Original Article

Utility of angiotensin-converting enzyme activity in aqueous humor in the diagnosis of ocular sarcoidosis

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Purpose: Many studies include elevated activity of angiotensin-converting enzyme (ACE) in serum in sarcoidosis and in ocular sarcoidosis as well, but there are only a few analyzing ACE activities in aqueous humor. The aim of this study is to illuminate the diagnostic value of ACE in aqueous humor in patients with ocular sarcoidosis. **Methods:** We analyzed twenty patients with ocular sarcoidosis and 18 patients with nonocular involvement. All patients have biopsy-positive sarcoidosis of the lungs and/or mediastinal lymph nodes. Blood samples for ACE serum levels were obtained from all patients. Aqueous humor samples were taken by paracentesis with a 25-gauge needle in local anesthesia. With appropriate statistical tests, we compared ACE activity in serum and aqueous humor in patients with and without ocular sarcoidosis. **Results:** The majority of our patients with ocular sarcoidosis were female (12/20), also in the group with systemic sarcoidosis and without ocular involvement (12/6). Mean age of the whole analyzed group of sarcoidosis patients was 45 ± 6 years. There is no statistically significant difference in ACE activity in serum between two groups of patients (with and without ocular sarcoidosis). There is statistically significant difference in ACE activity in aqueous humor among patients with ocular and nonocular sarcoidosis. ACE activity in aqueous humor is significantly higher in patients with ocular sarcoidosis. **Conclusion:** Increased ACE activity in aqueous humor can point to a diagnosis of ocular sarcoidosis, without the need for ocular biopsy.

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Sarcoidosis is a chronic multisystem, multiorgan, inflammatory disease of unknown etiology and unpredictable course. The disease is characterized by noncaseating granulomas that may affect any organ of the body including the eye, lungs, lymph nodes, skin, heart, liver, and muscles.[1]

The frequency of ocular involvement in patients with sarcoidosis varies among published clinical reports. This could reflect either real variations in the prevalence of eye disease among different populations or it may reflect the lack of screening of patients with sarcoidosis for ocular disease.[2]

According to one of the first publications on ocular sarcoidosis published by G. James in 1974, sarcoidosis is responsible for only about 4% of cases of uveitis; however, eye involvement, predominantly uveitis, occurs in about one-quarter of patients with systemic sarcoidosis. Ocular involvement is very debilitating location of sarcoidosis, and the early recognition may prevent patients from further suffering and even blindness.[3]

Later on, Italian authors estimated 30%–60% of patients with known sarcoidosis have ocular involvement at some points during the course of disease and 4%–6% of patients with diagnosed uveitis developed later clinical sarcoidosis.[4] According to other authors, ocular involvement occurs in 11%–83% of cases of sarcoidosis.[5]

Since there are no definitive diagnostic serological or radiological tests, the presence of noncaseating granulomas on tissue biopsy together with compatible clinical features is usually considered as a proof of the diagnosis of sarcoidosis.[6] However, intraocular biopsy is not commonly performed for this disease. One retrospective study of patients with biopsy-positive sarcoidosis and uveitis suggested serum angiotensin-converting enzyme (ACE) and lysozyme in combination with chest X-ray findings had high sensitivity to confirm the diagnosis of sarcoidosis (over 80% of patients). The success was even higher when serum ACE and lysozyme were combined with a thoracic computerized tomography (CT) scan (over 90%).[7]

The function of an ocular renin–angiotensin system (RAS) is not yet known. Its activation in the eyes of diabetic subjects with proliferative retinopathy may suggest that

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angiotensin II (ANG II) is involved in the development of neovascularization.[8,9] Because ANG II receptors have been detected in retinal blood vessels, a role for the RAS in the regulation of retinal vascular tone is also conceivable.[10] Finally, the fact that renin inhibitors and ACE inhibitors lower intraocular pressure suggests that an intraocular RAS may play a role in aqueous humor dynamics.[11] Danser *et al*.[12] measured the concentrations of angiotensins I and II (ANG I and II) in vitreous fluid and ocular tissues of anesthetized pigs and in human aqueous, vitreous, and subretinal fluid obtained during eye surgery. Results showed that in tissues obtained from normal porcine eyes (anterior uveal tract, neural retina, and retinal pigment epithelium + choroid), ANG I and II were 5–100-fold higher than could be accounted for by contamination with blood. ANG I and II in ocular tissues are, therefore, unlikely to be derived from the circulation. In porcine vitreous fluid, ANG I and II were close to the limit of detection. Previous studies in bovine and feline eyes[13,14] have shown that the blood plasma content of the retina, anterior uveal tract, and retinal pigment epithelium + choroid are 1%, 5%, and 20% of total tissue weight, respectively. One may assume that the plasma content of porcine ocular tissues is similar. Thus, in the presence of an intact blood–retinal barrier (BRB), little or no ANG I or II enters the vitreous compartment. In human ocular fluids obtained from diseased eyes, ANG I and II levels were readily measurable and correlated linearly with the level of serum albumin, indicating that after partial breakdown of the BRB, diffusion of ANG I and II from the circulation into the eye may occur. Results from these studies indicated that both ANG I and II are generated locally in ocular tissues with little leakage into ocular fluids.

