شبكة المعلومات الجامعية

التوثيق الإلكتروني والميكروفيلم

بسم الله الرحمن الرحيم

HANAA ALY

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جامعة عين شمس

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قسم

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها
علي هذه الأقراص المدمجة قد أعدت دون أية تغيرات

يجب أن

تحفظ هذه الأقراص المدمجة بعيدا عن الغبار

HANAA ALY

INTRODUCTION

Primary immunodeficiency disorders (PIDs) are a group of genetic defects characterized by abnormalities of one or more components of the immune system. While there have been several advances in diagnosis, management, and research in the field of PIDs, they continue to remain underdiagnosed, especially in the less affluent countries. More than 300 genetically defined single-gene inborn errors of immunity are being recognized as a cause of PID ***(Bousfiha et al., 2015).***

The prevalence of PID varies depending on the type of immunodeficiency. While selective IgA deficiency is common (1 in 223 to 1 in 1000), other immunodeficiencies such as severe combined immunodeficiency (SCID) are fortunately rare (1 in 58,000). As many immunodeficiencies are continually being discovered, the exact prevalence is unknown, though it is estimated to be low ***(Kwan et al., 2014).***

Chronic granulomatous disease (CGD) is an innate, inherited, heterogeneous, immunodeficiency disorder that renders patients susceptible to recurrent, severe pyogenic bacterial and/or fungal infections and excessive hyperinflammatory responses with eventual granuloma formation and premature death ***(Roos and de Boer, 2014).***

Genetic defects of nicotinamide adenosine dinucleotide phosphate (NADPH) oxidase components are known to cause CGD. These genetic defects lead to significantly decreased reactive oxygen species (ROS) production, which plays a pivotal role in microbial killing. Mutations may affect one of

the five genes (CYBB, CYBA, NCF1, NCF2, and NCF4) that encode structural components of the NADPH oxidase protein ($gp91^{phox}$, $p22^{phox}$, $p47^{phox}$, $p67^{phox}$, and $p40^{phox}$, respectively). The $gp91^{phox}$ and $p22^{phox}$ proteins are located in the cell membrane and together form the flavocytochrome b558 of the NADPH oxidase. During phagocytosis, the cytosolic NADPH oxidase subunits $p47^{phox}$, $p67^{phox}$, and $p40^{phox}$ translocate to flavocytochrome b558 in the phagosomal membrane, inducing a conformational change that allows NADPH to donate electrons to molecular oxygen. This leads to the generation of superoxide in the phagosome, from which other ROS such as hydrogen peroxide may be formed ***(Roos et al., 2013).***

CGD affects males more often than females. In North American and European studies, approximately, two-thirds of individuals have the disorder. Defects in $gp91^{phox}$ are an X-linked recessive (XLR-CGD) inheritance and are found in approximately 70% of CGD patients, who have the most severe phenotype. The autosomal recessive forms of CGD (AR-CGD) are due to mutations in the CYBA gene on chromosome 16 (5% of patients), the NCF1 gene on chromosome 7 (20%), the NCF2 gene on chromosome 1 (5%), or the NCF4 gene on chromosome 22, only one patient reported ***(Matute et al., 2009).***

AIM OF THE WORK

To diagnose the X-linked type of CGD in a group of Egyptian children by detection of Cytochrome b β subunit (CYBB) gene expression by reverse transcription polymerase chain reaction (RT-PCR). We also aimed to find out if levels of CYBB gene expression are correlated with values of phagocytic lytic index as a cheaper screening method for CGD.

CHRONIC GRANULOMATOUS DISEASE

Primary immunodeficiency (PID) refers to a heterogeneous group of disorders characterized by poor or absent function in one or more components of the immune system which predisposes affected individuals to increased frequency and severity of infection. They are about 300 genetically inherited disorders according to the American Allergy, Asthma and Immunology Organization ***(Picard et al., 2015)***.

