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ORIGINAL ARTICLE

Serum Anti-carbonic Anhydrase Antibodies and Oxidant–Antioxidant Balance in Patients with Acute Anterior Uveitis

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ABSTRACT

Purpose: To study the existence of anti-carbonic anhydrase antibodies (anti-CA-I&II) in acute anterior uveitis (AAU) patients and to analyze the relationship between the levels of these antibodies and the total antioxidant capacity (TAC), total oxidant capacity (TOC), oxidative stress index (OSI), and malondialdehyde (MDA) level.

Methods: Forty-five AAU cases and 43 healthy controls were enrolled in this prospective study.

Results: The average anti-CA I and II antibody levels were 0.433 ± 0.306 and 0.358 ± 0.261 IU/mL, respectively, in the AAU group and 0.275 ± 0.147 and 0.268 ± 0.108 IU/mL, respectively, in the control group ($p = 0.004$ and $p = 0.036$, respectively). In addition, it was found that the TOC, OSI, and MDA levels in the AAU subjects were statistically significantly higher than those of the control subjects.

Conclusions: These results suggest that autoimmune responses against CA I and CA II and an altered serum oxidant-antioxidant balance may be involved in the pathogenesis of AAU.

Keywords: Antioxidants, autoimmunity, carbonic anhydrases, oxidants, uveitis

Uveitis is a term for an autoimmune disease that covers many sight-threatening intraocular inflammatory conditions. This disease is rarely observed and, therefore, it often goes unnoticed.[1–3] Uveitis is classified as anterior, intermediate, posterior, or panuveitis depending on the part of the uvea affected by the inflammatory process.[2] The most frequently observed form of the disease is anterior uveitis. Anterior uveitis may lead to recurrent intraocular inflammation and may cause transient or permanent visual impairment and ocular complications. Delays in the diagnosis and treatment of this disease may result in damage to various ocular structures.[1,4,5]

Uveitis occurs when the normal state of ocular immune privilege is compromised.[4] Although the cause of this disease might be an underlying systemic disease, a significant proportion of affected individuals do not have clinical signs or symptoms of systemic disease. Therefore, an important proportion of cases represent idiopathic uveitis.[2]

The etiopathogenesis of uveitis is still not fully understood today. Various studies performed to investigate this disease have suggested that the following might be involved: human leukocyte antigens (HLAs); polymorphisms in certain immune response genes, such as tumor necrosis factor (TNF); major histocompatibility complex (MHC) class I polypeptide-related sequence A (MICA); interleukin-1 (IL-1); several chemokines; the costimulatory molecule known as cytotoxic T-lymphocyte antigen 4 (CTLA-4); and the complement system.[6–15] Polymorphisms in antioxidant enzyme genes have

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also been found to play important roles in the pathogenesis of uveitis.[16] Nevertheless, these past studies remain insufficient to elucidate the etiopathogenesis of uveitis.

Carbonic anhydrase enzymes (CAs, EC 4.2.1.1) are zinc-containing metalloproteins that catalyze the reversible hydration of carbon dioxide to bicarbonate and hydrogen ions. To date, 16 diverse CA isoenzymes have been found to play essential roles in many different physiological processes, and these enzymes have been classified according to their cellular localization and biophysical properties.[17] The important functions of these enzymes in tissues include gas exchange, ion transport, bone resorption, aqueous humor production, gluconeogenesis, lipogenesis, and ureagenesis.[17–19]

In various studies that have been performed in recent years, it has been emphasized that antibodies that develop against the CA I and CA II isoenzymes (anti-CA I and anti-CA II antibodies) may have roles in the etiopathogenesis of various autoimmune diseases.[20–23] However, the roles of these antibodies in the etiopathogenesis of uveitis, another autoimmune disease, have not been investigated previously. Consequently, this study was performed to assess the existence of anti-CA I and anti-CA II antibodies in patients with acute anterior uveitis (AAU), which is the most frequently observed type of uveitis, and to evaluate the possible roles of these antibodies in the etiopathogenesis of this disease. Another purpose of this study was to assess the oxidant–antioxidant balance and the level of oxidative stress in AAU patients. Thus, total antioxidant capacity (TAC), total oxidant capacity (TOC), oxidative stress index (OSI), and malondialdehyde (MDA) levels as an important indicator of oxidative stress were also assessed.

