Hematology

HYPOPHYSIS-ADRENAL SYSTEM AND IMMUNOLOGIC STATUS OF PATIENTS WITH IDIOPATHIC THROMBOCYTOPENIC PURPURA

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SUMMARY : A series of 62 patients, l7 males and 45 females with idiopathic thrombocytopenic purpura and 35 patients forming the control group were studied. All patients were given glucocorticosteroid hormones. The first group of patients sensitive to hormone revealed endogenic cortisol levels above normal before therapy. Hypercortisolemia was observed in the cases with severe, mild or intermediate forms of disease along with complete compensation under GCH therapy. These patients produced adequate reaction to stress, cortisol activation in pathologic process with mobilization of the preventation forces for elimination of unnecessary agents. In all ITP cases with response to hormone therapy (group I) endogenic cortisol values reached to immunodepressive levels.

Key words : Hypophysis-adrenal system, immunologic status, idiopathic thrombocytopenic purpura.

INTRODUCTION

Recently the role of hormonal and immune systems regarding pathogenesis of autoimmune blood diseases, in particular, idiopathic thrombocytopenic purpura (ITP) was widely studied. It is observed that ITP patients reveal several disturbances of the immune status with T-suppressors deficiency, considerable depression of cell immunity and humoral immunity activation that increases immunoglobulin synthesis and antibody production (1, 2). Hypofunctions of adrenal glands also play their definite roles in ITP pathogenesis (3).

Data of the reports published concerning the clinical and laboratory indices of ITP patients compared with those of normal cases. At the same time adequate evaluation of the indicators of therapeutic responses require further investigations. ITP therapy includes glucocorticosteroid hormones (GCH) as the main therapeutic agent. Under this treatment some patients reveal steady clinical and hematological compensation and general improvement while others remain refractory, even to relatively high doses of these hormones. For the latter group of patients splenectomy, immuno-depressants or plasma exchange (plasmapheresis) and immuno-absorption are advised.

One of the mechanisms of GCH may be through the immune system and specifically through immunosuppression. These considerations justify the study of the adaptation-protection responses and mechanisms of immunotrophic hormone function in ITP patients. Such mechanisms are of primary importance for the definition of role of adrenocorticotrophic hormones (ACTH), and cortisol in the adaptation system and their participation in homeostatic regulation. They are also important from the point of T-cells sub-populations involved in the immune response regulation and interaction of these lymphocytes with steroid hormones.

Proceeding from above mentioned considerations it seemed interesting for us to study hypophysis-adrenal system and immunologic status simultaneously in ITP patients.

MATERIAL AND METHODS

We observed 62 ITP patients (17 males, 45 females) between the ages of 16-54 with disease duration from 3 months to 7 years. ITP was diagnosed depending on typical clinical picture with hemorrhagic syndrome expressed in ecchymosis, petechiae, rashes, subcutaneous hemorrhages on body and limbs, nasal or uterine hemorrhages with normal size spleen or splenomegaly. Auto antibodies fixed on platelets were defined by immunofluorescent method (4). To

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this test positive reaction was observed in all patients included in this series. Detailed analyses of clinical and hematologic data permitted identification of symptomatic and heteroimmune ITP forms. Absence of clinical and/or hematological compensation or improvement after GCH course peros in doses equivalent to 1.0-1.5 mg/kg/day of prednisone during 4-6 weeks was considered as hormone-refractoriness. Sampling and evaluations were made at the moment of hospitalization and after therapy. At the moment of testing the patients both were hospitalized for the first time or had been receiving GCH from 1 to 4 months, venous blood samples were removed in post absorptive state in the morning. ACTH and cortisol levels were measured by radiommune assay using "Gamma-12" - counter, commercial sets "Instar" (USA), and "Steron-K ^{125}I" (USSR). Mean values of cortisol-refractory lymphocyte populations were determined using morpho-functional criteria depending on lymphocytotoxic GCH effect during long-term incubation (5). Number of lymphocytes in peripheral blood was evaluated by, method of spontaneous rosette formation (6); numbers of subpopulations of T-helpers (T_h-equivalent) and T-suppressors (T_s-equivalent) were evaluated by additional E-rosettes test using the ophilline (7); B-lymphocytes were evaluated by method of indirect complementary rosettes EAG (8); young immature B-lymphocytes of mouse-EM-rosettes (9); serum immunoglobines (Ig) by radial immuno-diffusion method (10); number of circulating immune complexes by precipitation in polyethylenglicole method (11). Control group considered of 35 healthy first time donors. Data obtained in this study was analyzed by variation statistics method of student with investigation of correlation dependences. Patients were retrospectively divided into 2 groups to evaluate therapeutic effects. The first group included 35 ITP patients taking GCH and revealing complete clinical-hematological compensation or improvement. Twenty-seven patients of the second group responded unfavorably to the same treatment revealing GCH refractoriness.

RESULTS AND DISCUSSION

The study showed (Table 1) that the level of ACTH in the first group patients was much higher than that in the second group ($p<0.001$) of patients or normal controls. ACTH values in the second group of ITP patients were comparable to those of normal controls. There is no evidence of change in ACTH values during the time of treatment in either groups of patients.

In the first group of patients the initial levels of cortisol were evaluated ($p<0.001$). In some cases hypercortisolamina (reaching to from 216.2 to 431.9 pg/ml) was observed while endogenic hormone values differed slightly influenced by GCH (variants from 207.8 to 331.2 pg/ml). Reduction of cortisol level was noted in the remainder of the first group of patients (31%). Final effect of the treatment expressed in reduction of cortisol level to normal.

