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Anti *Saccharomyces cerevisiae* Antibodies in Patients With Anti-β2 Glycoprotein I Antibodies

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Background: In this study, cross-reactive epitopes on β2 glycoprotein I and *Saccharomyces cerevisiae* have been described. The objective of our study was to determine the frequency of anti *S. cerevisiae* antibodies (ASCA) in patients with anti-β2 glycoprotein I antibodies (aβ2GPI). *Methods:* A retrospective study was conducted in 77 patients with aβ2GPI (aβ2GPI-IgG or aβ2GPI-IgA). Eighty blood donors were used as a control group. ASCA IgG and ASCA IgA were determined by Enzyme Linked Immunosorbent Assay (ELISA). *Results:* Thirteen patients among 77 had ASCA. ASCA (IgA or IgG) was significantly more frequent in patients than in healthy subjects (16.9% vs. 3.7%, $P = 0.01$). The positivity of both ASCA IgG and ASCA IgA is higher in patients than in control group (6.5% vs. 0%, $P = 0.02$). The frequency of ASCA IgG was significantly higher in patients than in the control group (15.6% vs. 2.5%, $P = 0.009$). In females, the frequency of ASCA IgG was significantly higher in patients than in control group (17.5% vs. 3.7%, $P = 0.03$). The average titer of ASCA IgG was significantly higher in patients than in the control group (9.7 ± 23 U/ml vs. 2.2 ± 2.8 U/ml; $P = 0.004$). ASCA IgG was significantly more frequent than ASCA IgA in all patients (15.6% vs. 7.8%, $P = 0.04$). *Conclusion:* The frequency of ASCA was significantly higher in patients with aβ2GPI than in the control group. J. Clin. Lab. Anal. **30**:818–822, 2016. © 2016 Wiley Periodicals, Inc.

Key words: anti-β2 glycoprotein I antibodies; anti *Saccharomyces cerevisiae antibodies*; Tunisia

INTRODUCTION

Beta2 glycoprotein I (β2GPI), a cofactor of anticardiolipin antibodies, plays an important role in blood clotting and in immune response (1). Anti-β2GPI antibodies (aβ2GPI) are potentially thrombogenic and responsible for several pathological complications (2, 3).

Anti *Saccharomyces cerevisiae* antibodies (ASCA), yeast commonly used in food industry, were considered as a serological marker for Crohn's disease (4). It has also been shown that ASCA had a high predictive value for inflammatory bowel disease (5). Additionally, elevated levels of ASCA had been found in patients with Behcet's disease (6), spondyloarthritis (7), coeliac disease (8, 9), intestinal tuberculosis (10), primary biliary cirrhosis (11, 12), autoimmune hepatitis (13), type 1 diabetes (14), and autoimmune thyroid disease (15, 16).

Recently we have demonstrated that ASCA were more frequent in patients with systemic lupus erythematosus (SLE) than in the control group (17). Furthermore, Krause et al. (18) described ASCA in primary antiphospholipid syndrome (APLS) and in patients with APLS associated with SLE. Moreover, these authors (18) have described cross-reactive epitopes on β2GPI and *S. cerevisiae.* So, the aim of our study was to determine the frequency of ASCA in the Tunisian patients who have aβ2GPI and in whom SLE was excluded.

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PATIENTS AND METHODS

Patients

In our retrospective and multicenter study, we collected 77 sera samples, via the database of our immunology laboratory, from 77 patients (14 males, 63 females, median age 41 years, age range 24–83 years) with a positivity of aβ2GPI. Sera were collected between 2012 and 2013 from nine hospitals in the Center of Tunisia. Patients were admitted for suspicion of APLS. We cannot confirm that patients had APLS because we did not have the second sample for anti-phospholipid antibodies (aPL) assay. SLE was excluded in all patients.

Sera of 80 blood donors (54 female and 26 male) were collected as normal controls. All sera were negative for aβ2GPI and were stored at −80°C until they were used. The study was approved by local Ethics Committee and all the patients gave their informed consent.