Serum ACE has been a biomarker of sarcoidosis activity for decades. The activity of this biomarker reflects the disease activity; as the ACE levels fluctuate with corticosteroid use, if high at the time of the diagnosis may contribute for the follow-up of these patients but are not specific enough for diagnostic purposes. High levels of serum ACE were also reported for other diseases (for example, tuberculosis, leprosy, and diabetes mellitus). Moreover, as serum ACE levels reflect the systemic burden of inflammation, normal serum ACE levels do not exclude the diagnosis of sarcoidosis, especially not in those with isolated ocular disease.[15]

Levels of ACE as a biomarker of sarcoid activity may be increased in serum, tears, aqueous humor, and cerebrospinal fluid as well. It is then reasonable to make an assumption that aqueous ACE activity of patients with various diseases (and sarcoidosis-associated uveitis as well) should be increased though its diagnostic value is not yet clearly determined. The goal of this study is to illuminate the diagnostic value of ACE in aqueous humor in patients with ocular sarcoidosis.

Methods

We analyzed twenty patients with ocular sarcoidosis and 18 patients with nonocular involvement. All patients have biopsy-positive sarcoidosis of the lungs and/or mediastinal lymph nodes. All patients included in this study were diagnosed in our clinic. Blood samples for ACE serum activity were obtained from all patients. Patients included in this study were not taking medications that interfere with the renin–angiotensin–aldosterone system, i.e., ACE inhibitors or ANG II receptor antagonists. Patients with comorbidities, especially diabetes mellitus, were also excluded from the study.

ACE activity was measured by the spectrophotometric method adapted in the laboratory utilizing synthetic tripeptide substrate N-[3-(2-furyl)acryloyl]-L-phenylalanylglycylglycine, using a commercial test kit (Trinity Biotech, St. Louis, USA), on an Olympus AU 2700 automated analyzer (Beckman Coulter Biomedical Ltd., laboratory reference value: 8–52 U/L).

Aqueous humor was collected in local anesthesia from the anterior chamber into a 1 ml plastic syringe using a 25-gauge needle and 100–150 µl of aqueous humor was aspirated. Following aqueous humor collection, balanced salt solution was injected to refill the anterior chamber.

The samples were frozen and stored at −80°C; the measurements were done after sample collection was done from all patients. The concentrations of ACE in aqueous humor were measured using ELISA test. Here, we had no reference value evaluable from the literature, but we considered for further analyses serum reference values measured in the laboratory.

The study protocol was approved by the Ethics Committee of the Faculty of Medicine University of Belgrade, and the written informed consent was obtained from all participants.

Statistical analysis was performed using Statistical Package for the Social Sciences version 15.0 software (IBM, Chicago, Illinois, USA).

Results

The majority of our patients with ocular sarcoidosis were female (12/20), also in the group with systemic sarcoidosis and without ocular involvement (12/6). Mean age of the whole analyzed a group of sarcoidosis patients was 45 ± 6 years. No statistical difference was found between the group with and without ocular sarcoidosis considering the age.

Regarding the stage of the lung disease, most of the patients were in Stage 1 in both groups as shown in Table 1. There were no statistical differences between ocular involvement and the stage of the lung disease.

Results of analyses of ACE activity in serum and aqueous humor are summarized in Table 2 and shown in Figs. 1 and 2.

There is no statistically significant difference in ACE activity in serum between two groups of patients (with and without ocular sarcoidosis, $P = 0.339$).

