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CHRONIC GRANULOMATOUS DISEASE – DIAGNOSTICS AND GENETIC COUNSELING EXPERIENCE

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ABSTRACT

The chronic granulomatous disease (CGD) is a heterogeneous group of primary immunodeficiencies of the phagocytosis. During our experience of the last 20 years 57 children with recurrent infections were studied. In seven cases X-recessive form of CGD was found. Besides the characteristic clinical picture, the application of nitroblue tetrazolium (NBT) test was essential for confirmation the diagnosis. The test was performed on isolated endotoxin stimulated granulocytes. Five of the mothers were carriers of CGD showing NBT (+) cells in the range of 28, 5% to 43, 5%. One of them is probably carrier of the novo arisen mutation. Patient's relatives were also studied and carriers of CGD were found among them. Genetic counseling was performed in all cases and prenatal diagnosis for sex determination in one family as well. The problems of genetic counseling in relation to the genetic heterogeneity of CGD are discussed.

Key words: primary immunodeficiency, phagocytic dysfunctions, recurrent infections, NBT test

INTRODUCTION

Chronic granulomatous disease (CGD) is a heterogeneous group of primary immunodeficiencies of phagocytosis, caused by mutations in genes encoding subunits of NADPH oxidase complex. The defective production of microbicidal oxidants O^{-}, H_2O_2 and HOCl, during the respiratory burst in phagocytes and impaired killing of pathogens is a cause for recurrent life-threatening infections with catalase-positive bacteria and fungi. The infections, mainly pneumonia, lymphadenitis, skin infections hepatic and perirectal abscesses, osteomyelitis, otitis media and others with less frequent localizations, begin usually in the first year of life (1, 2, 3). As a result of chronic inflammatory stimulation, lymphoadenopathy and hepatosplenomegaly with non-specific granulomas arise. Later complications such as granulomatous obstruction of different hollow organs are observed too.

First patients, identified in 1957, were male with apparent X-linked inheritance, but 10 years later females with CGD were also reported (4, 5, 6). This suggested that the disease is genetically heterogeneous which was supported by the observed intra- and inter-familial variability.

The investigations of different groups in USA and Europe during the last 15 years revealed the molecular basis of this heterogeneity (4, 5, 6, 7). The disease is caused by a defect of specific membrane-linked NADPH-oxidase.The NADPH-oxidase complex is composed of a heterodimeric membrane-bound flavocytochrome b558 (formed by gp91-phox and p22-phox) and four cytoplasmatic subunits: p47-phox, p67-phox, p40-phox and Rac2. Different genes located consequently on chromosome Xp21.1, 16q24, 7q11.23, 1q25 and 22q13.1 encode them. Mutations in any of the genes encoding NADPH-oxidase subunits can cause CGD. Various types of defects - deletions, insertions and point mutations were

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discovered that provide an explanation for the clinical and genetic heterogeneity of CGD (6, 7, 8).

Approximately two thirds of CGD cases result from mutations in X-linked gene, encoding gp91-phox subunit. Although the clinical picture of the disease is relatively typical, its diagnostics requires different specialized laboratory methods for evaluation of the respiratory burst activity, as measurement of O2 consumption and generation of O2-, or H2O2, chemiluminescence, cytochrom b558 spectrophotometric determination. One of the most convenient assays is the nitroblue tetrazolium (NBT) test.

In this report we present our experience in the diagnostics of several CGD patients and carriers by a simple, screening NBT method.

MATERIALS AND METHODS

During the last 20 years fifty-seven children with recurrent infections, suspected for primary immunodeficiency were studied for phagocytic dysfunctions.

The oxygen-dependent antimicrobial capacity of granulocytes was evaluated by cytochemical screening NBT test in modification (9, 10). Briefly, granulocytes from freshly clotted blood were isolated by adhesion on endotoxin-coated cover slip. The cover slip was then incubated with 0, 12% NBT solution in PBS (pH 7, 44), supplemented with pooled human serum, washed, fixed and stained with Safranin. Two hundred cells per patients were scored. Normally more than 90% of granulocytes became NBT positive (NBT+), appearing as large exploded cells with cytoplasm stained blue by the deposits of insoluble formazan, formed after the reduction of yellow NBT dye. Some patient's relatives were also examined by the NBT test.