Methods

Anti *S. cerevisiae antibodies*

ASCA IgA and IgG were detected by a commercially available ELISA kit (Orgentec®, Mainz, Germany). The antigen contained highly purified mannan from *S. cerevisiae*. Results were expressed as arbitrary units with a cut-off for positivity of 10 U/ml following the manufacturer's instructions.

aβ2GPI

IgG aβ2GPI and IgA aβ2GPI were determined by a commercial ELISA (Orgentec®) using a purified human β2-glycoprotein I. Results were expressed as arbitrary units with a cut-off for positivity of 8 U/ml following the manufacturer's instructions.

Statistical Analysis

The frequencies were compared using Chi-square or Fisher's exact test. A P-value less than 0.05 was considered significant.

RESULTS

Frequency of ASCA in Patients and in the Control Group

Compared to the control group, patients had a significantly higher frequency of ASCA (IgG or IgA; 16.9% vs. 3.7 %, $P = 0.01$) and ASCA IgG (15.6% vs. 2.5%, $P = 0.009$). ASCA IgA was higher in patients than in the control group, but reaching a borderline significance (7.8% vs. 1.2%, $P = 0.05$). The positivity of both ASCA IgG and ASCA IgA is higher in patients than in the control group (6.5% vs. 0%, $P = 0.02$; Table 1).

TABLE 1. Frequency of ASCA in Patients and in the Control Group

	Patients ($n = 77$)	Control group ($n = 80$)	P
ASCA IgG or IgA	16.9% (13/77)	3.7 % (3/80)	**0.01**
ASCA IgG and IgA	6.5% (5/77)	0 %	**0.02**
ASCA IgG	15.6%* (12/77)	2.5% (2/80)	**0.009**
ASCA IgA	7.8%* (6/77)	1.2% (1/80)	0.05

ASCA, anti *Saccharomyces cerevisiae* antibodies.
*$P = 0.04$.

Frequency of ASCA According to Gender

Thirteen patients among 77 had ASCA. Among these 13 patients, 12 were females. ASCA (IgG or IgA) was more frequent in females (19%) than in males (7.1%), but the difference was not statistically different. In females, the frequency of ASCA IgG was significantly higher in patients than the control group (17.5% vs. 3.7%, $P = 0.03$). The positivity of both ASCA IgG and ASCA IgA is higher in female patients than in the control group (7.9% vs. 0%, $P = 0.04$). In females, ASCA (IgG or IgA) were more frequent in patients than in the control group, but reaching a borderline significance (19.0% vs. 5.5%, $P = 0.05$; Table 2).

Comparison Between ASCA IgG and ASCA IgA

ASCA IgG was significantly more frequent than ASCA IgA in all patients (15.6% vs. 7.8%, $P = 0.04$; Table 1). ASCA IgG levels were significantly higher in patients than in the control group (9.7 ± 23 vs. 2.2 ± 2.8; $P = 0.004$), whereas ASCA IgA levels were almost equal in patients and the control group (Table 3).

Frequency of ASCA and aβ2GPI Isotypes in 77 Patients

In patients, ASCA IgG was significantly more frequent than ASCA IgA (15.6% vs. 7.8%, $P = 0.04$). In contrast, aβ2GPI IgA was significantly more frequent than aβ2GPI IgG (83.1% vs. 22%, $P \leq 10^{-6}$; Table 4).

Characteristics of 13 Patients With ASCA

Among 77 patients with aβ2GPI (IgG or IgA), 13 have ASCA (IgG or IgA). Characteristics of these 13 patients are shown in Table 5.

TABLE 2. Frequency of ASCA According to Gender

	Female patients ($n = 63$)	Female subjects of control group ($n = 54$)	P	Male patients ($n = 14$)	Male subjects of control group ($n = 26$)	P
ASCA IgG or IgA	19% (12 /63)	5.5% (3/54)	0.05	7.1% (1/14)	0%	NS
ASCA IgG and IgA	7.9% (5/63)	0%	**0.04**	0%	0%	NS
ASCA IgG	17.5% (11/63)	3.7% (2/54)	**0.03**	7.1% (1/14)	3.8% (1/26)	NS
ASCA IgA	9.5% (6/63)	1.8% (1/54)	NS	0%	3.8% (1/26)	NS

ASCA, anti *Saccharomyces cerevisiae* antibodies; NS, not significant.

