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Evaluation of Humoral Immune Function in Patients with Chronic Idiopathic Thrombocytopenic Purpura

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ABSTRACT

Coincidence of autoimmune diseases such as immune thrombocytopenic purpura (ITP) with immunodeficiencies has been reported previously in patients who suffered from primary antibody deficiency (PAD). But there is no original study on immunological profiles of ITP patients to find out their probable immune deficiency.

In this case-control study, ITP patients' humoral immunity was investigated for diagnosis of PAD in comparison with normal population. To evaluate the humoral immune system against polysaccharide antigens, patients' serum immunoglobulin levels were measured and a 23-valent pneumococcal capsular polysaccharide vaccine (PPV23) was administrated to evaluate the antibody response to vaccination.

In this study, 14 out of 36 patients (39%) were diagnosed with antibody mediated immune deficiency including 2 patients (5.5%) with immunoglobulin class deficiency and 4 (11%) with IgG subclass deficiency. The remaining patients suffered from specific antibody deficiency. The most frequent deficiency in ITP patients was specific antibody deficiency.

Therefore, immunological survey on ITP patients may be important especially for those who have undergone splenectomy.

Keywords: Idiopathic; Immunologic Deficiency Syndromes; Purpura; Splenectomy; Thrombocytopenic

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INTRODUCTION

Idiopathic thrombocytopenic purpura (ITP) is a hematologic disorder characterized by impairment of

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coagulatory system due to autoimmune destruction of sensitized platelets by IgG antibodies. Diagnosis of ITP is made when an isolated decreased platelets count (under 150,000/mL) is detected while other clinically apparent etiologies of thrombocytopenia like drugs and other causes are ruled out.[1-3] The etiologies of ITP are unclear; however some of probable causes are classified due to either genetic or acquired factors.[4-6] Acquired factors are most often immunological problems, like cross reactive antibodies against platelet membrane glycoproteins which are made during viral infections and loss of peripheral tolerance resulting in self-reactive antibody production which destroys platelets. Also immune system might have an abnormal function during autoimmune or lymphoproliferative diseases, such as systemic lupus erythematosis (SLE) and lymphoid leukemias. Splenectomy may be needed in some patients affected by chronic ITP.[7] Effective immunization against polysaccharide antigens which should be given before this surgery is of great importance in these patients since asplenic patients are at increased risk of severe infections such as post-splenectomy sepsis (PSS) caused by encapsulated bacteria.[8] Primary antibody deficiency (PAD) is a group of diseases with decreased human immunoglobulin levels and higher susceptibility to chronic and recurrent infections.[9] Approximately 20-22% of patients with definitive diagnosis of PAD develop autoimmune disorders with prominence of ITP and autoimmune hemolytic anemia (AIHA).[10,11] ITP seems to be the first manifestation of PAD diseases, such as common variable immunodeficiency (CVID), which may delay the diagnosis of PAD and lead to irreversible complications.[12-15] Notwithstanding these documents, there is no study on patients with ITP to investigate the concomitance of ITP with immune deficiencies. In this study, ITP patients' humoral immunity was investigated for diagnosis of PAD.

PATIENTS AND METHODS

Patients

Forty-two children and adult patients who were referred to our department with established diagnosis of chronic ITP were enrolled in this study during 2010-2011. Diagnosis of ITP was determined by guidelines of the American Society of Hematology.[16] According to exclusion criteria for all patients, we did not enter patients with following characteristic into this study: cases with previous pneumococcal vaccination within the past five years, receiving corticosteroids or immunosuppressive therapy in the previous six months, any treatments for allograft rejection, use of pooled immunoglobulin product within the last six months and splenectomy.

After exclusion, for each remaining 36 patients, sex- and age-matched healthy individuals were also evaluated as the control group. The control group, made up of 36 healthy donors, was also studied to establish a criterion for normal response to 23-valent pneumococcal vaccine. None of them had a history of primary or secondary immunodeficiency and recurrent infections.

This study was approved by the Ethics Committee of Tehran University of Medical Sciences; moreover written informed consents were also obtained from the adult patients and children's parent(s).

Methods

A questionnaire was designed to collect demographic information and medical histories for each patient by reviewing the patients' records. Patients' blood samples were taken from 36 patients to measure immunoglobulin isotype (IgA, IgM, and IgG) concentrations using nephelometry (Behring Nephelometer, Behringwerke, Marburg, Germany)[17] and IgG-subclasses by enzyme-linked immunosorbent assay method (ELISA).[18,19] Out of 36 patients, 25 (11 patients refused to receive vaccination) received single dose of 0.5 mL unconjugated pneumococcus polyvalent vaccine (PNEUMO 23 Aventis, Pasteur, France). Blood samples were taken before and 28 days after vaccination. In both patient and control groups, specific antibodies against whole pneumococcal antigens were measured using the protocol of the third-generation ELISA format.[20] Results were reported as end point titer determined by the highest dilution giving an optical density of 0.2 or higher. There are no universal criteria for adequate antibody response to pneumococcal specific antibody levels and each laboratory has to consider the response in normal healthy controls to generate criteria to define hyporesponsiveness to vaccine. All subjects showing an increase in specific antibody titers equal to or greater than lower limit of the 2-tailed 90% probability interval of post-immunization specific IgG of the healthy adults were defined as responders.[21] The median titers before and after vaccination titers in the

control group were 70 and 450 U/mL, respectively. The lower limit of the 2-tailed 90% probability interval of post-immunization specific IgG was 129 U/mL, using the minimum significant increase for adequate response in the patients' group.

