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ORIGINAL ARTICLE

Anti-*Saccaromyces Cerevisiae* antibodies (ASCA) are elevated in autoimmune thyroid disease ASCA in autoimmune thyroid disease

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Abstract Environmental factors have been implicated in the development of autoimmune thyroid disease (AITD). Anti-*Saccaromyces Cerevisiae* Antibodies (ASCA) were shown to be elevated in several autoimmune diseases. The aim of the study was to determine ASCA levels and their relationship with thyroid autoantibodies in patients with AITD. One-hundred and twelve patients with AITD (age 41.1 ± 12.8 years; F/M:96/16) and 103 healthy controls (38.5 ± 10.3 years; F/M:82/21) were included. Twenty-four patients had Graves disease (GD), and 88 had Hashimoto's thyroiditis (HT). ASCA IgA and IgG, TSH, free T4, anti-thyroglobulin, and anti-thyroid peroxidase antibody concentrations were determined. ASCA IgA positivity in patients with GD (16.6%) was similar to patients with HT (13.6%) and was higher than controls (5.8%). No significant difference was present between the frequencies of IgG positivity among GD (12.5%), HT (7.9%), and control groups (5.8%). The mean levels of ASCA IgA and IgG were comparable within the groups. No correlation of ASCA and anti-thyroglobulin and anti-thyroid peroxidase levels was observed. Increased IgA ASCA positivity is observed in patients with GD, suggesting a role of environmental stimuli in its pathogenesis. The role of ASCA in the etiology of AITD needs to be further examined.

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Introduction

Environmental factors besides genetic susceptibility may play an important role in the pathogenesis of autoimmune thyroid diseases (AITDs) [1]. The possible role of infection on autoimmunity, through bystander immune activation or molecular mimicry mechanisms, has previously been implicated [2, 3]. Some viral agents have been shown to trigger autoimmune thyroid disease in animal models [4]. Infections of the thyroid gland itself have also been demonstrated to be associated with thyroid autoimmunity [4]. High prevalence of *Y. enterocolitica* antibodies was observed in patients with AITD. Furthermore, in vitro studies showed TSH binding sites on *Y. enterocolitica* and their interaction with Graves immunoglobulins [5].

Anti-*Saccaromyces Cerevisiae* antibodies (ASCA) are antibodies against the oligomannan in the cell wall of *S. cerevisiae*, widely used in bread yeast [6, 7]. ASCA levels are elevated in Crohn's disease (CD) and have been used as serological marker to differentiate CD from ulcerative colitis (UC). Combining ASCA with p-anti-neutrophil cytoplasmic antibody (ANCA) increases its sensitivity in the differential diagnosis [7, 8]. ASCA levels are also increased in patients with celiac disease [9], autoimmune liver disease [10], primary biliary cirrhosis [11], Behçet's disease with gastro-intestinal involvement [12], and spondyloarthropathies [13].

The aim of our study was to determine ASCA levels and their relationship with thyroid autoantibodies in Turkish patients with AITD.

Results

Demographic findings, distribution of Graves disease (GD) and Hashimoto's thyroiditis (HT) patients, distribution of antibody positivity, and TSH free T4 (fT4) levels of the study groups are shown in Table 1. Eight patients in GD group and 28 patients in HT groups had a history of smoking. No patients were on immunomodulatory drugs, and no patient had any intercurrent infection at the time of analysis.

Comparison of ASCA levels

Mean ASCA IgA and IgG levels are shown in Table 2. While IgA and IgG levels were comparable among the patients with GD, HT, and controls, all were lower than the levels in CD group.

