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Erythrocyte Glucose-6-phosphate Dehydrogenase Isoenzyme Phenotypes in Leprosy[1]

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The enzyme glucose-6-phosphate dehydrogenase (G-6-PD) (Enzyme Commission Number 1.1.1.49) is an important critical component of biological systems since it catalyzes the first step of the pentose phosphate shunt. The actual biochemical reaction is oxidation of glucose-6-phosphate to 6-phosphogluconate using nicotinamide adenine dinucleotide phosphate (NADP) as the coenzyme ([18]). G-6-PD is widely distributed in nature, being found in the mammalian liver, in erythrocytes, yeasts, and bacteria ([18]). Much interest has centered around the G-6-PD of human erythrocytes because a deficiency of the enzyme leads to a hemolytic crisis when the individual with the defect consumes fava beans or is administred drugs, such as antimalarials, sulfonamides, analgesics, or others ([17]). In the erythrocyte, G-6-PD is required to provide NADPH; this keeps glutathione in the reduced state and so prevents the lysis of red cells ([18]). The usual method of studying erythrocyte G-6-PD is by standing a peripheral blood smear. It permits classification into absence (homozygous), partial deficiency (heterozygous), and normal. It would be appropriate to point out, however, that the diagnosis of G-6-PD deficiency, using the conventional methemoglobin reductase method, has the potential drawback of recording false-positive end point titrations ([3]). The phenotypes of G-6-PD have been described using polyacrylamide disc gel electrophoresis (PADGE) ([1, 8, 18]) and starch gel electrophoresis of hemolysates ([5]). Such analyses have shown two prominent, electrophoretically different, isoenzymes of G-6-PD on the basis of which two phenotypes A+ and B+ have been defined ([19]). Both isoenzymes have essentially identical molecular weights (240,000 daltons), amino acid compositions, immunological specificities, and subunit structures of the two subunits. The differences are that the isoenzyme of the phenotype A+ contains aspartic acid and moves in electrophoresis like adult hemoglobin; while B+ contains asparagine and has a slower electrophoretic mobility. Studies from the United States have described marked differences in Caucasians and Negroes ([18]). Besides these major isoenzymes, minor bands have also been noted in starch and polyacrylamide disc gel electrophoresis.

Leprosy is an unusual disease in which attributes of the host are more important than those of the parasite in determining both susceptibility and the clinical form of the disease. Therefore there is much continuing interest in genetic polymorphisms and the clinical varieties of leprosy. The genetic markers studied in leprosy have included the HLA antigens ([7]), pseudocholinesterase ([16]), haptoglobin ([14]), blood groups ([12]), beta-lipoproteins, group specific substances, transferrin ([10]), and the Au1 gene ([4]).

A careful scrutiny of the literature has disclosed only three reports of G-6-PD and leprosy ([2, 10, 11]). These have documented the occurrence of deficiency of the enzyme but not the phenotypes. We describe here a study of the G-6-PD isoenzyme phenotype obtained from hemolysates of patients of leprosy carefully classified according to the Ridley-Jopling scale ([13]).

MATERIALS AND METHODS

Subjects

Samples of blood were obtained from five, normal, healthy adult volunteers. Fifty cases of leprosy attending the Sir J. J. Group of Hospitals, Bombay, were included in the present study. A detailed history which included the duration of illness and the occurrence of any reaction was obtained. The duration and nature of treatment were doc-

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umented. All cases were under sulfone therapy for between two and 12 years. A detailed clinical examination was carried out and recorded. The Morphological Index (MI) and the Bacteriologic Index (BI) were determined. A cutaneous punch biopsy was studied after staining with hematoxylin and eosin and the Ziehl-Neelsen stain (Fite-Faraco modification). A lepromin test using armadillo lepromin was carried out. Each patient was then classified according to Ridley and Jopling ([13]). Twenty-four of the patients had lepromatous leprosy (LL)—18 males and 6 females; 5 had borderline lepromatous (BL)—5 males; 5 had borderline tuberculoid (BT)—3 males and 2 females; and 16 had tuberculoid leprosy (TT)—14 males and 2 females. A sample of blood collected in acid citrate dextrose (ACD) solution was obtained in each case to study the erythrocyte G-6-PD.