MATERIALS AND METHODS

This prospective study was performed in the departments of ophthalmology and biochemistry at the School of Medicine, Karadeniz Technical University, Trabzon, Turkey. The study followed the tenets of the Declaration of Helsinki. After receiving approval from the medical ethics committee, informed consent was obtained from all participants.

Inclusion and Exclusion Criteria for the Study

A total of 45 patients with signs of AAU identified during detailed ophthalmologic examinations and 43 healthy control subjects without signs of AAU were included in the study. Control subjects were recruited from the eye clinic and comprised patients with no eye problems other than refractive error. Because detailed ophthalmologic examinations were performed, the criterion for identifying active findings related to anterior uveitis (conjunctival hyperemia, keratic precipitate, cells in anterior chamber, flare, synechia) was taken into consideration in diagnosing AAU. Only those patients who had AAU for the first time were included in the study. The exclusion criteria were as follows: a history of renal disease, cardiovascular disease, deep vein thrombosis or pulmonary embolism; any autoimmune disease (such as Graves disease, Hashimoto disease, diabetes mellitus, systemic lupus erythematosus, rheumatoid arthritis, Sjögren syndrome, and anti-phospholipid syndrome); thrombophilia; a previous episode of uveitis; current use of anticoagulant, anti-inflammatory, antihypertensive or antiplatelet therapy; and refusal to participate in the study.

The demographic and clinical data for all subjects were recorded. Blood samples collected in plain tubes containing separation gel were obtained from the AAU patients and control subjects. The blood was allowed to clot for 15–30 min and then centrifuged. The serum samples were stored at −80 °C in a deep freezer until the analyses of the anti-CA I and anti-CA II antibody levels, the total antioxidant capacity (TAC), the total oxidant capacity (TOC), and the malondialdehyde (MDA) level.

Laboratory Analysis

Enzyme-linked immunosorbent assay (ELISA) for the serum anti-CA I and anti-CA II antibodies. Human CA I and CA II, electrophoretically purified from erythrocytes, were purchased from Sigma Chemical (St. Louis, MO). Serum anti-CA I and anti-CA II antibodies were detected by ELISA according to a previously described method.[23] Briefly, microtiter plates (high binding, flat-bottomed plates; Bioscience) were coated with 50 µL of 10-µg/mL CA I or CA II in carbonate buffer (0.05 mM, pH 9.6) and incubated overnight at 4 °C. The wells were washed 4 times with phosphate buffer (pH = 7.4) and blocked with 3% skim milk in phosphate buffer for 2 h at room temperature (RT). After being washed 4 times with phosphate buffer containing 0.05% Tween-20, the wells were incubated with 100 µL of serum diluted with 1% skim milk in phosphate buffer (1:200) for 2 h at RT. After washing, each well was incubated for 2 h at RT with 100 µL of a 1:2000 dilution of peroxidase-conjugated anti-human IgG anti-serum (Sigma) in dilution buffer. Following 5 washes with phosphate buffer containing 0.05% Tween-20, the wells were incubated with 100 µL of substrate solution for 20 min at RT. The reaction was stopped by the addition of 100 µL of 2 M H_2SO_4 to each well. The absorbance was read at 480 nm. Control wells that were not coated

with CA I or CA II were included in the ELISA for each serum sample studied.

All assays were performed in duplicate, and the specific binding of serum antibodies to CA I or CA II was calculated as follows: the average absorbance of the antigen-coated wells minus the average absorbance of the control wells (specific binding = $A_{coated} - A_{control}$).

