Allergol Immunopathol (Madr). 2014;42(6):580–585

Allergologia et immunopathologia

Sociedad Española de Inmunología Clínica, Alergología y Asma Pediátrica

www.elsevier.es/ai

ORIGINAL ARTICLE

Detection of inheritance pattern in thirty-three Mexican males with chronic granulomatous disease through 123 dihydrorhodamine assay

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Received 8 April 2013; accepted 28 July 2013
Available online 2 June 2014

KEYWORDS
Chronic granulomatous disease;
123 dihydrorhodamine;
Discoid lupus;
Chronic granulomatous disease carriers

Abstract
Background: There are two inheritance patterns, the X-linked recessive (XL) pattern and the autosomal recessive pattern. There is no information on the predominant inheritance pattern of male patients with chronic granulomatous disease (CGD) in Mexico.
Objective: The aim of this study was to determine the inheritance pattern in a cohort of Mexican male patients with CGD by means of the detection of an XL status carrier among their female relatives, and to describe the frequency of discoid lupus (DL) among carriers.
Methods: We detected the female relatives within the families of male patients with CGD, and carried out the 123 dihydrorhodamine (DHR) assay in all female participants. All carriers were questioned for current or past established DL diagnosis.

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[1] All the participants are members of the MEXID (Mexican group for immunodeficiencies).

http://dx.doi.org/10.1016/j.aller.2013.07.014

Results: We detected 33 families with one or more CGD male patients; we found an XL-CGD in 79% of the relatives from at least one female relative with a bimodal pattern. For the remaining seven relatives we were not able to confirm a carrier status by means of a DHR assay. Moreover, we detected one mother with CGD secondary to skewed X-chromosome inactivation. We also found 47 carriers, and only one carrier with DL among them.
Conclusion: We concluded that XL-CGD is the most frequent form of CGD in a cohort of CGD male patients in Mexico. DHR assay is a fast and practical tool to determine the CGD form in the Latin-American countries. Finally, DL frequency in Mexico is lower than that reported in the literature for other regions of the world.

Introduction

Chronic granulomatous disease (CGD) is a primary immunodeficiency caused by a defect in any of the five components of the NADPHoxidase that leads to a complete lack of, or significant decrease in, the production of microbicidal reactive oxygen metabolites. Mutations in the gp91phox gene (CYBB on Xp 21.1) produce the X-linked recessive form of the disease that affects about 70% of all CGD patients. As expected from genetics, the overwhelming majority of the X-linked patients are male. The remaining 30% of the cases are inherited in an autosomal recessive (AR-CGD) way affecting males and females equally. Mutations in genes of *NCF1, NCF2, NCF4* y *CYBA* are responsible for the autosomal recessive pattern. Individuals with X-CGD have a more severe clinical phenotype and higher mortality than those with AR-CGD.[1–4]

Lyonisation explains that there is a random inactivation of one X-chromosome in somatic cells during early foetus development. This results in two distinct populations of polymorphonuclear leucocytes: one with normal and the other with abnormal production of microbicidal reactive oxygen metabolites. Both populations can be detected by DHR assay, which shows a bimodal histogram for carriers.[5,6]

The objective of this study was to identify the inheritance pattern of the 33 Mexican males with CGD by looking for a carrier in their female relatives by means of a DHR assay. We also determined the presence, or the history, of discoid lupus in the carriers and offered them genetic counselling.

Patients and methods

Patients

The parents with one or more male patients with CGD and without an established inheritance pattern, from eight different hospitals throughout Mexico, were invited to participate in our study. We first traced the family tree in order to identify all the female relatives on the mother's branch, and then invited them to participate in the study. The protocol was reviewed and approved by the appropriate local Ethics and Research Committees in accordance to the International Conference on Harmonization Good Clinical Practice guidelines and the Declaration of Helsinki. All participants who agreed to participate signed the informed consent and/or the informed assent. All carriers were interrogated for current or past diagnosis of discoid lupus established by a physician as disc-shaped lesions, erythematous plaques of varying size, and containing areas of follicular hyperkeratoses. Again, genetic counselling was offered to all carriers.

Three millilitres of venous blood was obtained from each participant and collected in a lithium heparin vacutainer tube. The samples were processed immediately with the objective of avoiding cell damage caused by blood drawing, shipping, mechanical irritation or temperature variation which could cause a false X inactivation of some neutrophils.

Materials and methods

The working dilution of dihydrorhodamine 123 (DHR 123) (45 μg/ml) was prepared by adding 30 μl of DHR (DHR; Molecular Probes, Eugene, OR, USA) stock solution (5 mg/ml) to 3.33 ml of phosphate-buffered saline (PBS). In order to prepare the working dilution of phorbol-myristate-acetate (PMA) (50 ng/ml), we added 10 μl of PMA (Sigma Chemical, Munich, Germany) stock solution (1 mg/ml) to 1 ml of PBS.

Dihydrorhodamine flow cytometry assay

Three 100 μl samples were taken from each whole blood of possible carriers and placed in separate tubes. The tubes were labelled as stimulated,[1] resting,[2] and reagent blank tests.[3] Twenty-five microliters of working DHR solution (final concentration 1.125 μg/ml) was added to the stimulated[1] and resting samples.[2] All tubes were incubated at 37 °C for 15 min. Then 10 μl of PMA solution (final concentration 100 ng/ml) was added to the stimulated tubes.[3] After further 20-min incubation at 37 °C, all tubes were added with 1.0 ml of FACS lysing solution (Becton Dickinson, Heidelberg, Germany), allowed to rest at room temperature for 20 min, were then centrifuged. The supernatant was discarded and the cells were washed twice with 2 ml of PBS. After the second centrifugation, the supernatant was discarded and replaced with 0.25 ml of 1% paraformaldehyde.

By using the FACSAria I® (Becton Dickinson, Heidelberg, Germany) the parameters forward and side scatter were collected, as well as FL2. Twenty thousand events were acquired in the established granulocyte gate for each tube. Data analysis was performed using FlowJo 7.2.4 software (Tree Star, Inc., Ashland, OR, USA). The oxidative index (NOI)