Characterization of isoenzymes and phenotypes of G-6-PD

Preparation of hemolysate. The venous blood collected in ACD was centrifuged at 3000 × *g* for 10 min and the supernate discarded. The cells were washed twice with normal saline and hemolyzed by treating with cold distilled water (4°C).

Polyacrylamide disc gel electrophoresis (PADGE). Each hemolysate was studied in duplicate in PADGE, one of the samples being pretreated with NADP. The hemolysates were electrophoresed in PADGE ([6]) using a miniaturized system ([15]). The sample volume was 0.05 ml mixed with an equal quantity of 40% w/v sucrose with a trace of 1% w/v aqueous bromophenol blue as a marker. The buffer was tris glycine (pH 8.3, 0.1 M). A constant current of 1 mA per gel was used and electrophoresis continued until the marker reached the end of the capillary. The gels were removed and stained for G-6-PD activity.

Staining for G-6-PD (tetrazolium-formazan method). A staining mixture of the sodium salt of glucose-6-phosphate (substrate), NADP (coenzyme), $MgCl_2$ (co-factor), phenazine methosulfate (PMS, electron transferrer) and nitroblue tetrazolium (NBT, indicator) was prepared ([1]). The gels were immersed in the mixture and incubated at 37°C for 1 hr in the dark. The presence of G-6-PD in the gel was indicated by a purple color of formazan formed by the reduction of NBT. With each gel the number of major and minor purple bands and their relationships to the band of hemoglobin were noted. This permitted phenotypic differentiation into A+ and B+.

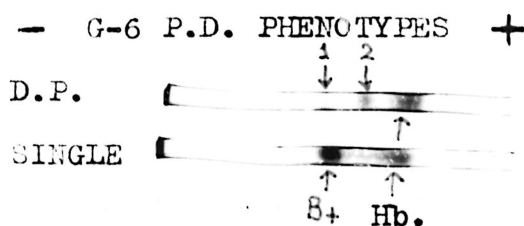
RESULTS

None of the patients or the controls showed a deficiency of the enzyme G-6-PD in the hemolysates. All the samples containing NADP revealed a single, well-defined, intense, purple-colored formazan band indicating enzymatic activity. In all duplicate hemolysates without NADP there were two prominent bands in contrast to the single band obtained with NADP. Again, in all samples (from both patients and controls) the position of the band of G-6-PD was behind the hemoglobin band indicating the phenotype to be B+ (The Figure).

DISCUSSION

The literature contains three references concerning G-6-PD and leprosy. Pettit and Chin ([11]) studied the qualitative occurrence of G-6-PD in patients with leprosy. They suggested the possibility that a deficiency of red cell G-6-PD predisposed to the development of lepra reactions. Lechat, *et al.* ([10]) in an extensive study of several polymorphisms in serum globulins among leprosy patients at Cebu (Phillipines) also looked for G-6-PD deficiencies using a spot test. They did not notice any differences between controls and patients. Banait and Junnarkar ([2]) used the methemoglobin reductase method ([9]) for studying the enzyme levels in peripheral blood smears of leprosy patients. As many as 25.7% (26 of 101 cases) showed a deficiency of erythrocyte G-6-PD. A majority of the patients in lepra reaction (71.4%, 10 of 14 cases) showed the deficiency; while the corresponding figures for nonreactional cases were: lepromatous 29.9% (14 of 52 cases); indeterminate, 25% (8 of 32 cases); and tuberculoid, 23.5% (4 of 17 cases). However, this study was carried out in the central part of India (Nagpur), an area where both leprosy and the gene for G-6-PD deficiency (Gd gene) are very common. None of the three studies referred to described the electrophoretic characterization of the A+ and B+ phenotypes of G-6-PD.