Table 1: Stage of the lung disease in patients with and without ocular sarcoidosis

Chest X-ray	Number of patients	
	No ocular sarcoidosis (18 patients)	Ocular sarcoidosis (20 patients)
Stage 0		1
Stage 1	9	9
Stage 2	5	3
Stage 3	3	5
Stage 4	1	2

Table 2: Angiotensin converting enzyme in serum and in aqueous humor in patients with and without ocular sarcoidosis

	ACE serum activity (U/L) (reference value 8-52 U/L)		ACE aqueous humor activity (U/L) (reference value not evaluable)	
	Without ocular sarcoidosis	With ocular sarcoidosis	Without ocular sarcoidosis	With ocular sarcoidosis
Minimum	21	15.6	1.1	64.2
Maximum	95	124	13.4	15.7
Median	55.05	45	2.65	88.2
Mean±SD	56.37±22.79	50.21±27.46	3.79±3.24	91.48±20.36

SD: Standard deviation, ACE: Angiotensin-converting enzyme

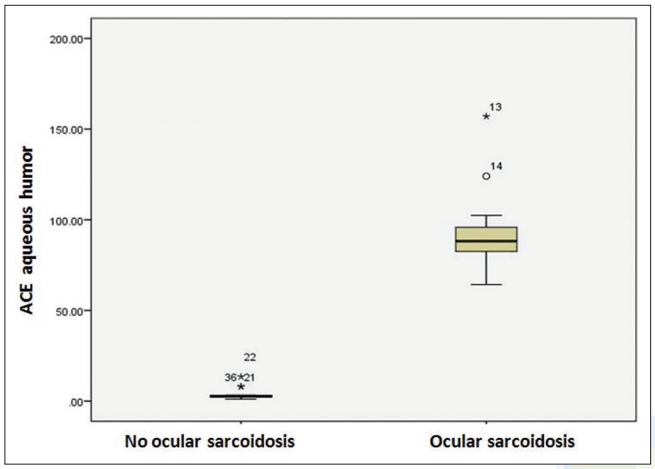

Figure 1: Angiotensin-converting enzyme activity in humor aqueous in patients with and without ocular sarcoidosis

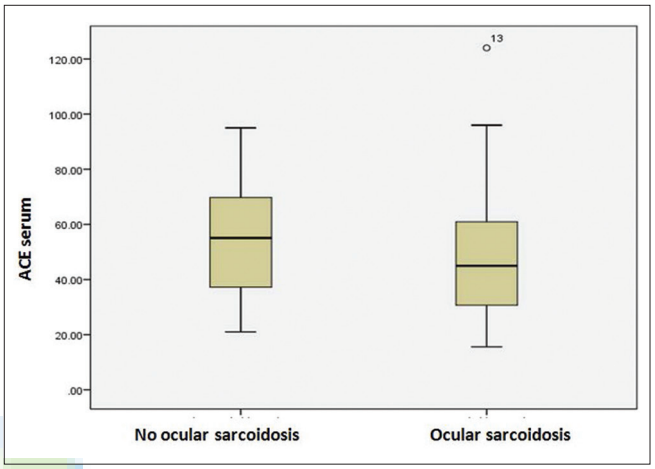

Figure 2: Angiotensin-converting enzyme serum activity in patients with and without ocular sarcoidosis

There is statistically significant difference in ACE activity in aqueous humor among patients with ocular and nonocular sarcoidosis as shown in Mann–Whitney U- value (P = 0.000). ACE activity is significantly higher in patients with ocular sarcoidosis.

Discussion

Studies made in animal models (bulls' and pigs' eyes) were highly suggestive that ANG I and ANG II are generated locally in ocular tissues. Therefore, it was reasonable assumption that ACE is also present in human aqueous humor and its' activity reflects various physiological and pathological conditions involving the eye and RAS.[8-14]

