3. J Project congress Dedeman Hotel, 4-7 December 2019, Konya, Turkey

Chronic granulomatous disease and diagnostic algorithm

Prof Dr. Mustafa Yavuz Köker

Erciyes University, Medical School "Department of Immunology Kayseri, Turkey

Özet

Kronik granülomatoz hastalık (KGH) görülme sıklığı 1/100-200 bin doğum olan, nötrofil fonksiyonun bozukluğu ile karakterize bir nadir primer immün yetmezlik hastalığıdır. En sık görülen fenotip X-bağlı formdur ve NADPH oksidaz kompleksinin en büyük bileşeni olan gp91-phox ünitesi eksiktir. KGH ın diğer dört formu otozomal resesif (OR) karakterli olup (p22-phox, p47-phox p67-phox, ve p40-phox) birisinin eksikliği sonucu oluşur. X-KGH dünyada KGH hastalarının %65'ini oluştururken, ülkemiz ve bölgemizde bu oran %40 düzeyindedir. Diğer taraftan akraba-arası evlilikler kaynaklı doğumların fazla olması nedeniyle OR-KGH yakın coğrafyamızda daha sık (%50-60) ortaya çıkmaktadır.

Introduction

Chronic granulomatous disease (CGD) a primary immunodeficiency and characterized with inability to killing microorganisms by the neutrophils and phagocytes. It is rare neutrophil function disorder. Although the incidence is at 1 / 100-200.000 births, it may vary in different country. One of the component of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase complex is defective in this disorder. Most of the mutation is observed in *CYBB* gene, which encodes gp91-phox, and result in X-linked CGD. The other four forms of autosomal recessive (AR) is characterized by defect in *CYBA, NCF1, NCF2, NCF4* genes encoded proteins (p22-phox , p47-phox, p67-phox and p40-phox). While X-CGD cases 65% of patients in western country, this rate is around 40% in our country and region. On the other hand, AR-CGD occurs more frequently (60%) in our nearby geography due to the high births between consanguineous marriages (1).

In the basic pathology of CGD, oxidase complex cannot occur and superoxide anion ($-O_2$) cannot be formed enough. In this case, the pH level in the phagosome cannot decrease to the level that will enable the activation of lytic enzymes (pH 4.5). Also, catalase produced by intracellular microorganisms neutralizes some of the existing oxygen radicals and raises the pH level (pH 6↑). In these cases, the activation of lytic enzymes in the phagosome can not be fully achieved and microorganism destruction cannot take place. Infections characterized by the inability to kill Staf aureus, Burkholderia cepacia, Serratia marcescens, Nokardia and some fungi (Aspergillus), especially catalase-positive microorganisms, are observed. The system's inability to cope with especially inoculation and exposure situations creates clinical presentations accompanied by deep tissue infections.

Clinical presentation: Recurrent bacterial and fungal infections that involve the lungs, lymph nodes, liver and other visceral organs. Granuloma formation may occur in the tissues of the infection area, depending on the prolongation of infections in KGH. Hypergammaglobulinemia (IgG ↑), hepato-splenomegaly and enteritis are frequently observed. The X-CGD form, which is seen especially in boys, usually appears before the age of 1 and is more severe. Mild phenotypes, especially autosomal recessive form (p47-phox deficiency) may occur at a later age. The patient and healthy neutrophil population, which constitute the carrier character in mothers in X-linked form, are observed. Mild clinical signs can be observed in approximately

50% of mothers. In some rare cases, CGD symptoms can be observed in women due to x-ch inactivation.

Diagnosis and monitoring

KGH is very important for early diagnosis, prevention of permanent tissue damage and maintenance of comfortable life. The first test used for this purpose is the NBT smear test. With the flow cytometry and DHR test becoming widespread in the last two decades, an increase in the number of cases has been observed. Today, in the CGD diagnostic tests, the laboratory diagnosis of the disease is confirmed with the DHR 123 test at the first stage. In the DHR test, neutrophil stimulation is performed via the protein kinase C (PKC) pathway via phorbol myristat acetate (PMA) (2). Live neutrophils without stimulation are interpreted in favor of CGD. In addition, maternal carriage can be determined in X-KGH by DHR test, so phenotypic distinction can be made roughly. In the DHR test, the stimulation index (SI) is used to show the amount of neutrophil stimulation and how many times the neutrophil activity increases. While normally an increase of 70-100 times is expected, in some cases, values between SI: 3-10 can be taken depending on the residual activity. These cases are investigated for variant forms or p47-phox deficiency. In addition, the expression of intracellular and surface molecules forming NADPH oxidase is measured by flow cytometry with specific antibodies and subgroup determination is performed. The diagram we use in the diagnosis studies of CGD is attached (figure 1).