In parallel to exclude other phagocytic defects the phagocytic activity was determined by latex bead ingestion test (11), myeloperoxidase and G6PD activity on peripheral blood smears and by fluorescent blot method respectively (12, 13)

RESULTS AND DISCUSSION

Out of the 57 children with recurrent infections studied, 8 patients from 7 families showed clinical signs for CGD. All these 8 patients were males, aged from 2 months to 7 years. They suffered from different severe infections: mainly pyodermia, started immediately after birth, pneumonias and suppurative lymphadenitis. Four of them have also developed perirectal abscesses, two – enterocolitis and otitis media. In most of the cases Staphylococcus aureus, E. coli and Klebsiella were the isolated pathogens.

The investigation of phagocytosis in these patients revealed normal ingestion of latex particles and myeloperoxidase staining on blood smears. The normal G6PD activity in the patients excludes a rare "pseudo" variant of CGD with G6PD deficiency. However, the application of the NBT slide test didn't show any deposits of formazan in the granulocytes, i.e. they all were NBT negative **(Fig. 1b)**

In 5 of the families the NBT test was performed to the parents. In the fathers more than 90% of cells were NBT (+) **(Fig. 1a)** whereas in the mothers two populations of cells were observed – NBT (+) and NBT (-). The NBT (+) cells were in the range from 28,5% to 43,5% **(Fig.1c)**. There was no overlap between the carrier state and the normal range. This confirmed that they are carriers of CGD gene mutations and hence the X-linked inheritance of the disease is proved.

The severity of infections and the NBT data in 7 of our cases strongly suggested an X91° subtype of CGD, according to the recently published disease classification (3, 8) In one male patient only the autosomal recessive form of CGD could not be excluded, as the parents have not been tested.

In order to check the relatives for heterozygocity, the NBT test was performed in four of the families. In one of them (T.P./94) the parents have not be studied, but the grandmother was proved to be a carrier, which suggests a X-linked inheritance **(Fig. 2)**.

In the second family (A.M./76; Ts.T./86) only the mother was found to be carrier. The normal NBT scores of her parents supposed a de novo arisen germ line mutation in one of them **(Fig. 3).** In the third family studied (P.M./78) the 2 months' old sister of the proband was a carrier as a mother **(Fig. 4)**. In the fourth family (D.D./94), where two boys, children of the mother's aunt have died from recurrent infections, 5 female relatives heterozygotes were discovered **(Fig. 5).**

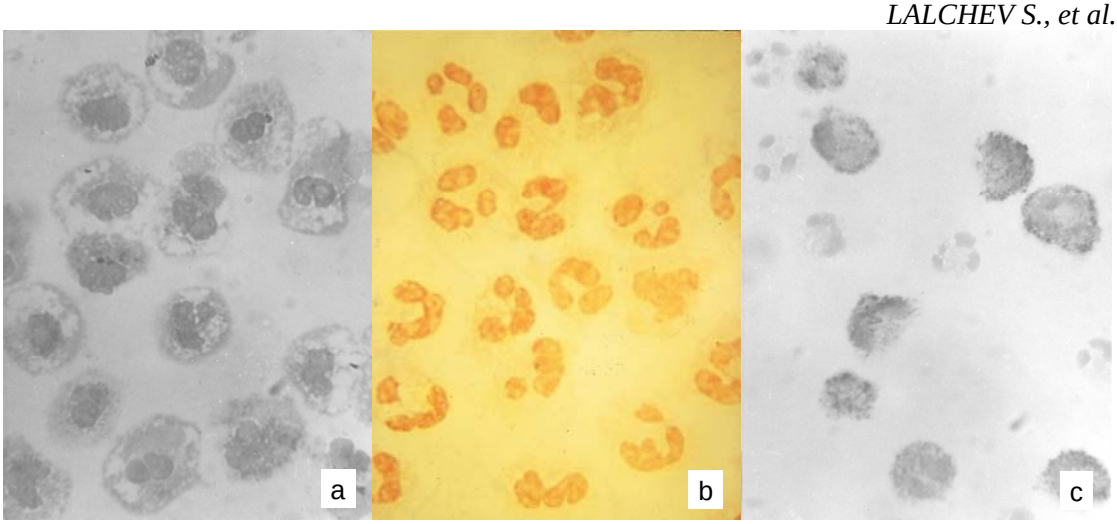

Figure 1. NBT slide test - a. control (NBT+ cells); b. patients with CGD (NBT- cells); c. female carriers (two populations of cells: NBT+ and NBT-)