TABLE 3. ASCA IgG and IgA Levels in Patients and Control Group

	Patients ($n = 77$)	Control group ($n = 80$)	P
ASCA IgG (U/ml)	9.7 ±23	2.2 ± 2.8	**0.004**
ASCA IgA (U/ml)	5.6 ± 15.8	3.9 ± 0.3	NS

ASCA, anti *Saccharomyces cerevisiae* antibodies; NS, not significant.

TABLE 4. Frequency of ASCA and aβ2GPI Isotype in Patients

	aβ2GPI	ASCA	P
IgG or IgA	100%	16.9% (13/77)	$<\mathbf{10^{-6}}$
IgG and IgA	6.5% (5/77)	6.5% (5/77)	NS
IgG	22% (17/77)*	15.6% (12/77)**	NS
IgA	83.1% (64/77)*	7.8% (6/77)**	$<\mathbf{10^{-6}}$

*$P < 10^{-6}$.
**$P = 0.04$.
ASCA, anti *Saccharomyces cerevisiae* antibodies; aβ2GPI, anti-beta 2 glycoprotein I antibodies; NS, not significant.

Discussion

The APLS is characterized by the presence of aPL that binds target molecules mainly via β2GPI (19, 20). The infectious origin of APLS has proved to be one of the explanations for generation of aβ2GPI (21). Indeed, among the environmental factors that trigger APLS, it is the bacteria, viruses, and yeast that were most described (21).

We have previously determined the frequency of ASCA in SLE (17). Krause et al. (18) determined the frequency of ASCA in patients with a primary APLS and in patients with APLS associated with SLE. In the present study, we determined the frequency of ASCA in patients with aβ2GPI and without SLE. In this study, ASCA (IgA or IgG) were significantly more frequent in patients than in healthy subjects (16.9% vs. 3.7%, $P = 0.01$). This frequency was similar to that found in the study by Krause et al. (20%) (18). Krause et al. explained this significant ASCA positivity in patients with APLS by cross-reactivity between the two epitopes of antigens, namely phosphopeptidomannan of the yeast *S. cerevisiae* and the β2GPI. Recently, Rinaldi et al. (22) confirmed the result of Krause et al. (18) by consulting the protein database of the National Center for Biotechnology Information (NCBI) and they found a structural similarity of 39% between phosphopeptidomannan of the yeast *S. cerevisiae* and β2GPI.

In the study by Krause et al. (18), the frequency of ASCA (20%) was lesser than that of aβ2GPI (68.4%). Even in our study, ASCA were less frequent than aβ2GPI (16.9% vs. 100%). Indeed, only a subpopulation of aβ2GPI is specific to the glycosylated site of the β2GPI molecule that cross-reacts with phosphopeptidomannan of *S. cerevisiae* (18). Furthermore, in our previous study, we determined the frequency of ASCA in SLE patients, and compared this frequency with that of aβ2GPI. The results showed a significantly lower frequency of ASCA (IgG or IgA) than that of aβ2GPI (IgG or IgA; 31.9% vs. 54.3%) (17).

The presence of cross-reactive epitopes on β2GPI and *S. cerevisiae*, demonstrated in the study by Krause et al., suggests a pathogenic role of ASCA in Crohn's disease associated thrombosis (18). On the other hand, a significantly higher frequency of aβ2GPI has been reported in Crohn's disease patients compared with healthy subjects (23). However, it was not clear whether the patients with positive ASCA had or not an increased risk of thrombosis.

One patient, among 13 having ASCA, presented a myocardial infarction. It has been similarly reported between the sequence of phosphopeptidomannan and ICAM-1. ICAM-1 and *P*-selectin are crucial molecules for transendothelial migration of leukocytes, playing an important role in the process of atherogenesis, especially in patients with a chronic systemic inflammatory autoimmune diseases (22). In a case-control study whose purpose was to investigate the possible role of ASCA in atherosclerosis, high levels of IgA and IgG ASCA have been found in patients with acute myocardial infarction, suggesting that ASCA could be a useful marker of unstable atherosclerotic plaque (24).