Measurement of the total antioxidant capacity (TAC). The total antioxidant status was determined using the method developed by Erel.[24] The serum TAC was calculated in mmol Trolox equivalents/L.

Measurement of the total oxidant capacity (TOC). The TOC was determined using the method previously described by Erel.[25] The serum TOC was calculated in μmol H_2O_2 equivalents/L.

Calculation of the oxidative stress index (OSI). The TOC:TAC ratio was used as the OSI. To perform the calculation, the units of the TAC, mmol Trolox equivalents/L were converted into μmol Trolox equivalents/L, and the OSI was calculated as follows: OSI = [(TOC, μmol H_2O_2 equivalents/L)/(TAC, μmol Trolox equivalents/L) × 100].[26]

Measurement of the serum malondialdehyde (MDA) level. The level of lipid peroxidation in serum samples was determined as the MDA concentration using the method described by Yagi.[27] Briefly, 0.3 mL of serum was mixed with 2.4 mL of 0.042 M H_2SO_4 and 0.3 mL of 10% phosphotungstic acid. After being allowed to stand at room temperature for 5 min, the mixture was centrifuged at 1600*g* for 10 min. The supernatant was discarded and the sediment was suspended in 4 mL of distilled water. Subsequently, 1 mL of 0.67% thiobarbituric acid was added and the mixture was heated in boiling water for 60 min. The mixture was centrifuged at 1600*g* for 10 min. The absorbance of the organic layer was then read at 532 nm. Tetramethoxypropane was used as a standard, and the MDA levels were calculated as nmol/mL.

Statistical Analysis

The results were expressed as the means ± standard deviation. Statistical analyses were performed using SPSS 13.0.1 (SPSS, Chicago, IL; License no: 9069728, KTU, Trabzon, Turkey). The normality of the data was assessed using the Kolmogorov-Smirnov test. A comparative analysis of the serum anti-CA I and MDA levels between the groups was performed using the Mann-Whitney test. An independent samples *t*-test was used for the other parametric comparisons. The chi-square test was used for the comparison of the sex distribution. The correlations between the serum anti-CA I and anti-CA II levels and the TAC, TOC, OSI, and MDA levels were examined in all study groups using Spearman correlation analysis. $p < 0.05$ was regarded as statistically significant.

RESULTS

The average age of the 45 patients in the AAU group, 21 (46.7%) of whom were female, was 40.13 ± 10.46 years (18–56 years). The average age of the 43 healthy individuals who constituted the control group, 20 (46.5%) of whom were female, was 39.14 ± 9.74 years (18–53 years). There was no statistically significant difference between the two groups in terms of sex or age ($p = 0.988$ and $p = 0.646$, respectively).

The comparison of the mean serum anti-CA I and anti-CA II antibody levels—TAC, TOC, OSI, and MDA—between the uveitis and control groups is shown in Table 1. The data in Table 1 indicate that the levels of anti-CA I and anti-CA II antibodies in the uveitis patients were significantly higher than those in the control subjects. No significant difference between the two groups was found in terms of the mean TAC. However, the mean TOC, mean OSI, and mean MDA level were statistically significantly higher in the uveitis group.

In this study, any absorbance value higher than 0.569 (the mean absorbance + 2 SD of healthy the control group) was defined as positive for anti-CA I antibodies. Anti-CA I antibody positivity was detected in 9 of the 45 patients with AAU (20%) and 2 of the 43 healthy subjects (4.7%) (Figure 1).

In this study, any absorbance value higher than 0.484 (the mean absorbance + 2 SD of the healthy control group) was defined as positive for anti-CA II antibodies. Anti-CA II antibody positivity was detected in 7 of the 45 patients with AAU (15.6%) and 2 of the 43 healthy subjects (4.7%) (Figure 2). When separate calculations were performed after removing outliers (as seen in Figure 2) in the disease group, the significant difference between AU and control patients with respect to the anti-CA II antibody levels diminished ($p = 0.897$).