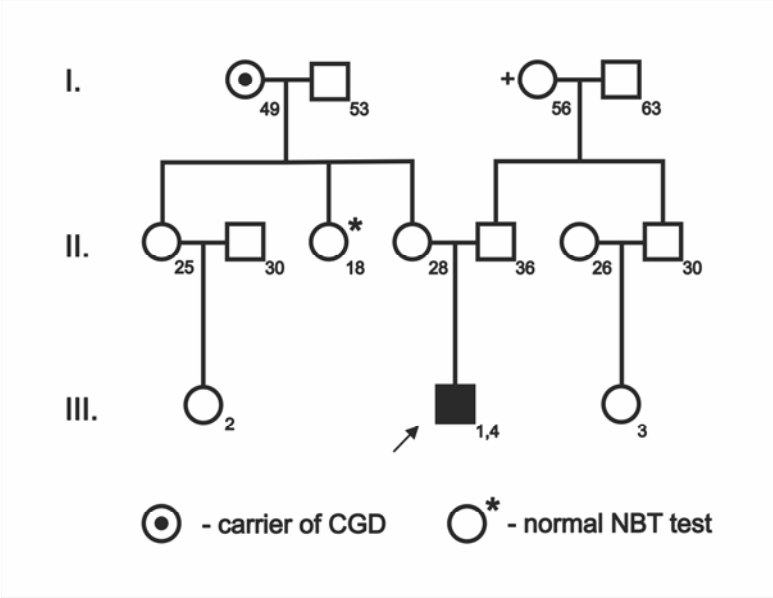

Figure 2. Pedigree of family T.P.(suspected X-linked form)

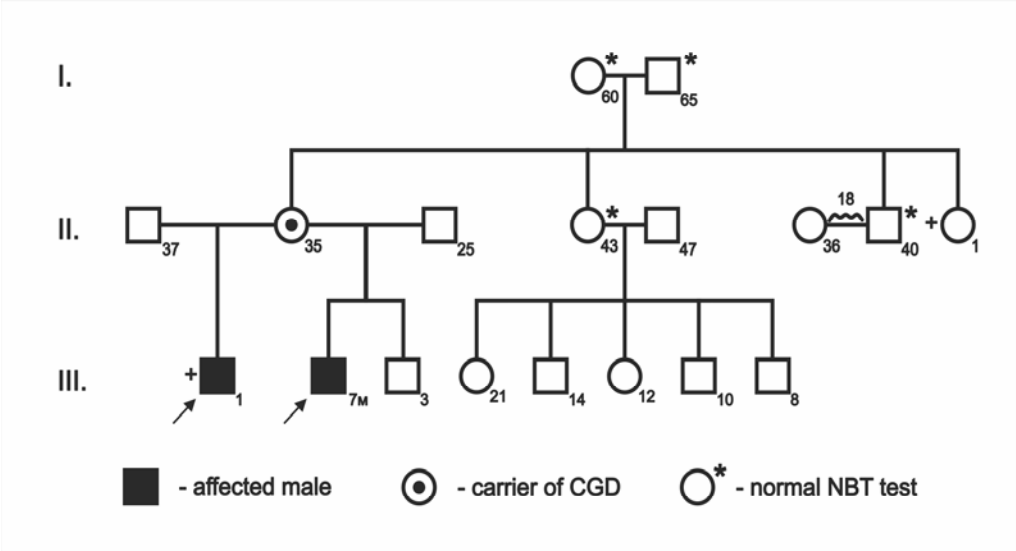

Figure 3. Pedigree of family A.M./Ts.T. (X-linked form -de novo arisen mutation).