PID should be suspected in patients with recurrent sinus or ear infections or pneumonias within a one year period; failure to thrive; poor response to prolonged use of antibiotics; persistent thrush or skin abscesses; or a family history of PID ***(McCusker et al., 2018).***

It is important to note that PIDs are distinct from secondary immunodeficiencies that may result from other causes, such as viral infections [e.g., human immunodeficiency virus (HIV)] or bacterial infections, malnutrition, protein losing states (e.g., nephrotic syndrome and severe burns) which results in immunoglobulin (Ig) loss, or treatment with drugs that induce immunosuppression as immunosuppressive, anti−inflammatory, and biological drugs, particularly in the context of hematopoietic stem cell transplantation (HSCT) and organ transplantation, and in lymphoproliferative malignancy [e.g., multiple myeloma (MM), chronic lymphocytic leukemia (CLL) and lymphoma] ***(Srivastava and Wood, 2016).***

The International Union of Immunological Societies (IUIS) Expert Committee on Primary Immunodeficiency updated the classification of human PIDs into major groups:

(Bousfiha et al., 2020)

1- Immunodeficiencies affecting cellular and humoral immunity.

2- Combined immunodeficiencies with associated or syndromic features.

3- Predominantly antibody deficiencies.

4- Diseases of immune dysregulation.

5- Congenital defects of phagocyte number, function, or both.

6- Defects in Intrinsic and Innate Immunity.

7- Autoinflammatory disorders.

8- Complement deficiencies.

9- Bone marrow failure disorders.

10- Phenocopies of PID.

CGD is an inherited disorder of the innate immune system characterized by a defective oxidative burst of phagocytes and subsequent impairment of their microbicidal activity. Mutations in one of the NADPH oxidase (NOX) components may affect gene expression or function of this system, leading to the phenotype of CGD ***(Agudelo-Florez et al., 2004).***

I- Epidemiology of CGD:

A- Prevalence:

The prevalence rate of CGD varies among the population based on social, religious and cultural factors that influence birth rates and frequency of consanguinity. It is about 1 case per 160,000 individuals ***(Arnold and Heimall, 2017).***

Given the annual US birth cohort of nearly 4 million infants, approximately 20 children each year are born with CGD (prevalence 1:200,000). By comparison SCID occurs in approximately 40 live births per year in the United States. Estimates of the incidence of CGD in Europe and Asia are similar although some populations are affected more often, including the Arab population of Israel, in which the incidence is estimated to be 1.5 per 100,000 live births ***(Rider et al., 2018).***

In a review of a cohort of Egyptian CGD patients, the incidence of AR forms was found to be high (76%) attributed to the high consanguinity rate, while the incidence of the X-linked type was much lower (24%) ***(El-Hawary et al., 2016).***

In a retrospective epidemiologic population-based study done in Eastern Province of Saudi Arabia by ***Suliman et al. (2009),*** they found that the incidence rate of CGD was 5.2 cases per 100,000 person-year and was exclusively the autosomal recessive inherited rather than the X-linked inherited form.

B- Race:

Chronic granulomatous disease affects persons of all races ***(Roos and De Boer, 2014).***

C- Sex:

Approximately 80% of patients with CGD are males, because the main cause of the disease is a mutation in an X-linked gene. However, defects in autosomal genes may also underlie the disease and cause CGD in both males and females ***(Wolach et al., 2005).***

In Egypt, the prevalence of the AR forms was found to be higher because of the higher consanguinity rate (82%), and the prevalence of $p22^{phox}$ deficiency was (46%) while the prevalence of the X-linked type was much lower ***(El-Hawary et al., 2016).***

II- Clinical Manifestations of CGD:

A- Recurrent Infections:

Recurrent infections that do not respond to the usual antibiotic treatment is the typical presentation of CGD which may be bacterial or fungal, though some patients may present with failure to thrive, multiple granulomas, or inflammatory complications. The disease is usually diagnosed in infancy, childhood and sometimes in early adulthood depending on the extent of the respiratory burst affection ***(Antachopoulos et al., 2007).***

These infections are usually life threatening. Typical infections include purulent bacterial infections presenting as pneumonia, sinusitis or liver abscess, or necrotizing fungal infections of deep tissue or bone. Most common pathogens are Staphylococci and catalase positive bacteria, while the most

common fungal organisms are Aspergillus, Nocardia and Candida. Other less common bacteria include Burkholderia species and Mycobacterial species ***(Alsultan et al., 2006).***

