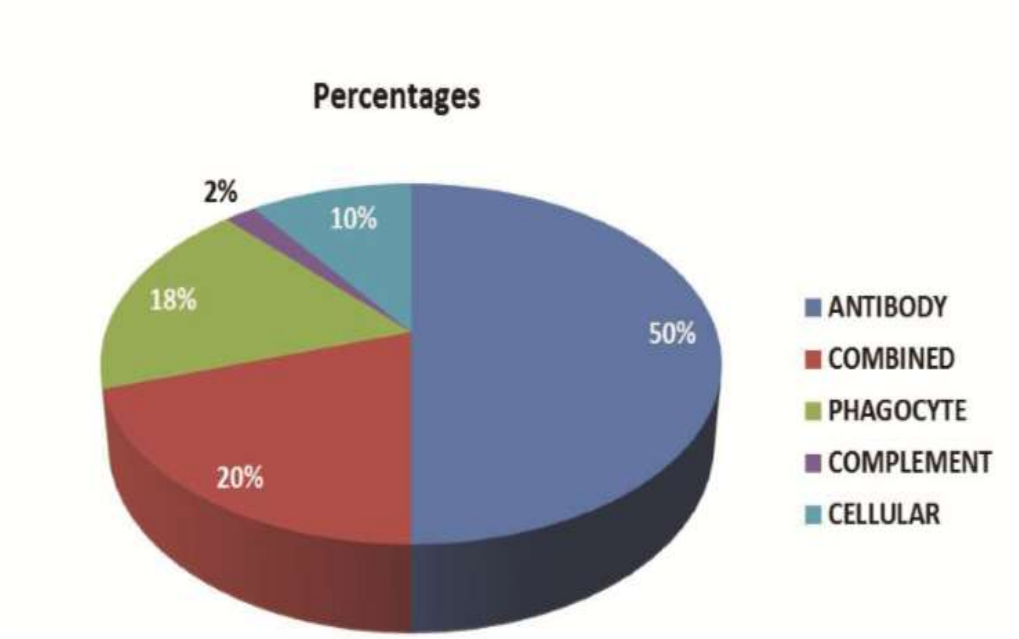

Figure (1): Distribution of Common Immunodeficiencies. Primary immune deficiencies can involve either the adaptive (T- and B- lymphocyte deficiencies) or the innate (phagocyte, complement or other defects) immune response. Of these, defects in phagocyte function constitute only about 18%, with the larger portion of the defects seen in the B cell/antibody and/or T cell components of immunity ***(Song et al., 2011)***.