Uveitis is a frequent (20%–50%) and early feature of sarcoidosis.[3] Typical sarcoid uveitis presents with mutton-fat keratic precipitates, iris nodules, and anterior and posterior synechiae. Posterior involvement includes vitritis, vasculitis, and choroidal lesions. Cystoid macular edema is the most important and sight-threatening consequence. Histologic proof from a biopsy is the gold standard for the diagnosis of ocular sarcoidosis. However, the patient with sarcoidosis can have uveitis of other etiology, overlap systemic, and/or other diseases. An international workshop has recently established diagnostic criteria for sarcoidosis uveitis when biopsy is unavailable or negative: these are based on a combination of ophthalmological findings and laboratory tests.[16] The laboratory investigations or investigational procedures that were judged to provide value in the diagnosis of ocular sarcoidosis in patients having the above intraocular signs included[1] negative tuberculin skin test in a Bacillus Calmette–Guérin-vaccinated patient or in a patient having had a positive tuberculin skin test previously,[2] elevated serum ACE levels and/or elevated serum lysozyme,[3] chest X-ray revealing bilateral hilar lymphadenopathy (BHL),[4] abnormal liver enzyme tests, and[5] chest CT scan in patients with a negative chest X-ray result. Four levels of certainty for the diagnosis of ocular sarcoidosis (diagnostic criteria) were recommended in patients in whom other possible causes of uveitis had been excluded: (1) biopsy-supported diagnosis with a compatible uveitis was labeled as definite ocular sarcoidosis; (2) if biopsy was not done, but chest X-ray was positive showing BHL associated with a compatible uveitis, the condition was labeled as presumed ocular sarcoidosis; (3) if biopsy was not done and the chest X-ray did not show BHL, but there were three of the above intraocular signs and two positive laboratory tests, the condition was labeled as probable ocular sarcoidosis; and (4) if lung biopsy was done, and the result was negative, but at least four of the above signs and two positive laboratory investigations were present, the condition was labeled as possible ocular sarcoidosis.[17] Many studies include elevated values of ACE in serum in sarcoidosis and in ocular sarcoidosis as well, but there are only a few analyzing ACE activities in aqueous humor.[18] In a study of Birnbaum *et al*. in a total of 63 patients with uveitis 40%–42% had elevated levels of ACE activity in serum.[7] Weinreb *et al*. measured serum ACE levels in ten patients with chronic granulomatous uveitis with suspected ocular sarcoidosis without evidence of systemic disease and compared it to levels in ten patients with other forms of uveitis and healthy controls. The authors reported that serum ACE levels were higher than 2 standard deviation above mean

in five of ten patients with suspected ocular sarcoidosis and only one patient in the other uveitis groups. Healthy controls had no high serum ACE values. These authors were the first to document that the association of an elevated serum ACE with a chronic granulomatous uveitis suggested the diagnosis of ocular sarcoidosis, and serum ACE was a useful ancillary test for diagnosing ocular sarcoidosis in patients having chronic diffuse granulomatous uveitis.[18] A study by Sharma and Vita conducted in 1983 on 25 patients.[19] found that seven out of ten patients with eye sarcoidosis had increased ACE activity in tears. In our study, all patients with sarcoid uveitis had increased ACE activity in aqueous humor. Activity of ACE in tears can represent ocular surface and lacrimal apparatus involvement in sarcoidosis. Furthermore, in this study, ACE levels in tears were decreasing on prednisone therapy. Serum ACE activity in studies on chronic sarcoidosis did not correlate significantly with prognosis, clinical picture severity, and response to therapy, maybe due to ACE II receptor gene polymorphism.[20-22]

RAS controls fluid volume, electrolyte balance, and blood pressure homeostasis.[23] Many peptides and enzymes of RAS have already been detected in the human eye, and they are even suggested to have a role in the pathogenesis of different ocular diseases.[8-10]

Renin is involved in the conversion of angiotensinogen to ANG I, and the ACE is involved in the conversion of ANG I to ANG II. ANG II, which is the active substance in RAS, has vasoconstrictive effects.[24-26] ANG I and ANG II are produced locally in ocular tissues and that little of these locally produced angiotensins leaks into the ocular fluids. Only when the blood –retinal barrier (BRB) is disrupted, angiotensins can reach the vitreous fluid compartment in concentrations high enough to be detected by assays.[26] The similar is happening with blood–brain barrier which is very comparable to the BRB. Studies have shown that neither ANG I nor ANG II is able to pass the blood–brain barrier.[27,28]

The ACE detected in the aqueous humor in sarcoidosis is definitely the product of granulomatous inflammation of the eye tissue. In our study, it was significantly higher in patients with ocular sarcoidosis and even higher compared with serum ACE in these patients. This finding is in tune with the fact that an activated intraocular RAS is the result of active ocular sarcoidosis.[28,29] The limitation of the study is that ACE activity reference values for aqueous humor are not yet standardized.

Conclusion

Our study showed statistically significant higher levels of ACE activity in aqueous humor in ocular sarcoidosis in comparison with the levels of ACE activity in aqueous humor in patients without ocular manifestations. This finding can be important in distinguishing the etiology of uveitis when uveitis is the first hallmark of disease. Although elevated ACE activity in serum is one of the criteria for the diagnosis of ocular sarcoidosis, this study shows that increased ACE activity in humor aqueous can point to diagnosis of ocular sarcoidosis, without the need for ocular biopsy.

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Conflicts of interest

There are no conflicts of interest.

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