Most of these patients present with systemic manifestations among which hepato-splenomegaly, osteomyelitis (particularly that caused by Aspergillus species), lung abscesses and empyema (as a complication of recurrent pneumonias), multiple granulomas, gastrointestinal manifestations as malabsorption, perianal abscesses and fistulae, oral ulceration, characteristic obstructive lesions associated with granulomatous infiltration ***(Marciano et al., 2004),*** hepatic, perihepatic abscesses and perirectal abscesses that can persist for years despite aggressive antimicrobial therapy and fastidious local care ***(Carnide et al., 2005).***

Many central nervous system (CNS) complications have been also encountered in patients with CGD. Brain abscess has been well described, Salmonella enterica, and Aspergillus were the most isolated causative organisms ***(Patiroglu et al., 2010).*** Other complications associated with CGD include CNS granulomatous disease and leptomeningeal, and focal brain infiltration by inflammatory macrophages ***(Turgut, 2010).***

B- Inflammatory Complications:

Not only the infections complicate CGD, but also inflammations were registered as complications in patients with CGD, and the most common were those affecting the gastrointestinal tract (GIT). Extensive GIT ulcerations, obstructions, inflammatory bowel disease (IBD)-like colitis

(Fig.1), multiple granulomas formation and lymphadenopathy were encountered ***(Chiriaco et al., 2016).***

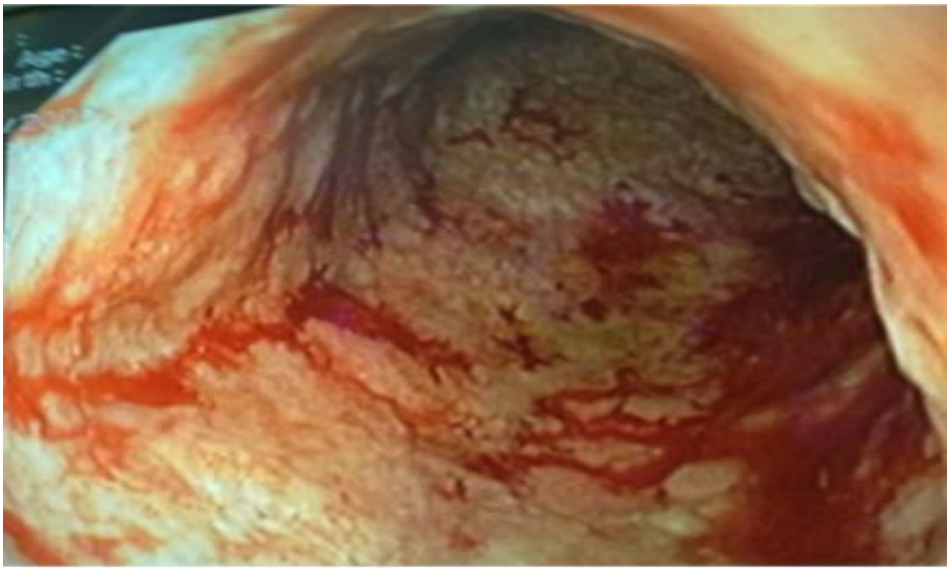

Figure (1): IBD-like colitis in a CGD patient.

Macroscopic appearance: colonic mucosa is hyperemic, edematous, congested with micro- and macro ulcerations; spontaneous bleeding ***(Chiriaco et al., 2016).***

III- Diagnosis of CGD:

The first and most important step in the diagnosis of CGD is raising the suspicion while the patient is presenting by recurrent severe infections in different sites with different organisms. Then diagnosis of CGD is established by demonstrating absent or markedly reduced oxidase activity in stimulated neutrophils. Screening for CGD is accomplished by nitroblue tetrazolium test (NBT) or by flow cytometry dihydrorhodamine (DHR) assay, then the diagnosis is established on the molecular genetic basis ***(Rezvani et al., 2005).*** The following diagram shows the approach for reaching the accurate diagnosis of CGD **(Fig. 2)** ***(Rossouw et al., 2018).***

