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Original Article

# Evaluation of Aqueous Flare in Graves' Ophthalmopathy and its Relationship with Thyroid Hormones, Antibodies, and Clinical Activity Score

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## Abstract

**Objectives:** The aim of this study was to assess intraocular inflammation in patients with active and inactive Graves' ophthalmopathy (GO) using an aqueous laser flash meter and to assess its relationship with thyroid hormones, antibodies, and clinical activity score (CAS).

**Methods:** Forty patients (29 females and 11 males) were included in the study. The patients were divided into two groups according to CAS; patients with CAS <3 (inactive) were included in Group 1 and patients with CAS ≥3 (active) were included in Group 2. The laser flare meter was used to measure the flare of aqueous humor. Each patient's ocular findings, thyroid hormone, and antibody levels were also recorded.

**Results:** The mean age of patients was 46.88±11.79 years in Group 1 and 44.50±12.59 years in Group 2 (p=0.555). The mean CAS was 0.88±0.65 in Group 1 and 3.57±0.85 in Group 2 (p<0.001). The mean aqueous flare was 6.5±2.2 ph/ms in Group 1 and 7.0±6.4 ph/ms in Group 2 (p=0.73). Hertel exophthalmometry, intraocular pressure (IOP), antithyroglobulin antibody, and thyroid stimulating hormone receptor antibody (TRAb) levels were similar in both groups (each p>0.05). There was no correlation between aqueous flare value and CAS, Hertel exophthalmometry, IOP, thyroid hormone, and antibody levels (each p>0.05). There was a significant correlation between CAS and antibody levels (each p<0.05).

**Conclusion:** Flare values that are not much above the normal range may be an indication that intraocular inflammation is not elevated in GO patients. This suggests that the damage to the blood-aqueous barrier in these patients is not severe enough to increase intraocular inflammation.

**Keywords:** Aqueous flare, blood-aqueous barrier, clinical activity score, graves' ophthalmopathy, laser flare photometry, thyroid stimulating hormone receptor antibody

## Introduction

Graves' ophthalmopathy (GO) is the most common extrathyroidal manifestation of Graves' disease, a chronic autoimmune disorder often associated with hyperthyroidism (1). Although the underlying pathogenic mechanisms have not been fully elucidated, the most likely hypothesis is that the thyroid gland and the orbit tissues have similar antigenic properties, and that immune responses are elicited against these antigens. Antibodies to the thyroid stimulating hor-

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mone (TSH) receptor (TRAb) have been shown to play an important role in the pathogenesis and course of orbitopathy, in addition to thyroid gland diseases (2,3). The course of the disease is described as follows: (1) Active phase: An active inflammatory process, leading to histopathological changes in the orbit (enlargement of the fibro adipose tissue, increase in the volume of the extraocular muscles, increased production of hydrophilic glycosaminoglycans by the fibroblasts), (2) plateau phase: The process by which the disease stabilizes as inflammation begins to subside, and (3) inactive phase: The process by which the disease gradually improves as the inflammation burns off (4).

Accurate assessment of disease activity and severity is very important in the management of GO. The most up-to-date and reliable scoring methods are the clinical activity score (CAS) and the disease severity classification proposed by the European Group on Graves' Orbitopathy (5). Clinical manifestations of pathological changes in the orbit include eyelid edema, soft-tissue changes such as conjunctival hyperemia and chemosis, exophthalmos, diplopia, and compressive dysthyroid optic neuropathy. The CAS with the presence/absence of these findings is the best-validated scoring system (6).

The blood-aqueous barrier (BAB) is in the non-pigmented epithelium of the ciliary body and the wall of the iris capillaries. It prevents blood cells and plasma proteins from passing into the aqueous humor (7). In inflammatory conditions affecting the anterior segment of the eye, the integrity of the BAB is disrupted, and the concentration of proteins and cells in the aqueous humor increases. Laser flare photometry is a non-invasive, objective, and quantitative method that allows measuring aqueous protein concentration with very high reproducibility (8). This method can be used to detect subclinical changes in the BAB. Laser flare photometry has made it possible to investigate the effect of surgery, drugs, laser procedures, and various systemic diseases on intraocular inflammation.

The aim of this study was to evaluate the intraocular inflammation in active and inactive GO patients and to investigate the relationship between aqueous flare and thyroid hormones, antibodies, and CAS using an objective measurement method, the laser flare meter.