When considering the correlations of the parameters for the entire study group, it was observed that there were statistically significant relationships between the anti-CA I and anti-CA II antibody levels ($r = 0.563$, $p < 0.0001$), between the MDA level and the TOC ($r = 0.629$, $p < 0.0001$), and between the MDA level and the OSI ($r = 0.603$, $p < 0.0001$).

DISCUSSION

Uveitis is defined as intraocular inflammation that is considered to be triggered by diverse etiologies. The available data indicate that both innate and adaptive immune responses may be predominant mechanisms involved in the development of uveitis.[7] The induction of an inflammatory cascade and the activation of an immune response cause the clinical signs of the disease to be revealed in some way.[4,11] The intraocular expression of various proinflammatory cytokines and

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TABLE 1. Comparison of the anti-carbonic anhydrase I and II antibody levels (anti-CA I and II), total antioxidant capacity (TAC), total oxidant capacity (TOC), oxidative stress index (OSI), and malondialdehyde (MDA) level between the study groups.

Parameters	Uveitis group	Control group	*p* levels
Anti-CA I (IU/mL)	0.433 ± 0.306 (0.126–1.382)	0.275 ± 0.147 (0.038–0.93)	0.004*
Anti-CA II (IU/mL)	0.358 ± 0.261 (0.079–1.397)	0.268 ± 0.108 (0.063–0.537)	0.036**
TAC (mmol Trolox equiv/L)	0.626 ± 0.132 (0.405–0.968)	0.623 ± 0.178 (0.389–1.165)	0.922**
TOC (mmol H_2O_2/L)	44.237 ± 21.924 (11.409–100.845)	28.268 ± 13.149 (10.931–63.641)	<0.0001**
OSI (%)	7.299 ± 3.491 (1.542–16.248)	4.77 ± 2.393 (1.242–10.752)	<0.0001**
MDA (nmol/mL)	1.28 ± 0.822 (0.48–4.85)	0.838 ± 0.426 (0.198–1.995)	<0.0001*

*Mann –Whitney test.
**Independent samples *t*-test.

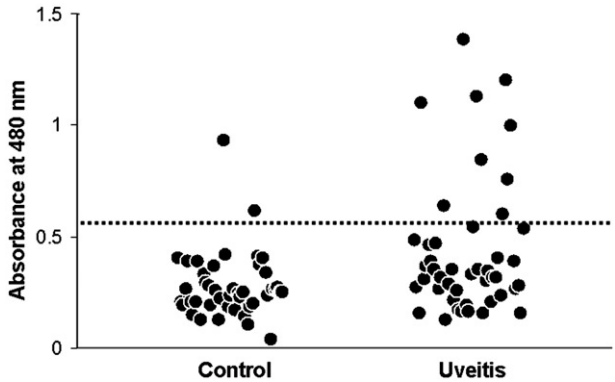

FIGURE 1. Anti-CA I antibody levels in patients with uveitis and healthy controls. The dotted line indicates the mean + 2 SD value for the healthy controls (A 480 = 0.569).

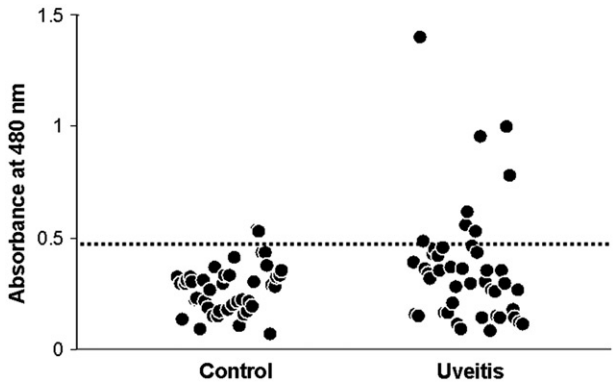

FIGURE 2. Anti-CA II antibody levels in patients with uveitis and healthy controls. The dotted line indicates the mean + 2 SD value for healthy controls (A 480 = 0.484).