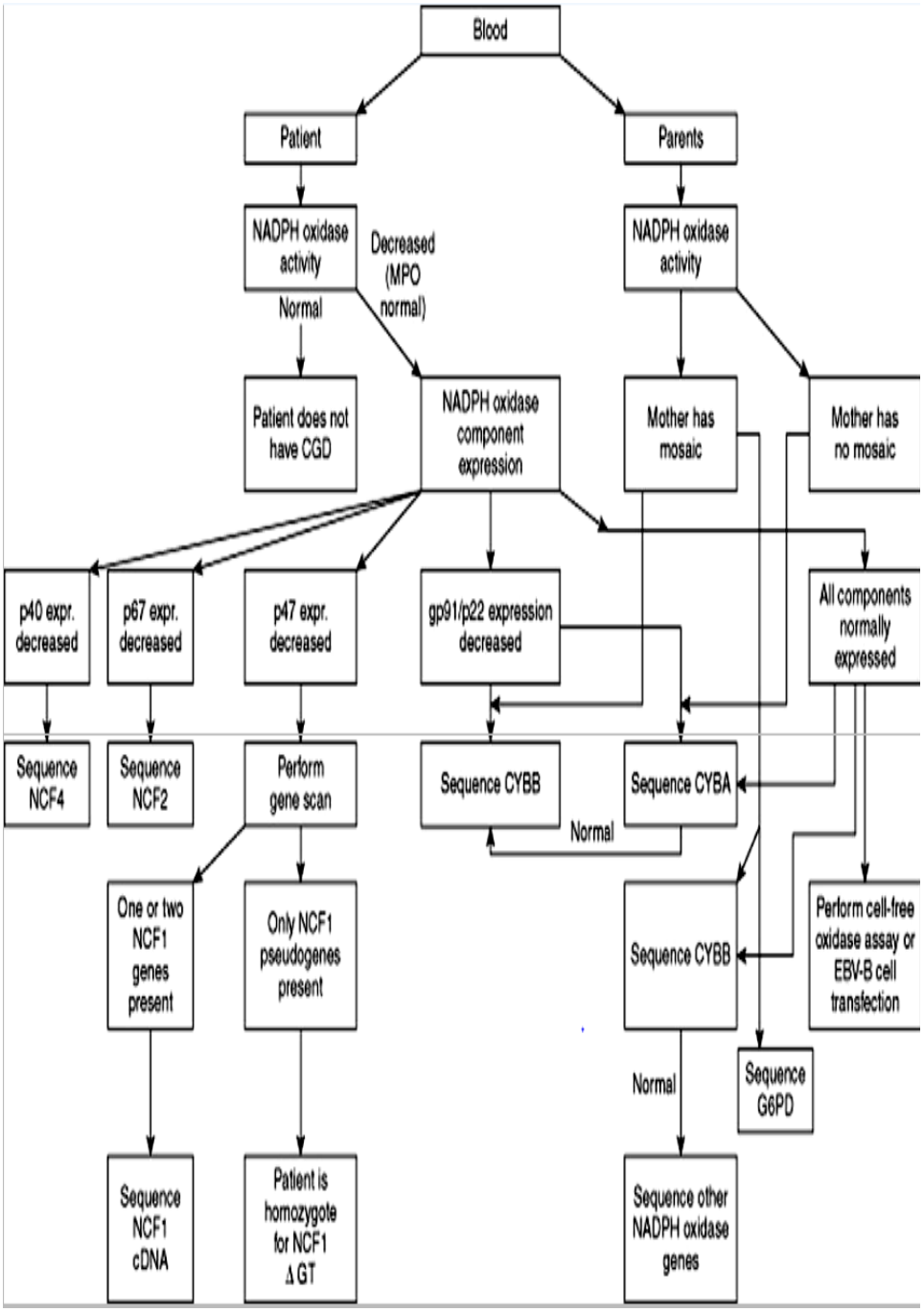

Figure (2): Approach for diagnosis of CGD ***(Rossouw et al., 2018).***

A- At the Cellular Level:

i- Nitroblue Tetrazolium Test (NBT):