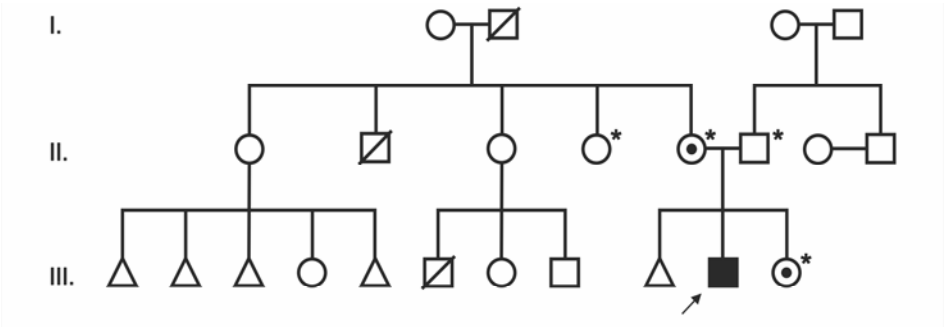

Figure 4. Pedigree of family P.M. (X-linked form. Proband's sister is a carrier).

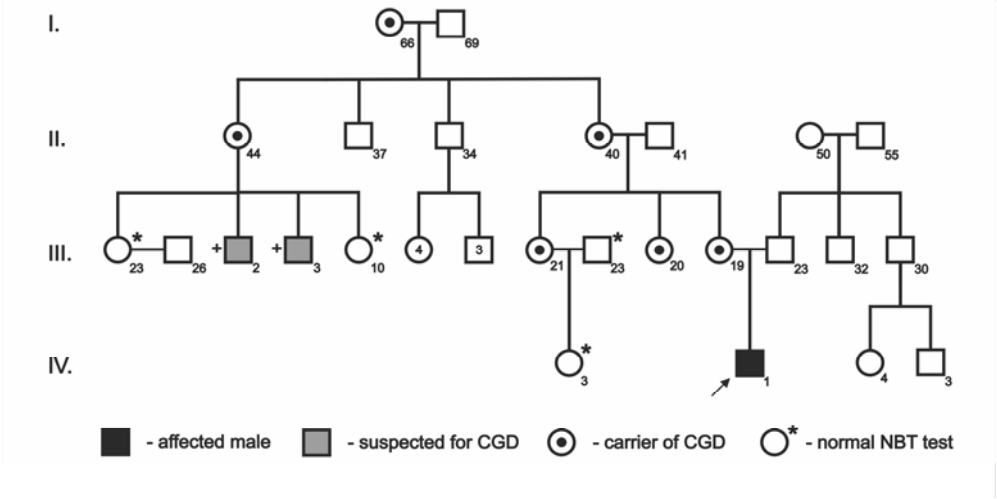

Figure 5. Pedigree of family D.D.(X-linked form, transmitted through 4 generations).

Genetic counseling was carried out in 5 of the families. In one of them (Fig.3) as the risk for boys with CGD is high as 50%, prenatal diagnosis for sex determination was performed. The chromosomal analysis on amniocytes established a male fetus. Unfortunately the mother refused to interrupt the pregnancy. Thus a second boy was born with CGD, confirmed by the NBT test performed hours after the delivery.

The results from our study indicate that the NBT slide test is a sensitive, simple and inexpensive sreening method for CGD. Recently a modification of this method with phorbol myristate acetate stimulation has been applied for detection of CGD patients and heterozygote carriers (14). The authors have had positive result with the NBT test using cord fetal blood and their suggestion is that the NBT test may be use for antenatal diagnosis of the disease.

Now existing DNA analysis makes it possible to provide an exact earlier prenatal diagnosis by CVS, but preliminary characterization of the CGD mutations is necessary since more than 90% of the families have their unique mutations (3, 8).

CONCLUSIONS

The NBT slide test is a simple and useful method for diagnostics of CGD, as well as for determination of carriers of X-linked form of the disease.

In all cases with CGD familial studies have to be performed in order to identify as more as possible carriers to whom in due time prospective genetic counseling and consequent prenatal diagnostics should be proposed.

International collaboration in the investigation of the nature of CGD mutations will give information about the more frequent ones in our population, as well as about their relation to the heterogeneity of the disease.

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