

ISONIAZID ACETYLATED PHENOTYPE IN PATIENTS WITH PARACOCCIDIOIDOMYCOSIS AND ITS RELATIONSHIP WITH SERUM SULFADOXIN LEVELS, GLUCOSE-6-PHOSPHATE DEHYDROGENASE AND GLUTATHIONE REDUCTASE ACTIVITIES.

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The authors evaluated the isoniazid acetylating phenotype and measured hematocrit, hemoglobin, glucose-6-phosphate dehydrogenase and glutathione reductase activities plus serum sulfadoxin levels in 39 patients with paracoccidiodomycosis (33 males and 6 females) aged 17 to 58 years. Twenty one (53.84%) of the patients presented a slow acetylating phenotype and 18 (46.16%) a fast acetylating phenotype. Glucose-6-phosphate dehydrogenase (G6PD) activity was decreased in 5 (23.80%) slow acetylators and in 4 (22.22%) fast acetylators. Glutathione reductase activity was decreased in 14 (66.66%) slow acetylators and in 12 (66.66%) fast acetylators. Serum levels of free and total sulfadoxin were higher in slow acetylators ($p < 0.02$). Analysis of the results permitted us to conclude that serum sulfadoxin levels are related to the acetylator phenotype. Furthermore, sulfadoxin levels were always above 50 µg/ml, a value considered therapeutic. Glutathione reductase deficiency observed in 66% of patients may be related to the intestinal malabsorption of nutrients, among them riboflavin, a FAD precursor vitamin, in patients with paracoccidiodomycosis.

Key-words: Isoniazid acetylating phenotype. Paracoccidiodomycosis. Glucose-6-phosphate dehydrogenase (G6PD). Glutathione reductase. Sulfadoxin.

The therapeutic conduct adopted by us thus far^{6 18} for paracoccidiodomycosis is divided into two stages, i.e. acute and maintenance treatment. Several drugs have been employed for acute treatment, among them sulfa drugs alone¹⁷ or in combination with trimethoprim^{5 7 18}. For maintenance, the use of slow-excretion sulfa drugs is recommended for a period of at least two years¹⁸. These drugs, because of their oxidizing action, may cause serious side effects in patients with genetic red blood cell defects^{1 3 4 6 9 14 21}. Among such defects are glucose-6-phosphate dehydrogenase (G6PD) and glutathione reductase deficiency^{3 14}. Glutathione reductase deficiency also may be an acquired defect due to deficiency of riboflavin, a FAD precursor vitamin^{9 14}. These

defects may cause attacks of hemolytic anemia in patients treated for paracoccidiodomycosis, with consequent severe worsening of clinical signs and prognosis.

In addition, the literature is controversial with respect to the effect of isoniazid acetylating phenotype on serum levels of sulfones, sulfonamides and isoniazid. Some investigators^{8 10 27} believe that the acetylating phenotype is intimately related to the serum levels of these drugs, whereas others²⁴ disagree.

In view of the above considerations, the objectives of the present study were to determine the isoniazid acetylating phenotype and to evaluate correlation of it with G6PD and glutathione reductase activity and with serum levels of free and total sulfadoxin in patients with paracoccidiodomycosis.

MATERIAL AND METHODS

The present study was conducted at the Paracoccidiodomycosis Outpatient Clinic of the Division of Infectious and Parasitologic Diseases of the School of Medicine of Botucatu/UNESP, São Paulo, Brazil, from 1987 to 1989. Thirty-nine patients (6 females and 33 males, aged 19 to 64 years) with a confirmed diagnosis of paracoccidiodomycosis were studied. The clinical form of the disease¹³ was chronic in 29 and subacute progressive in 10 patients. All patients

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were under maintenance treatment with one tablet (500 mg) of sulfadoxin twice a week. As recommended by Mendes¹⁸, the medication was taken on Mondays and Thursdays and blood samples were collected on Wednesdays at about 2:00 p.m.. All patients had been under maintenance treatment for at least one month and followed medical prescriptions rigorously. They were tested for isoniazid acetylating phenotype and G6PD and glutathione reductase activities and submitted to measurement of hematocrit, hemoglobin and serum levels of free and total sulfadoxin.