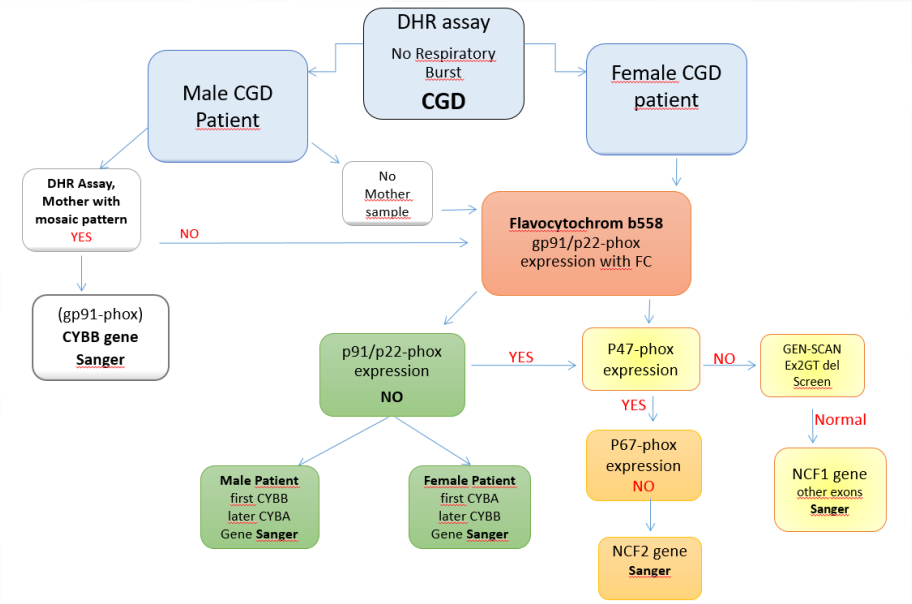

Fig 1. Diagnostic Algorithm Applied in Chronic Granulomatosis Disease

In the second stage of the diagnosis of CGD, genetic diagnosis is achieved by mutation scanning with gene expression and Sanger sequence analysis after DNA extraction from patient samples. In the molecular diagnosis of CGD, scans have been used in recent years with the next generation sequencing (NGS) method instead of Sanger sequence analysis. Gene-scan analysis method is preferred in *NCF1* gene mutation scans, where residual activities are frequently seen, because of the fact that "pseudogene" is found and the detection of "hot spot" mutations in this gene is faster and cheaper (2).

With the developing laboratory infrastructure, it has been understood that there are intermediate forms (p40-phox defect and Eros defect) with low NADPH oxidase activity in recent years. This situation raised new discussions in the diagnosis of CGD and the diagnostic studies have become complicated.

Prophylaxis including antibiotics and antifungal should be applied lifelong after the diagnosis. It can be added to the interferon-gamma treatment protocol during periods of serious infection. Informing the family after genetic diagnosis and providing counseling for new child requests is a preventive approach that will reduce families' having new sick children.
Treatment approaches

KGH clinical follow-up and annual controls are very important. Sufficient doses of regular antibiotics / antifungal prophylaxis applied in the follow-up of KGH can provide long-term survival without infection. Successful results have been achieved in recent years, with the opportunity to access bone marrow transplantation. Especially transplants made from 10/10 compatible donors reach long survival periods.

Other studies on NADPH oxidase
The NADPH oxidase enzyme has a lot to do with many areas of life. It is known that especially due to overwork of the NADPH oxidase enzyme and excessive destruction of exogenous antigens taken into the cell, carcinogenic products that are effective in the development of cancer (lung cancer) can occur, some drugs and chemotherapeutic agents interact with NADPH oxidase enzyme. It is thought that the autotoxic effects of some chemotherapeutic agents are associated with NADPH oxidase, and that antibiotics such as aminoglycosides cause ototoxic hearing loss caused by tissue damage due to NOX3 (NADPH oxidase tissue isomer) over activity in cochlea "hair" cells (4,5). In addition, there is information that the genomic polymorphism existing in the NADPH oxidase enzyme may be a factor on the background of different responses to individual drug treatments and that these effects of the NADPH oxidase enzyme are closely related to aging. Hereby, we anticipate that research on determining the individual NADPH oxidase index will in the future.

Acknowledgement: *This study supported by Erciyes University for BAP project TSA-2019-8322.*

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