Comparison of ASCA positivity

In the whole study group, ASCA IgA positivity was significantly higher than the control group (14.2% in AITD vs. 5.8% in controls). On the other hand, the frequency of IgG positivity was comparable between the groups (9.8% in AITD vs. 5.8% in controls, $P = 0.02$). When the groups were analyzed separately, ASCA IgA positivity in patients with GD (16.6%) was similar to patients with HT (13.6%) and was higher than controls (5.8%). On the other hand, ASCA IgA positivity in HT patients was increased compared to controls, but reaching a borderline significance ($P = 0.09$). Similarly, ASCA IgG positivity in patients with GD, HT, and controls was comparable (Fig. 1).

No correlation was observed between ASCA positivity and either anti-thyroid peroxidase (anti-tpo) or anti-thyroglobulin (anti-tg) levels. Multivariate analysis performed to determine the predictors of ASCA IgA positivity did not also reveal any effect of anti-tpo or anti-tg antibodies.

Discussion

To our knowledge, this is the first study investigating ASCA levels in AITD patients. Although mean antibody levels are similar, our results demonstrated increased number of patients with ASCA IgA positivity compared to controls. This is mostly due to the elevated presence in GD patients, since ASCA IgA positivity in HT patients only reached a borderline significance. Although both classified under the heading of AITD, there is known phenotypic and genetic heterogeneity between GD and HT. The more prominent increase of IgA positivity in GD patients may be due to the different immunological processes underlying these diseases.

ASCA was originally used as a serological marker to make a differential diagnosis between CD and UC [7, 8]. Later, it has been demonstrated in some other autoimmune diseases involving the GI tract, such as celiac disease [9], autoimmune liver disease [10], primary biliary cirrhosis [11], and Behçet's disease with gastro-intestinal involvement.

A recent letter by Theiss et al. reported that a substantial fraction of patients with nontreated celiac disease, and treated celiac disease and CD, had positive ASCA IgA and IgG titers. Eight of the 36 patients with CD had clinical thyroid disease, out of whom six had positive anti-tpo antibodies [14].

The role of ASCA in the pathophysiology of inflammatory bowel disease (IBD) needs to be further clarified. Since there is a wide distribution of oligomannosides among different microorganisms, it is not clear whether ASCA is specific for *S. cerevisiae* or any other microorganism in the gastrointestinal tract [15]. Cross-reactivity with various mannose-containing molecules has been suggested to induce a hypersensitivity reaction during inflammatory process [16]. Mpofu et al. analyzed *S. cerevisiae* mannan for its effects on human peripheral blood

Table 1 Demographic findings, TSH, and free T4 levels of the study groups

	GD ($n = 24$)	HD ($n = 88$)	Control group ($n = 103$)	*P*
Age	40.3 ± 13.5	42.8 ± 12.8	38.5 ± 10.3	NS
Gender (M/F)	2/22	8/80	9/94	NS
BMI (kg/m^2)	23.6 ± 5.6	26.1 ± 4.9	24.2 ± 6.7	NS
Antibody positivity				
Anti-tpo	20/24	79/88	–	
Anti-tg	9/24	62/88		
Ophthalmopathy	8	0	–	
Dermopathy	–	–	–	
TSH	0.35 ± 0.23	3.97 ± 3.78	1.09 ± 0.77	<0.0001
fT4	1.72 ± 1.81	1.47 ± 0.99	1.43 ± 0.28	NS

ANOVA for age, BMI, TSH, and fT4. *GD* graves disease, *HT* Hashimoto's thyroiditis, *BMI* body mass index, *anti-tpo* anti-thyroid peroxidase, *anti-tg* anti-thyroglobulin, and *fT4* free T4

Table 2 ASCA IgA and IgG levels in GD, HT, control groups, and CD patients

	GD ($n = 24$)	HT ($n = 88$)	CD ($n = 23$)	Controls ($n = 103$)	P
IgA (U/ml)	5.2 ± 6.4	5.1 ± 18.2	29.7 ± 83.9	3.7 ± 1.8	0.002
IgG (U/ml)	6.1 ± 7.9	10.1 ± 19.4	34.7 ± 45.9	8.3 ± 10.0	0.0002

$P > 0.05$ for GD versus HT, GD versus control, and HT versus control. (Mann–Whitney U)