In the present study, among 13 patients with ASCA, one was diagnosed with coeliac disease and having made several spontaneous abortions. In coeliac disease, there is an increased intestinal permeability that could be implicated in the genesis of ASCA (25). In fact, we and others

TABLE 5. Characteristics of 13 Patients With ASCA

			ASCA levels (U/ml)		aβ2GPI levels (U/ml)		
N	Sex	Age (years)	IgG	IgA	IgG	IgA	Clinical manifestations
1	F	30	100	35	14	68	Venous thrombosis
2	F	34	13	12	0	56	Recurrent spontaneous abortions
3	F	37	96	0	0	34	Recurrent spontaneous abortions
4	F	34	17	15	0	12	Recurrent spontaneous abortions
5	M	50	15	0	0	19	Hypertension, stroke several times, and myocardial infarction
6	F	37	22	6.3	0	18	Recurrent spontaneous abortions
7	F	58	20	8	0	13	Purpura
8	F	32	15	2.9	0	17	Recurrent spontaneous abortions
9	F	37	124	15	11	46	Venous thrombosis, known with coeliac disease
10	F	37	14	5	0	11.5	Venous thrombosis
11	F	42	32	134	0	70	Venous thrombosis
12	F	44	48	3.5	11	0	Quadripyamidal syndrome, sphincter disorders
13	F	29	4.1	28	0	27	Recurrent spontaneous abortions

ASCA, anti *Saccharomyces cerevisiae* antibodies; aβ2GPI, anti-beta 2 glycoprotein I antibodies.

TABLE 6. ASCA IgG and IgA in Different Autoimmune Diseases

Autoimmune diseases	ASCA IgG (%)	ASCA IgA (%)	*P*
Coeliac disease (9)	24.8	8.8	$<\mathbf{10^{-6}}$
Primary biliary cirrhosis (12)	18.9	1.6	NS
Type 1 diabetes (14)	21	9.8	**<0.002**
Graves' disease (16)	11.8	0.8	**0.001**
SLE (17)	57.5	7.5	$\mathbf{10^{-3}}$
Present study	15.6	7.8	**0.04**

NS, not significant.

TABLE 7. aβ2GPI IgG and IgA in Different Autoimmune Diseases

	aβ2GPI IgA (%)	aβ2GPI IgG (%)	*P*
SLE (17)	50.9	19.8	$\mathbf{<10^{-6}}$
Coeliac disease (26)	14.3	1.6	**0.008**
Primary biliary cirrhosis (28)	62.5	12.5	$\mathbf{<10^{-6}}$
Present study	83.1	22	$\mathbf{<10^{-6}}$

(8, 9) demonstrated a high frequency of ASCA in coeliac disease. Furthermore, in another study we demonstrated a high frequency of aβ2GPI IgA in coeliac disease (26).

In the present study, the frequency of ASCA (IgG or IgA) was higher in female patients than in male (19% vs. 7%). Similarly, in our previous study on SLE, the frequency of ASCA (IgG or IgA) was higher in female patients (33%) than in male (23.1%) (17). These results could be explained by the fact that autoimmune diseases are more common in women because estrogen is implicated in the genesis of autoantibodies related to these pathologies (27).

In the present study, we found that the frequency of ASCA IgG was significantly higher than that of ASCA IgA (15.6% vs. 7.8%; $P = 0.04$) according to our previous studies (9, 12, 14, 16, 17) (Table 6).

In the present study, aβ2GPI IgA were significantly more frequent than aβ2GPI IgG (83.1% vs. 22%, $P < 10^{-6}$). The same result of the predominance of the IgA isotype was found in our previous studies (17, 26, 28) (Table 7). Indeed, it has been reported that IgA is the predominant isotype of aPL in African Americans (29).

In conclusion, we and other described a high frequency of ASCA in many autoimmune diseases and in patients with autoantibodies. It remains to ascertain if ASCA is the cause or the consequence of these autoimmune diseases.

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CONFLICT OF INTEREST

None of the authors have conflicts of interest to declare.

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