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Evaluation of Phagocytic Component of the Immune System in Patients with Organic Acidemia

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Abstract

Background: There are several reports of recurrent infections in patients with organic acidemia. Almost all studies on the immune system of these patients have investigated the immune system in the acidotic phase of the disease. In the present study, the phagocytic component of the immune system was evaluated in patients with organic acidemia.

Methods: After exclusion of patients in the acidotic phase of the disease, 31 patients with organic acidemia were included in the study. All patients completed the written informed consents, and the study was approved by the ethics committee of Mofid Children's hospital. Information including age, sex, type of organic acidemia, and history of hospitalization due to infection was recorded. Screening tests of phagocytic component of the immune system, including total and differential white blood cell (WBC) count and nitroblue tetrazolium (NBT) test, were performed for all the patients.

Results: The prevalence of neutropenia was high among patients (22.6%); it was even more frequent in patients younger than 3 years (42.6%). On the other hand, in most patients (93.5%), neutrophils showed normal function on the NBT test.

Conclusions: Neutropenia in the nonacidotic phase of organic acidemia can be the cause of recurrent infections in these patients. It can be independent of bone marrow suppression, caused by reduced production of these cells as a result of reduced pH in the acidotic phase of the disease.

Keywords: Organic Acidemia, NBT Test, Neutropenia, Phagocytic Cells, Metabolic Disease

1. Background

Inborn errors of metabolism (IEMs) include a large category of disorders, which result from congenital abnormalities in the activity of enzymes, involved in the metabolism of carbohydrates, fatty acids, and amino acid cycles (1). Organic acidemia refers to a category of congenital metabolic disorders, resulting from defective activities of enzymes, involved in the metabolism of branched essential amino acids (leucine, isoleucine, and valine). It notably includes maple syrup urine disease (MSUD), isovaleric acidemia (IVA), methylmalonic acidemia (MMA), propionic acidemia (PPA), and glutaric aciduria (GA) (2). This group of disorders is characterized by the presence of organic acids in the urine and clinical presentations in newborns, including feeding difficulties, vomiting, acidosis, and dehydration (2, 3).

Pancytopenia and neutropenia have been reported as the most common immunological and hematological problems in patients with acidemia (4, 5). According to multiple reports of infections caused by different pathogens in patients with organic acidemia, there is a possibility of accompanying immunodeficiency; therefore, exploring the components of the immune system seems valuable (6-10). According to a guideline presented in 2014, evaluation of serum C-reactive protein and blood culture is essential in the acute phase of these diseases, as the patients are often immunocompromised (11).

In several studies, the immune system of patients with organic acidemia has been evaluated only in the acidotic phase of the disease (8, 12). Therefore, we aimed to evaluate the screening parameters of the immune system in 31 patients with organic acidemia in the nonacidotic phase of the disease. In our previous study, we evaluated the complement and humoral components of the immune system in these patients, including serum IgA, IgG, IgE, IgM, C3, C4, CH50, isohemagglutinin titer, antitetanus IgG, and antidiphtheria IgG (13). In the present study, we investigated

the phagocytic arm of the immune system, using standard screening tests.

2. Methods

2.1. Study Population

In this study, 31 patients with organic acidemia were included. The diagnosis of organic acidemia was established, based on the patients' clinical findings and organic acids in the body fluids. The patients' clinical findings were compatible with the pathologically high levels of organic acids in the urine organic acid profile (studied by gas chromatography/mass spectrometry) and diagnostic acylcarnitine profile (studied by tandem mass spectrometry). The subjects were recruited based on their medical records in Mofid children's hospital. Patients in the acute phase of the disease (acidosis), those with other diseases (e.g., infectious diseases), and those receiving corticosteroids or other immune suppressive drugs were excluded from the study. All the patients followed the required diet and received treatment of organic acidemia.

2.2. Patient Information Questionnaire

A questionnaire including the patients' sex, age, type of organic acidemia, history of hospital admission and recurrent infections, and family history of metabolic diseases was completed according to the patients' medical records. Patients with a history of at least 5 hospital admissions within the past year were regarded as frequently admitted patients (14). Recurrent infections were defined as 2 or more severe infections in 1 year, 3 or more respiratory infections (e.g., sinusitis, otitis, and bronchitis) in 1 year, or need for antibiotics for 2 months/year (15).

2.3. Ethical Considerations

This study was approved by the Ethics Committee of Mofid Children's hospital, and written informed consents were obtained from all the participants.

2.4. Sample Collection

For this purpose, 1 mL of whole blood was obtained for nitroblue tetrazolium (NBT) and complete blood cell (CBC) tests with plastic 2 mL syringes, used for venipuncture with EDTA as the anticoagulant (1.5 - 2.0 mg of EDTA per mL of blood).

2.5. WBC Count

CBC and leukocyte differential counts were performed for each patient. Absolute neutrophil count (ANC) in the range of 1500 - 8000/mm^3 was considered normal, and patients with ANC less than 1500 were considered neutropenic. Severity of neutropenia was defined as mild (ANC, 1000 - 1500/mm^3), moderate (ANC, 500 - 1000/mm^3), and severe (ANC $\leq$ 500/mm^3) (2).