Isoniazid acetylating phenotype was determined before the beginning of treatment by the colorimetric test of Eidus et al¹² and Hodgkin et al¹⁵ and the patients were classified as fast acetylators when acetylated isoniazid was more than 65% and as slow acetylators when acetylated isoniazid was less than 65%, according these authors^{12 15}.

G6PD and glutathione reductase activities were determined by the technique standardized by Barraviera et al³. The normal values were 221.10 ± 21.06 $\mu\text{g}/\text{min}$ for G6PD activity, and 81.27 ± 9.54 $\mu\text{g}/\text{min}$ for glutathione reductase. Hematocrit levels were determined by the microhematocrit method and hemoglobin levels by the cyanomethemoglobin method²⁰.

Serum sulfadoxin levels were measured by the technique of Bratton & Marshall¹¹ which is used to evaluate free and total sulfadoxin levels.

Two study groups were proposed for evaluation of the results: Group 1: slow acetylators; and Group 2: fast acetylators.

Data were analyzed statistically using the paired "t" test²⁵. To determine the interrelationship of the variables, the linear correlation coefficient between pairs of variables was calculated.

RESULTS

Analysis of the results presented in Table 1 showed that 21(53.84%) patients were slow acetylator and 18(46.16%) fast acetylators.

Hematocrit and hemoglobin levels were reduced only among male patients, 5 of whom (27.77%) were fast acetylators and 6(28.57%) slow acetylators.

G6PD activity was decreased in 5(23.80%) slow acetylators and 4(22.22%) fast acetylators. Glutathione reductase activity was decreased in 14(66.66%) slow acetylators and in 12(66.66%) fast acetylators.

Serum levels of free and total sulfadoxin were significantly higher among slow acetylators ($p < 0,05$ and $p < 0,02$, respectively). There was a negative correlation between slow acetylating phenotype and serum level of free sulfa ($r_{0,01} = -0,45$).

DISCUSSION

Isoniazid acetylating phenotype and G6PD and glutathione reductase activities are genetically determined traits^{3 6 8 10 12 15}. The first is determined by major autosomal genes and is related to the acetylating capacity of hepatic acetyl transferase. One of the functions of this enzyme is to inactivate sulfones, sulfonamides and isoniazid^{10 12 15}. Population studies^{19 26} have demonstrated two types of acetylators, i.e., fast and slow, the frequency of each varying from population to population. American Indians, Japanese and Eskimos most often present a fast acetylating phenotype²⁶. In Brazil, Beiguelman et al¹⁰ evaluated the acetylating phenotype of Caucasian and Black patients with tuberculosis and detected 57% slow acetylators among Caucasians and 50% among Blacks. In the present study, the prevalence of acetylating phenotype was quite similar to that found by these investigators¹⁰.

G6PD activity is determined by genes located on the X chromosome^{14 21}. Thus, only females can manifest homozygous or heterozygous trait. In the present study, 3 patients heterozygous for G6PD deficiency were detected. Among the six G6PD-deficient male patients, only two developed anemia. It should be pointed out that reduced number of patients

Table 1 - Distribution of acetylating phenotype, hematocrit, hemoglobin, glucose-6-phosphate dehydrogenase (G6PD) and glutathione reductase (GR) activities and serum levels of total and free sulfadoxin.

Acetylating phenotype	Hematocrit (%) $\bar{X} \pm s$	Hemoglobin (g%) $\bar{X} \pm s$	G6PD ($\mu\text{g}/\text{min}$) $\bar{X} \pm s$	GR ($\mu\text{g}/\text{min}$) $\bar{X} \pm s$	Total Sulfadoxin ($\mu\text{g}/\text{ml}$) $\bar{X} \pm s$	Free Sulfadoxin ($\mu\text{g}/\text{ml}$) $\bar{X} \pm s$
Slow (n=21)	49.61 ± 7.54	14.46 ± 2.21	208.42 ± 38.46	62.73 ± 21.08	190.24 ±* 54.10	154.04 ±** 41.54
Fast (n=18)	48.88 ± 7.01	13.76 ± 1.95	214.03 ± 31.01	64.26 ± 16.24	152.98 ± 37.91	125.04 ± 32.39

Statistical analysis: * $t=2.45$; $p < 0.02$.

** $t=2.40$; $p < 0.05$; differences significatives in respect to the fast acetylating group.

with anemia detected among deficient individuals under the effect of oxidizing drugs such as sulfadoxin can be explained by the type of deficiency predominating in Brazilian populations^{1 4}. The deficiency encountered here is possibly of the African type, with a consequent tolerance of red cells to oxidizing drugs^{3 4 6 14 21}.