chemokines and adhesion molecules, as well as the selective recruitment of inflammatory cells from the systemic circulation to the eye, play roles during the development of uveitis. Nevertheless, the initial immune mechanisms that compromise the ocular immune privilege are not known.[4,7]

CAs widely exist in many tissues and organs in human body.[28] In previous studies, various levels of autoantibodies against the CA I and CA II isoenzymes were found in individuals with several autoimmune and idiopathic diseases, and it was reported that these autoantibodies might be responsible for the etiopathogenesis of the diseases. These diseases include acute tubulointerstitial nephritis,[20] rheumatoid arthritis,[21] autoimmune pancreatitis,[22] Graves disease,[23] systemic sclerosis,[29] ulcerative colitis,[30] idiopathic recurrent pregnancy loss,[31] polycystic ovary syndrome,[28] systemic lupus erythematosus, and Sjögren syndrome.[32] In our study, the serum anti-CA I and anti-CA II antibody levels in AAU patients were higher than those in the healthy control group. This is the first report describing significant increases in the serum anti-CA I and anti-CA II antibody concentrations in patients with AAU. These findings provide support for the hypothesis that the pathophysiology of AAU is autoimmune based. These antibodies may also play a role in the development of inflammation in the eye, being an organ containing CAs.

In our study, the oxidative stress index (OSI) values in AAU patients were significantly higher than those in the control subjects. Oxidative stress and reactive oxygen species (ROS) also play a role in AAU pathogenesis,[33,34] and interventions targeting oxidative stress and ROS products reduce ocular inflammation.[33] ROS lead to reversible or irreversible destruction in biological tissues. As a result of oxidative cellular damage, membrane lipids become oxidized and important breakdown products such as MDA are generated. Accordingly, the serum MDA level is widely used as a measure of oxidative stress.[33,35,36] MDA production was observed in our study as well, and a statistically significant positive correlation between the OSI values and the serum MDA levels was found.

Satici et al.[37] reported that increases in the MDA level in the aqueous humor might play a role in the pathogenesis of endotoxin-induced uveitis. In another study, Ozdamar et al.[15] found that the increase in the serum MDA level was related to the development of posterior uveitis. Likewise, in our study, the serum MDA levels in the AAU group were significantly higher than those in the healthy control group.

The increase in the serum MDA levels might be related to the development of antibodies against CA isoenzymes.[18,21] However, in our study, there was no

significant relationship between the serum MDA levels and the anti-CA I and anti-CA II antibody levels. This situation might have arisen from the complexity and heterogeneity of autoimmunity development, as the mechanism of autoantibody production is not fully understood. It has been suggested that this situation developed as a result of genetic predisposition, cross-reactivity between foreign and host antigens, or increased random B-cell activity. Foreign antigens, however, are considered to have developed as a result of infection, inflammation, environmental factors, drug administration, free radicals, and other modifying agents. ROS attack DNA, causing changes in its structure at the macromolecular level, and the resulting DNA damage appears to be a primary causative factor of autoantibody production.[38–40]

Our study has a number of limitations specific to our analysis, including involvement of a small number of patients, lack of identification of certain causes underlining the patients admission with AAU, and lack of data on long-term follow-up results on the course of anti-CA I and anti-CA II antibodies or MDA levels. Additionally, the significant differences between AU and control patients with respect to the anti-CA II antibody levels were diminished when separate calculations were performed after removing outliers in the disease group.

In conclusion, in this study, increased serum anti-CA I and II antibody levels and lipid peroxidation abnormalities, along with the disruption of the serum oxidant–antioxidant balance, were found in AAU patients. The results that we obtained verified the existence of oxidative stress in uveitis patients. It is not known whether the autoantibodies that develop against CA isoenzymes are a cause of uveitis or a result of this disease. More comprehensive and controlled studies are needed to determine the roles of these autoantibodies in uveitis etiopathogenesis.

DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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