2.6. NBT Test

For this purpose, 50 μL of EDTA blood samples was mixed with 50 μL of NBT solution and incubated in a bain-marie at 37°C for 30 minutes. After incubation, the tubes were centrifuged for 3 minutes at 1500 g, and the supernatant was carefully removed. The remaining sediment was prepared as a slide and examined with a microscope after Giemsa staining. One-hundred neutrophils were counted, and the percentage of neutrophils with formazan sediments was reported. NBT more than 90% was considered normal.

2.7. Statistical Analysis

SPSS version 23 was used for statistical analyses, and correlations were examined with Spearman's correlation test at a significance level of $\leq$ 0.05.

3. Results

In this study, a total of 31 patients (11 patients with MSUD, 10 patients with MMA, 5 patients with IVA, 4 patients with GA, and 1 patient with PPA) were studied, among whom 14 were male and 17 were female. The mean age of the patients was 49 $\pm$ 37 months. In total, 58% of the patients had experienced hospital readmission, and 41.9% had a history of recurrent infections. Moreover, 22.6% of the patients had parents with a family history of metabolic diseases, and 74.2% had consanguineous marriages. In Table 1, the demographic data and test results of the patients are presented.

Based on the findings, 7 (22.5%) patients had neutropenia, 4 (12.9%) had mild neutropenia, and 3 (9.6%) had moderate neutropenia. Neutropenia was more prevalent in patients younger than 3 years (42.6%; $P < 0.01$). As the findings revealed, 2 (6.45%) patients had abnormal NBT test results.

4. Discussion

Organic acidemia comprises a group of IEMs, associated with several clinical symptoms, including infections suggestive of immunodeficiency (16-19). In a previous

Table 1. The Demographic Data and Test Results of the Evaluated Patients

Patients	Sex	Age, mo	Type of Organic acidemia	WBC, cell/μL	ANC, cell/μL	NBT, %
1	F	52	MMA	17200	7224	100
2	M	45	MSUD	6400	4672	80
3	M	60	IVA	7600	3648	100
4	F	83	IVA	10100	7171	95
5	F	17	MSUD	9500	3610	100
6	M	102	IVA	5900	2478	95
7	M	41	MMA	8300	4482	100
8	F	60	MSUD	11800	8496	95
9	M	9	MMA	8300	2324	95
10	M	13	MMA	6700	1340	95
11	F	37	MMA	6100	3294	90
12	F	4	MSUD	6800	680	94
13	M	20	PA	4500	900	97
14	M	30	IVA	9300	3348	100
15	M	19	MMA	7000	1890	99
16	M	144	MMA	9800	4900	100
17	F	21	MSUD	8800	5456	100
18	M	12	MSUD	9300	4092	100
19	F	66	MSUD	6000	2760	100
20	F	75	MMA	7800	6084	100
21	M	51	MSUD	5900	1239	100
22	F	84	MSUD	7300	2774	100
23	F	46	MMA	5100	1275	100
24	F	15	IVA	9200	1840	95
25	M	90	MMA	13100	7860	100
26	F	9	GA	8900	1424	99
27	M	49	MSUD	11400	7296	100
28	F	30	GA	8100	972	70
29	F	42	MSUD	7200	2304	100
30	F	144	GA	8800	5984	92
31	F	36	GA	6000	3540	89

study, to evaluate humoral immunity in patients with organic acidemia, serum IgA, IgM, IgG, and IgE, antitetanus, antidiphtheria IgG, and isohemagglutinin titer were measured. Moreover, to evaluate the components of the complement system, serum C3, C4, and CH50 were measured; however, no defects were detected in these parameters (13).

In the present study, the phagocytic component of the immune system was evaluated in patients with organic acidemia in the nonacidic phase, since neutropenia with or without platelet deficiency or even pancytopenia, besides infection, has been shown to be a characteristic of organic acidemia in the acidotic phase (20, 21). In multiple studies, neutropenia has been reported in some patients with organic acidemia in the acidotic phase, and many hypotheses have been proposed to explain infections and neutropenia in these patients (7, 22). In a study conducted on 33 adults and 18 newborns with PPA in the acidotic phase, neutropenia was reported in 42% and 11% of

patients, respectively (21).

Similarly, in vitro studies have been performed to investigate the possible mechanisms of neutropenia in patients with organic acidemia. Inoue S. et al. in 1961 reported the dose-dependent inhibition of growth and development of bone marrow-committed stem cells in the presence of methylmalonic acids (23). In a similar study, Hutchinson RJ and colleagues revealed that acetate, propionate, and methylmalonate (to a lesser extent) induce inhibitory effects on the granulopoiesis of bone marrow granulocyte/macrophage progenitor cells (24). Meanwhile, neutropenia in the nonacidotic phase was frequent in this study, although it retained the normal function of neutrophils. In this regard, Trevani A. S. et al. reported that the acidic extracellular environment stimulates the activation of human neutrophils (25).

As the most important screening measures, CBC and NBT tests were applied to evaluate the phagocytic arm of the immune system. However, confirmation of NBT results with a more precise test (e.g., dihydrorhodamine test) would be preferable. Neutropenia in the nonacidotic phase of organic acidemia can be the cause of recurrent infections. It can be independent of bone marrow suppression, caused by reduced production of these cells as a result of reduced pH in the acidotic phase of the disease. Further studies are required to investigate the effect of pH and acidic environment on neutrophils and to identify the main cause of neutropenia in patients with organic acidemia.

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