GD graves disease, *HT* Hashimoto's thyroiditis, *CD* crohn disease

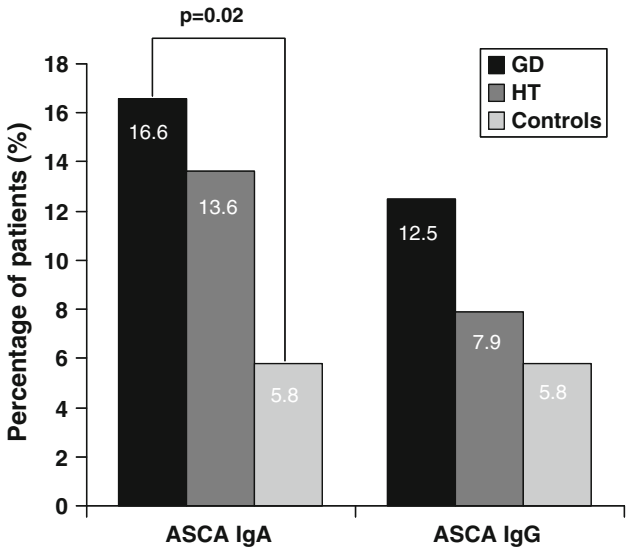

Fig. 1 Frequency of ASCA IgA and IgG positivity in the study groups. (Fischer's exact test for GD vs. controls, HT vs. controls, and GD vs. HT)

neutrophils, adherent monocytes, and monocyte-derived macrophages and demonstrated that mucosal phagocyte function is suppressed by microbial mannans, possibly of Mycobacterial origin. [17]. Loss of intestinal barrier function is suggested to play an important role for the development of autoimmune diseases of the GI tract [18, 19]. Impaired intestinal permeability provides a route for the enterance of environmental pathogens and may increase their exposure to the immune system. In other autoimmune diseases, increased permeability possibly results in an increased antigen delivery, triggering a multiorgan involvement, and autoimmune response [20].

The role of infections as environmental causes of AITD is extensively investigated. Bystander immune effects and molecular mimicry mechanisms have been suggested to activate auto-antibody production, but cannot alone explain the role of infections in the pathogenesis. Continuous stimulation with non-self antigens is suggested to be necessary to trigger the autoimmune process [21]. Mannose receptors have been shown in vitro to participate in autoimmune responses to Tg and TSHR, but not TPO [22]. The production of mannan-binding lectin, a key protein in activating the complement system, by human hepatocyte lines was increased by T3 and T4 hormones both clinically and in vitro [23].

Autoimmune thyroid disease may present as the extra-intestinal manifestation of IBD [24]; therefore, we questioned our patients for the presence of bowel symptoms. No diagnosed IBD was present in any of the patients, except for a few patients with non-specific bowel symptoms. Our population seemed to be presenting de novo as AITD, instead of having extraintestinal manifestations of inflammatory bowel disease. However, colonic examination was not performed to rule-out IBD clearly.

There have been case reports about the coexistence of AITD with both CD and UC [24–27]. There is no data on the level of ASCA in the patients that present with this coexistence. Common or similar environmental pathogens may be effective in stimulating the autoimmune process in all these polygenic disorders. The concept of increased intestinal permeability perpetuating the enterance of pathogens may be an acceptable mechanism. Screening large series of AITD for CD and UC may give some insight. Also simultaneous determination of thyroid autoantibodies and ASCA in large series of CD and UC patients may be helpful to find out the role of ASCA as a link between AITD and IBD.

In conclusion, our study demonstrates a low, but a significant presence of ASCA positivity in patients with AITD, mainly GD. Since increased intestinal permeability has been implicated in the pathogenesis of autoimmune diseases with ASCA positivity, studies about intestinal permeability of infectious agents and the role of *S. cerevisiae* infection in AITD needs to be determined with further studies.