Statistical Analysis

The data were analyzed using SPSS 13.0 software package. Specific antibody titers were expressed as the geometric mean. Comparisons between groups were performed using student T test; Mann- Whitney U test were used when the distribution was not normal for the selected variable.

RESULTS

Patients' Characteristics

Thirty six patients (17 males and 19 females) aged between 3 to 51 years (mean age 17.8±4.5 years) with diagnosis of chronic ITP were evaluated for humoral immunity during this study. The age of patients at the time of the diagnosis of ITP was 16.0±12.1 years and they were under follow up for 4.7±2.9 years. None of the patients had medical history of recurrent infections or suffering from other complications of PAD diseases. The mean serum levels of Immunoglobulins of cases did not significantly differ from controls (Table 1). Other immunological data of patients are presented in Table 1. Low serum IgA levels were observed in 2 patients (5.5%) including a 7-year-old female (P10) and a 13-year-old male (P11). These cases had isolated IgA deficiency with normal IgG subclasses and sufficient response to vaccine antigens.

Immunoglobulin G Subclass Deficiency

Isolated IgG subclass deficiency was detected in 4 patients (11%). The first one (p1) had IgG2 subclass deficiency and impaired response to the specific antigen. The second case (P12) was a 31–year-old female who had isolated IgG1 subclass deficiency with normal specific antigen response. The third patient (P13), 8 year-old female had isolated IgG4 subclass deficiency while having normal responses to antigen. The last patient (P14) was a 5-year-old female with isolated IgG4 subclass deficiency.

Specific Antibody Deficiency

Among all patients, 25 individuals received unconjugated pneumococcus polyvalent (PCP) vaccine in whom antibodies against PCP antigens were measured before and 28 days after vaccination. In contrast to control group, 9 out of 25 patients (36%) were found to be hypo-responsive to vaccine and had specific antibody deficiency (SAD) against PCP vaccination (Table 2). Although one of hyporesponsive vaccinated patients (4%) showed an association with isolated IgG2 subclass deficiency, remaining 8 patients (32%) had SAD with normal serum immunoglobulin and IgG subclass levels. The post-immunization serum IgG level against PCP antigen was 222.3±102.4 U/ml in patients which was significantly lower than controls (312.9±114.9U/ml; p=0.02).

Table 1. Comparison of characteristics of 36 patients with chronic ITP and their age- and sex-matched controls.

Parameters	Cases	Controls	*p*-value
IgG (mg/dL)	1213.2±533.1	1472.4±393.5	0.48
IgG1 (mg/dL)	702.7±129.3	923.5±147.2	0.23
IgG2 (mg/dL)	296.0±114.2	305±87.3	0.74
IgG3 (mg/dL)	102.9±72.8	127.1±63.9	0.21
IgG4 (mg/dL)	33.9±12.6	32.0±14.9	0.52
IgA (mg/dL)	159.0±76.2	174.9±32.3	0.07
IgM (mg/dL)	135.7±72.2	144.0±61.9	0.12
Pre-immunization	116.7±90.8	100.7±54.7	0.29
Post-immunization	222.3±102.4	312.9±114.9	0.02*
Increase	253.1±134.0	266.8± 129.3	0.14
Fold Increase Ratio	12.7±2.7	14.3± 3.7	0.09

* Significant difference

Table 2. Characteristics of patients with defects in antibody mediated immunity (n=14).

No.	Sex	age	Type of deficiency	IgG Before vaccination* (µg/mL)	IgG after vaccination** (µg/mL)	Increase (µg/mL)	Response to PPV23
1	M	11	SAD+IgG2 def	15	130	115	Hypo
2	F	51	SAD	150	190	40	Hypo
3	F	15	SAD	23	24	1	Hypo
4	M	17	SAD	22	32	10	Hypo
5	M	28	SAD	180	200	20	Hypo
6	F	22	SAD	80	150	70	Hypo
7	M	11	SAD	398	408	10	Hypo
8	F	26	SAD	184	179	5	Hypo
9	F	27	SAD	247	282	35	Hypo
10	F	7	IgA def	65	1000	935	Normal
11	M	13	IgA def	14.6	613	598.4	Normal
12	F	31	IgG1 def	25	450	425	Normal
13	F	8	IgG4 def	109	325	216	Normal
14	F	5	IgG4 def	-	-	-	NA

Normal immunoglobulin and subclass levels are not shown in this table.