The results of the present study are neg-



THE FIGURE. Duplicate separations of a hemolysate with (bottom) and without the addition of NADP (top) in disc electrophoresis stained for G-6-PD. Note two bands marked 1 and 2 (dissociation products) behind the hemoglobin band (marked Hb) in the upper gel. These have fused to form a single band characteristic of the B+ phenotype in the lower separation.

ative but certainly of some interest. All the cases and the controls belonged to the B+ phenotype. This observation can be interpreted to indicate the common phenotype of G-6-PD in our country. In this respect we have the phenotype which has been recorded in Americans of Caucasian origin ([18]). Observations in America have shown that American Negroes, although commonly of the B+ phenotype, have the other variant, A+, in as much as 19% of the population.

The patients in the present study had all been treated with dapsone (DDS) for between two and 12 years. This drug is known to precipitate hemolytic crises of varying grades of severity in individuals showing a deficiency of erythrocyte G-6-PD. It is perhaps needless to point out that none of the patients studied had overt evidence of hemolysis, and they did not suffer a deficiency of the enzyme.

In the present study all sera treated with NADP showed a single band of G-6-PD. In contrast, untreated parallel samples showed two bands (The Figure). This has been ascribed to covalent binding of G-6-PD by NADP so that the separate bands fuse to form a single one ([1, 18]).

SUMMARY

Hemolysates from 50 cases of leprosy and five controls were studied for glucose-6-phosphate dehydrogenase (G-6-PD) isoenzyme phenotypes by polyacrylamide disc gel electrophoresis using a miniaturized system. The cases were classified as lepromatous (24), borderline lepromatous (5), borderline tuberculoid (5), and tuberculoid (16) according to the Ridley-Jopling scale. All had been on treatment with dapsone for between two and 12 years. None of the cases showed deficiency of erythrocyte G-6-PD. All the cases and the controls showed the G-6-PD phenotype to be of the B+ variety. Therefore this genetic marker does not appear to be related to susceptibility to leprosy or to the clinical variety of the disease in our situation.

RESUMEN

Se estudiaron los fenotipos de las isoenzimas de la glucosa-6-fosfato deshidrogenasa (G-6-PD) en hemolisados de 50 casos de lepra y de 5 controles, por electroforesis vertical en gel de poliacrilamida. Los casos se clasificaron como lepromatosos (24), intermedios lepromatosos (5), intermedios tuberculoides (5) y tuberculoides (16) de acuerdo a los criterios de Ridley y Jopling. Todos los pacientes habían estado en tratamiento con dapsona por 2 to 12 años. Ninguno de los casos mostró deficiencias de la G-6-PD eritrocítica. El fenotipo de la G-6-PD en todos los pacientes y controles fue de la variedad B+. Por lo anterior, este marcador genético no parece estar relacionado con la susceptibilidad a la lepra o con la variedad clínica de la misma.

RÉSUMÉ

On a étudié les phénotypes de l'isoenzyme glucose-6-phosphate déhydrogenase (G-6-PD), en soumettant à l'électrophorèse sur disque d'un gel de polyacrylamide, en un système miniaturisé, des hémolysats provenant de 50 cas de lèpre et de 5 témoins. Les cas ont été classés en lépromateux (24), lépromateux dimorphes (5), tuberculoides dimorphes (5) et tuberculoides (5), selon la classification de Ridley-Jopling. Tous ces malades avaient été en traitement par la dapsone pour une période s'étendant de 2 à 12 ans. Aucun des cas n'a montré une déficience des érythrocytes en G-6-PD. Tous les sujets, tant cas que témoins, ont révélé un phénotype G-6-PD de la variété B+. Dès lors, dans les conditions de l'étude, cet indicateur génétique ne paraît pas être en relation avec la susceptibilité à la lèpre ou avec la variété clinique de la maladie.

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