Materials and methods

The patient group was randomly selected among patients who had been followed for the diagnosis of HT and GD. HT was diagnosed according to TSH levels being greater than 10 IU/l, with either anti-tpo or anti-tg antibody positivity and ultrasonographic findings of HT. GD was diagnosed by TSH levels being less than 0.1 IU/l; free T3 and T4 levels being above the normal range, elevation of either

anti-tpo or anti-tg or positivity of anti-TRAB antibodies and diffuse uptake on the thyroid scan.

The control group was randomly selected among a healthy population, having TSH levels, anti-tpo, and anti-tg levels within the normal range and having a normal thyroid examination.

A group of Crohn disease patients were included as positive controls for the assay. Medical history was obtained, and physical examination was performed in all the patients and controls. History of smoking, immunomodulatory drugs, and intercurrent infections were determined. Ophthalmopathy was assessed by NOSPECS classification. Dermopathy was determined by examination of the skin. Blood samples were collected from all the subjects after 8 h of fasting to determine ASCA, TSH, fT4, anti-tpo, and anti-tg levels. The samples were immediately centrifuged and stored at −70°C until assayed.

Biochemical parameters

ASCA

ASCA IgA and IgG were detected using the commercial kit, BINDAZYME ™ EIA Kit (The Binding Site Ltd, Birmingham, UK), as described previously [13]. The quantitative ASCA IgA and IgG results were obtained in U/ml units, and 7 U/ml units for IgA, and 22 U/ml units for IgG were accepted as cut-off values by the manufacturer's instructions.

Free T4, TSH, anti-tpo and anti-tg antibodies

Free T4 and TSH levels were determined by electrochemiluminescence immunoassay intended for use on the Roche Elecsys 2010 immunoassay analyzer (Roche Diagnostics). The within-run precision was 1.8% for a mean concentration of 3.96 mIU/ml, and the total precision was 3.6% for the same concentration. The measuring range of fT4 was 0.74–1.79 ng/dl. Serum anti-tg and anti-tpo antibody concentrations were measured by electrochemiluminescence immunoassay (Elecsys 2010; Roche Diagnostics). The measuring range of anti-Tg was 10–4000 IU/ml, and for anti-TPO, the range was 5–600 IU/ml. The within-run and between-run precisions of anti-Tg measurements were 4.6–5.6% for mean serum concentrations of 290–2894 IU/ml. Within-run and between-run precisions of anti-Tg measurements were 4.2–7% for mean serum concentrations of 15.3–269 IU/ml.

Statistical analysis

All statistical calculations were performed with SPSS (Statistical Package for Social Sciences) for Windows 15.0 software (SPSS, Chicago, IL, USA). ANOVA was used to compare age, BMI, TSH, and fT4 in the study groups. Comparisons between the groups in terms of IgA and IgG levels were done by ANOVA and Mann–Whitney *U* tests. The comparison of percentage positivity was performed by Fischer's exact test. Pearson correlation test was used for correlation analysis. Logistic regression method was used for multivariate analysis. Level of statistical significance was set at $P < 0.05$. All results were expressed as mean ± SD.