NA: not assessed; SAD: specific antibody deficiency; hypo: hypo responsive; def: deficiency

* Total IgG level (µg/mL) to PPV 23 Before vaccination

**Total IgG level (µg/mL) to PPV 23 four weeks after vaccination

Table 3. Characteristics of patients with chronic ITP according to antibody deficiency. Data are presented as geometric mean ±SD.

Topics	Normal specific antibody production (n= 16)	Specific antibody deficiency (n=9)	*p*-value
Age	14.0± 6.8	23.11±8.4	0.03*
Male / Female	7/9	4/5	0.64
IgG (mg/dL)	1096.4± 324.8	1340.3± 235.7	0.20
IgG1 (mg/dL)	602.8± 126.9	816.0±228.4	0.08
IgG2 (mg/dL)	264.2±93.8	392.2±192.3	0.13
IgG3 (mg/dL)	79.3±34.9	137.5±39.2	0.07
IgG4 (mg/dL)	28.3± 17.0	50.9±39.2	0.01*
IgA (mg/dL)	139.5±72.8	213.6±118.4	0.42
IgM (mg/dL)	120.4±69.5	172.4±79.7	0.31
Pre-immunization	63.3± 25.8	149.0±87.2	0.23
Post-immunization	441.2±157.2	177.4± 64.2	0.001*
Increase	385.3± 179.0	36.4±27.3	0.001*
Fold Increase Ratio	18.26±11.9	2.06 ±1.3	0.001*

* Statistically significant difference

ITP patients were also divided into two groups based on their response against pneumococcal vaccination and comparison was performed on their Immunological characteristic which is shown in Table 3. Post-immunization specific antibodies against whole pneumococcal antigens were significantly lower in the hypo-responder group comparing to normal-responders (177.4±64.2 *vs.* 441.2±157.2; p=0.001). They also showed lower increase in specific antibodies after vaccination (36.4±27.3 *vs.* 385.3±179.0; p=0.001) and 8 fold increase ratio (2.06 ±1.3 *vs.* 18.26±11.9; p=0.001).

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DISCUSSION

Immune dysregulation phenomenon in PAD diseases leads to anti-platelet antibody production and autoimmune destruction of platelets. According to results of this study, 38% of chronic ITP patients showed signs of primary immunodeficiency.

Selective IgA deficiency is a common immunologic abnormality which affects approximately 1 in 300 to 700 (0.2%) individuals,[22,23] but this proportion was significantly higher in population of chronic ITP patients in our study (5.0%). About two thirds of patients with IgA deficiency are asymptomatic requiring no treatment. [22,23]

IgG subclass deficiency is a disorder defined by significant decrease in serum levels of IgG subclasses. Up to 20% of healthy population may have reduced IgG subclasses and need no therapy and should not be marked as immunodeficient. However, this disorder should be investigated in presence of another immunoglobulin type deficiency, such as presence of unresponsiveness to polysaccharide antigens, or a history of recurrent infections. In our study, 4 patients (11.1%) showed low levels of IgG subclasses, only in one of them (with reduced IgG2 levels), antibody response to pneumococcal vaccine was insufficient. So, these results of lower levels of IgG subclasses in these patients are not sufficient to identify an immune deficiency disease for them.

SAD is an immunodeficiency which is defined as poor response to polysaccharide antigens in individuals with normal total immunoglobulin levels and IgG subclasses.[24] The etiology and immunopathogenesis of SAD as a primary immunodeficiency is still unclear.[25] Our study showed significant low increase in antibody response levels to 23 valent PPV in ITP patients in comparison to other normal responders. To our knowledge, there is no other study regarding antibody response to antigens in patients with ITP. Furthermore, our study presented SAD as the most frequent underlying primary immune deficiency in patients with ITP for the first time.

In design of our study, we excluded ITP patients who were splenctomized, although ablation of spleen is necessary for persistent hemorrhagic symptoms of chronic ITP.[26,27] This exclusion was performed because splenectomy brings confounding effects and induces immune dysfunction leading to increased risk of infections with encapsulated bacteria mostly in children younger than 4 years.[28]

However, the results of this study on existence of spectrum of PAD in ITP patients may recommend immunological study or immunization in ITP children especially in patients who undergo splenectomy for avoiding the decline of immune system. Measurement of post-vaccination antibody levels in ITP patients can be used to identify the poor responders who most likely are at increased risk of pneumococcal infection after splenectomy in future events with the conventional vaccination against pneumococcus which they receive before splenectomy. Since ITP patients with underlying SAD have impaired antibody response to presplenectomy immunization by pneumococcal vaccines, they may experience certain life threatening complications after splenectomy, like PSS.

The patients with SAD need to be followed up because of their impaired antibody response to the antigen and should be monitored for probable post splenectomy infections. They can be offered other prophylactic methods such as IVIG therapy, long term antibiotics and immunization with pneumococcal conjugate vaccines.

Nowadays by identifying the role of immune system in developing such situations, immunoglobulin replacement therapy like IVIG can be considered for therapy and management of ITP with both replacement and immunemodulatory effects. Moreover, IVIG may prevent the progression of other impositions of PID such as recurrent infections, autoimmune diseases and malignancies.

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