Table 1: Indices of ACTH, endogenic cortisol and cortisol-refractory lymphocytes populations of ITP patients during the treatment (M±m).

Index	ITP patients		Control group n=35
	Group 1 n=35	Group 2 n=27	
ACTH (pg/ml)			
Before treatment	96.6±3.5*,**	43.5±5.1	
After treatment	100.9±2.7*,**	47.3±3.5	48.4±5.2
Cortisol (ng/ml)			
Before treatment	170.3±9.5*,**	71.9±3.5*	
After treatment	124.8±9.4**	76.9±3.1*	127.9±6.8
Cortisol refractory lymphocytes populations			
Before treatment	51.3±0.9**	73.3±1.5*	
After treatment	60.8±1.8**	82.6±1.3*	54.5±5.1

* : Statistic authenticity of indices compared with control;

** : Statistic authenticity between the first and the second group of patients.

The second group of patients refractory to GCH revealed initial cortisol levels significantly lower than those of the control group, and that of the first group of patients ($p<0.001$). These differences were maintained during the period of treatment.

Hormone indices of the first and second groups of patients revealed several trends. If GCH-refractory patients compared to control cases the initial value of endogenic cortisol was significantly lower, the ACTH remained within the normal limits, while in the first group of patients these parameters were significantly higher.

Testing cortisol refractory lymphocyte populations we noted an increase in number of similar lymphocytes in hormone refractory patients in comparison with hormone sensitive ($p<0.001$) and control ($p<0.001$) groups. During the treatment we observed a significant increase of cortisol refractory lymphocyte populations in both groups of patients ($p<0.002$; $p<0.001$). Comparison of the immunologic indices (Table 2) uncovered differences between two groups of patients. GCH-refractory patients had T-cells deficiency and EAC-rosettes mean lower, EM-rosettes higher than that in the first group. EM-rosettes value were in both groups higher compared to the control group, but in the second group authentically higher than in

Table 2: Immunologic indices of ITP patients before the treatment (M±m).

Index		ITP patients		Control group n=35
		Group 1 n=35	Group 2 n=27	
T-lymphocytes				
E-rosettes	%	45.5±2.0*,**	40.1±1.3*	61.1±0.7
	abs	748.1±70.1	695.1±39.7	1265.0±61.6
T_h-equivalent	%	34.3±2.5	30.7±1.8	40.8±0.7
	abs	540.1±30.1	505.1±48.7	946.2±92.1
T_s-equivalent	%	11.2±0.9	9.4±0.8	20.5±0.1
	abs	208.1±15.1	190.0±6.7	478.5±51.0
T_h/T_s		3.1±0.4	3.3±0.5	2.0±0.3
B-lymphocytes				
EAC-rosettes	%	22.8±1.0	19.6±0.4	19.0±0.3
	abs	405.1±18.0	380.0±18.1	391.8±17.8
EM-rosettes	%	10.1±0.5**	22.1±1.1*	8.8±0.26
	abs	190.0±10.0	465.1±35.0	179.4±8.4
Immunoglobulins g/l				
IgA		2.01±0.1	2.0±0.1	1.84±0.09
IgG		20.12±0.7*,**	14.4±0.8	12.42±0.76
IgM		1.71±0.2	1.7±0.1	1.35±0.01
Circulating immune complexes unity of optimal strength		0.145±0.018*,**	0.201±0.02*	0.066±0.002

* : Statistic authenticity of indices compared with control;

** : Statistic authenticity between the first and the second group of patients.

first one ($p<0.001$). The rise of number of B-cells in patients in response to GCH was followed by a significant elevation of all classes of immunoglobulins and IgG level. It was significantly higher than that in patients refractory to GCH ($p<0.001$). Number of circulating immune complexes in blood serum of ITP patients was higher than the control indices, but in the second group it was authentically higher than that of the first group of patients ($p<0.05$). In both groups a significant reduction of T_h and T_s subpopulations of lymphocytes was observed in comparison with control rates, that was followed by misbalance of their ratio, and we noted an elevation of immunoglobulin levels, T_h/T_s index to 3.1-3.3 as a result (rate limit -2.0). We identified a correlation in refractory cases between reduction of T_s number which, obviously have suppressive function and rising circulating immunocomplexes levels ($r= -0.402$, $p<0.05$).

Big stock of circulating immunocomplexes along with autoimmune disease not correlated with antigen stimulus and level of immunoglobulins may depend on distraction of circulating immunocomplexes elimination mechanisms. At the same time circulating immunocomplexes may be attached on circulating blood cells damaging them and thus causing immune cytopenias.

It has earlier been mentioned that endogenic cortisol may be a factor preventing autoimmunity processing (12). Hence we can consider that reduction in endogenic hormone levels observed in our study may play a significant role in pathogenesis of this disease. In some researches it was shown that on the peak of immune response the GCH value in blood increases until it reaches a level which may be immuno-depressive. This elevation may be connected with lymphoid cells ability to produce corticosterone inducting and ACTH-shaped factors. These humoral lymphocyte factors may influence the glucocorticosteroid function both through hypothalamohypophysis (corticosterone-inducting factor increases the ACTH level in blood) or directly through adrenal glands (ACTH-shaped factor), i.e. through the "long" or "short" regulation arc (13).

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