References

1. L.J. DeGroot, J. Quintans, Endocr. Rev. **10**, 537–562 (1989)
2. M. Samarkos, G. Vaiopoulos, Curr. Drug Targets Inflamm. Allergy **4**, 99–103 (2005)
3. J.K. Olson, J.L. Croxford, S.D. Miller, Viral Immunol. **14**, 227–250 (2001)
4. Y. Tomer, T.F. Davies, Endocr. Rev. **14**, 107–120 (1993)
5. P. Heyma, L.C. Harrison, R. Robins-Browne, Clin. Exp. Immunol. **64**, 249–254 (1986)
6. M.H. Floch, J. Clin. Gastroenterol. **36**, 5–6 (2003)
7. J.F. Quinton, B. Sendid, D. Reumaux, P. Duthilleul, A. Cortot, B. Grandbastien, G. Charrier, S.R. Targan, J.F. Colombel, D. Poulain, Gut **42**, 788–791 (1998)
8. R.K. Linskens, R.C. Mallant-Hent, Z.M. Groothuismink, L.E. Bakker-Jonges, J.P. van de Merwe, H. Hooijkaas, B.M. von Blomberg, S.G. Meuwissen, Eur. J. Gastroenterol. Hepatol. **14**, 1013–1018 (2002)
9. R.Ch. Mallant-Hent, B. Mary, E. von Blomberg, Z. Yüksel, P.J. Wahab, C. Gundy, G.A. Meyer, C.J. Mulder, Eur. J. Gastroenterol. Hepatol. **18**, 75–78 (2006)
10. K.R. Reddy, J.F. Colombel, D. Poulain, E.L. Krawitt, Am. J. Gastroenterol. **96**, 252–253 (2001)
11. W. Sakly, M. Jeddi, I. Ghedira, Dig. Dis. Sci. **53**, 1983–1987 (2008)
12. I. Krause, Y. Monselise, G. Milo, A. Weinberger, Clin. Exp. Rheumatol. **20**, S21–S24 (2002)
13. S.Z. Aydin, P. Atagunduz, M. Temel, M. Bicakcigil, D. Tasan, H. Direskeneli, Rheumatology (Oxford) **47**, 142–144 (2008)
14. P.M. Theiss, L.M. da Silva Kotze, S.R. da Rosa Utiyama, R.M. Nisihara, I.G. Silva, P.G. Kotze, M. Olandoski, J. Clin. Gastroenterol. **44**, 309–310 (2010)
15. S. Suzuki, Curr. Top. Med. Mycol. **8**, 57–70 (1997)
16. G.R. Barclay, H. McKenzie, J. Pennington, D. Parratt, C.R. Pennington, R. Scand, J. Gastroenterol. **27**, 196–200 (1992)
17. C.M. Mpofu, B.J. Campbell, S. Subramanian, S. Marshall-Clarke, C.A. Hart, A. Cross, C.I. Roberts, A. McGoldrick, S.W. Edwards, J.M. Rhodes, Gastroenterology **133**, 1487–1498 (2007)
18. A. Fasano, T. Shea-Donohue, Nat. Clin. Pract. Gastroenterol. Hepatol. **2**, 416–422 (2005)
19. I. Fresko, V. Hamuryudan, M. Demir, N. Hizli, H. Sayman, M. Melikoglu, R. Tunc, S. Yurdakul, H. Yazici Ann, Rheum. Dis. **60**, 65–66 (2001)
20. T. Watts, I. Berti, A. Sapone, T. Gerarduzzi, T. Not, R. Zielke, A. Fasano, Proc. Natl. Acad. Sci. **102**, 2916–2921 (2005)
21. A. Fasano, Curr. Opin. Gastroenterol. **22**, 674–679 (2006)
22. G.D. Chazenbalk, P.N. Pichurin, J. Guo, B. Papoport, S.M. McLachlan, Clin. Exp. Immunol. **139**, 216–224 (2004)
23. C.M. Sorensen, T.K. Hansen, R. Steffensen, J.C. Jensenius, S. Thiel, Clin. Exp. Immunol. **145**, 173–182 (2006)

24. U. Monsén, J. Sorstad, G. Hellers, C. Johansson, Am. J. Gastroenterol. **85**, 711–716 (1990)
25. J.A. Snook, H.J. de Silva, D.P. Jewell, Q. J. Med. **72**, 835–840 (1989)
26. T. Inokuchi, Y. Moriwaki, S. Takahashi, Z. Tsutsumi, T. Ka, T. Yamamoto, Intern. Med. **44**, 303–306 (2005)
27. S.A. Shah, M.A. Peppercorn, J.A. Pallotta, Am. J. Gastroenterol. **96**, 1925–1926 (2001)