NBT test has been used to measure superoxide generation. In this test, neutrophils are stimulated with phorbol myristate acetate (PMA) in the presence of NBT dye. After stimulation, the yellow-colored dye is reduced by the NADPH oxidase complex in normal neutrophils to formazan, which is a dark-blue or purple precipitate that is retained within the cell **(Fig. 3).** The cells then are visually analyzed by microscopy for this color change. The majority of normal neutrophils will be blue or dark in color, but neutrophils that lack a functional NADPH complex fail to change color. The NBT test sometimes can diagnose X-linked female carriers, because they should have a mixed population of positive and negative cells as a result of the random X-chromosome inactivation known as lyonization. However, the NBT test is semiquantitative, and often, carriers of X-linked mutations or the AR mutations are not well identified. In addition to its semiquantitative nature, it is a very subjective test, its variability depends on lab personnel experience, and it may yield false positive or false negative results ***(Roos and de Boer, 2014).***

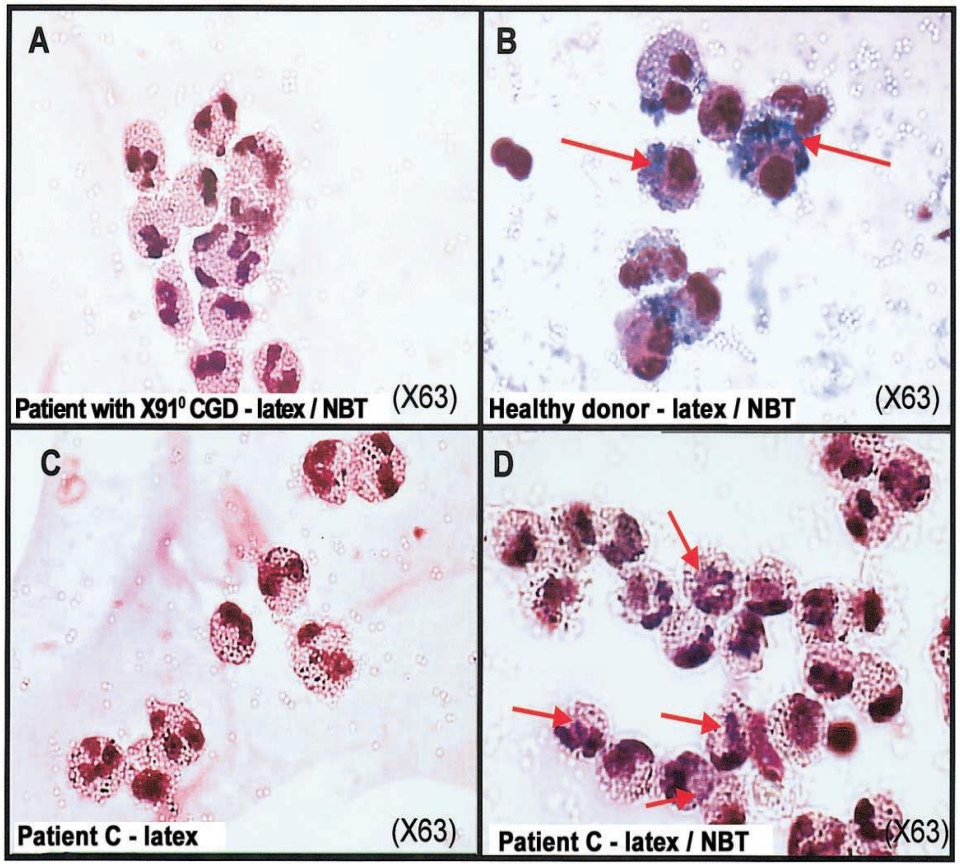

Figure (3): Normal and abnormal NBT test.

It shows a diagrammatic NBT test: the positive result with the purple formazan deposits and the negative results with the yellow deposits ***(Stasia et al., 2003)***

ii- Dihydrorhodamine 1,2,3 (DHR) Flow Cytometric Assay:

The DHR test has then widely replaced the NBT in the diagnosis of CGD. It can be performed quickly using small volumes of anti-coagulated blood. The interpretation of the DHR test results is less subjective, and the test is able to differentiate between X-linked CGD patients and carriers. Although the DHR test has become the standard method for diagnosis of CGD, the test has some limitations. The relatively short lifespan of neutrophils implicates that samples must arrive