From a genetical viewpoint, glutathione reductase deficiency follows the autosomal recessive model, the detection of deficient individuals being a very infrequent occurrence^{1 14}. For perfect functioning, this enzyme needs NADPH generated through the pentose pathway^{3 14 21} and FAD, a phosphorylated riboflavin derivative^{2 9}. Barraviera et al², in a study of individuals from the Brazilian Amazon region, detected decreased glutathione reductase activity due to riboflavin deficiency caused by the peculiar dietary habits of that region. In addition, the elevated rate of helminth infestation detected among Amazon populations may strongly contribute to the consumption of this vitamin¹⁶. The elevated prevalence of glutathione reductase observed in the present study may have been related to the intestinal malabsorption of nutrients, among them amino acids and vitamins, observed among patients with progressive paracoccidioidomycosis^{7 17}.

On the other hand, individuals chronically using sulfamide derivatives are known to suffer stimulation of glutathione synthetase which causes increased synthesis of reduced glutathione²³. These individuals eventually show an increase in glutathione reductase activity. The present findings showed exactly the contrary, i.e., a decrease in glutathione reductase activity, strengthening the hypothesis that the nutritional deficiency of riboflavin may be the limiting factor in glutathione reductase activity. It should be pointed out that all anemic patients presented decreased glutathione reductase activity. It has been reported^{1 6 9 14 21} that the same drugs that cause anemia among G6PD-deficient individuals can cause anemia among glutathione reductase-deficient subjects. In this case, the nutritional deficiency on the one hand, the chronic effect of sulfadoxin on the other, may have strongly contributed to the onset of anemia.

The relationship between acetylating phenotype and serum levels of fast-excretion sulfamide derivatives are controversial^{24 27}. Vree et al²⁷ and Barraviera et al⁸, when evaluating the serum sulfadiazine levels of patients using these drugs and their relationship with the acetylating phenotype, found more elevated drug levels and decreased renal function among slow acetylators. The present results agree with those reported in these studies^{8 27} and clearly demonstrate the difference in serum sulfadoxin levels between slow and fast acetylators. On the other hand, serum levels of free sulfadoxin were

always above 50 µg/ml in all patients studied, levels considered therapeutic by Padilha-Gonçalves²². Thus, the posology used was appropriate since it maintained satisfactory serum levels of the drug even among fast acetylators.

Finally, determination of acetylator phenotype and of serum sulfadoxin level as well as their relationships with G6PD and glutathione reductase activities represents an important and very useful parameter since it permits better clinical follow-up of the patients, with early observation of the possible onset of anemia and of therapeutic faults due to insufficient circulating levels of the drug.

RESUMO

Os autores avaliaram o fenótipo acetilador da isoniazida, hematócrito, hemoglobina, atividade da glicose-6-fosfato desidrogenase, glutathione reductase e os níveis séricos de sulfadoxina de 39 doentes com paracoccidioidomycose, sendo 33 do sexo masculino e 6 do feminino, com idades compreendidas entre 17 e 58 anos. Vinte e um (53,84%) doentes apresentaram fenótipo acetilador lento e 18 (46,16%) rápido. A atividade da glicose-6-fosfato desidrogenase (G6PD) esteve diminuída em 5 (23,80%) acetiladores lentos e 4 (22,22%) rápidos. A atividade da glutathione reductase esteve diminuída em 14 (66,66%) acetiladores lentos e 12 (66,66%) rápidos. Os níveis séricos de sulfadoxina livre e total foram maiores nos acetiladores lentos ($p < 0,02$). A análise dos resultados permite concluir que os níveis séricos de sulfadoxina relaciona-se com o fenótipo acetilador. Além disso, os níveis estiveram sempre acima de 50 µg/ml, níveis estes considerados terapêuticos. Por outro lado, a deficiência de glutathione reductase pode estar relacionada com a má absorção intestinal de nutrientes, entre eles riboflavina, vitamina precursora de FAD.

Palavras-chaves: Fenótipo acetilador da isoniazida. Paracoccidioidomycose. Glicose-6-fosfato desidrogenase (G6PD). Glutathione reductase. Sulfadoxina.

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