## Methods

The study was approved by the Ethics Committee of Istanbul Training and Research Hospital (Approval date and number: 2023-113) and adhered to the tenets of the Declaration of Helsinki. The medical records of patients who were diagnosed with GO at Beyoglu Eye Training and Research Hospital, Oculoplastic Surgery Clinic between 2022-2023 were retrospectively reviewed. Forty patients with laser flare measurements and simultaneous thyroid hormone and antibody levels were included in the study. Patients with known ocular disease, a history of uveitis attack, ocular surgery, and corticosteroid treatment in the past 6 months were excluded from the study.

A complete ophthalmologic examination including a dilated fundus examination was performed in all controls. Hertel exophthalmometry measurements, eye movements including nine gaze positions, diplopia examination, and CAS values were recorded. The CAS is composed of seven substances; spontaneous retrobulbar pain, pain on attempted upward or downward gaze, redness of eyelids, redness of the conjunctiva, swelling of the caruncle or plica, swelling of the eyelids, and swelling of the conjunctiva (chemosis). A ten-item CAS, including ≥2 mm increase in exophthalmos, ≥8° decrease in eye movements in any direction of gaze, and ≥1 line decrease in visual acuity (Snellen) over a period of 1–3 months. While the CAS was assessed using seven parameters in the first examined patients, ten parameters were used to assess the patients at the follow-up visit. (CAS <3 = inactive GO; CAS ≥3 = active GO) (5). Patients were divided into two groups according to CAS; inactive patients included in Group 1 and active patients included in Group 2.

After ophthalmological examination, the aqueous flare was measured 30 min after tropicamide instillation in both eyes using a laser flare meter (FC-700, Kowa Co. Ltd, Tokyo, Japan). Five separate validated measurements were taken by the same clinician who was unaware of the GO severity of the patients, and the mean value was recorded. Since there was no significant difference between the flare values of both eyes, the right eye of each patient was included in the analysis for standardization. Simultaneous thyroid hormone levels (TSH, fT4, and fT3) and antibody (TRAb, antithyroperoxidase antibody, and antithyroglobulin antibody (TGAb)) levels were evaluated at the same visit. The smoking habits of the patients were recorded.

### Statistical Analysis

Statistical analysis was performed using SPSS 26.0 software (IBM Corp., Armonk, NY, USA). Descriptive statistics were used to describe the characteristics of the study population. Continuous data were expressed as mean, standard deviation, and minimum-maximum values. The normality of the distribution of continuous variables was tested using the Kolmogorov–Smirnov test. Independent Samples t-test and Chi-Square test were used to compare clinical parameters between groups. Pearson correlation test was performed to evaluate the relationship between aqueous flare value and CAS, intraocular pressure (IOP), thyroid hormone, and antibody levels. $P<0.05$ was considered statistically significant.

## Results

Forty eyes of 40 patients with GO were included in the study. There were 26 patients in Group 1 and 14 patients in Group 2. The mean age of the groups was similar (p=0.555). While more women were affected in Group 1 (Female/Male [F/M] = 22/4), both genders were equally affected in Group 2 (F/M = 7/7) (p=0.019). The CAS values of the patients were as follows: CAS = 0 in seven, CAS = 1 in 15, and CAS = 2 in four patients in Group 1; CAS = 3 in nine, CAS = 4 in two, and CAS = 5 in three patients in Group 2. The baseline characteristics of the groups are shown in Table 1.

In assessing oculomotor function, oculomotor limitation, and diplopia in at least one gaze position were significantly more common in Group 2 (p<0.05). The concurrent CAS scores, the aqueous flare measurements, and the immunological data of the patients are shown in Table 2.

The correlation analysis showed no significant correlation between aqueous flare and CAS, IOP, Hertel exophthalmometry, thyroid hormones, and antibody levels. However, there was a significant correlation between the CAS and the level of antibodies (Table 3).

## Discussion

GO is characterized by an initial active phase, followed by a plateau phase, and then an inactive phase with a tendency to improve. In GO patients, mononuclear cell infiltration (CD4 and CD8 T lymphocytes, B lymphocytes, plasma cells, and macrophages) has been shown in the orbital fat, extraocular muscles, and lacrimal gland. TNF, IL-2, IFN-γ, and other inflammatory cytokines released by these cells are particularly potent in the active phase, whereas cytokines such as IL-1, IL-6, and TGF-β are released by fibroblasts and adipocytes in the later stages (9,10). In addition to the effects of GO on

**Table 1.** Baseline characteristics

| | Group 1 (inactive GO) n=26 | Group 2 (active GO) n=14 | p |
|---|---|---|---|
| Age, years | 46.88±11.79 | 44.50±12.59 | 0.555 |
| Gender, f/m | 22/4 | 7/7 | 0.019* |
| BCVA, Snellen | 0.81±0.22 | 0.92±0.23 | 0.194 |
| IOP, mmHg | 16.69±3.36 | 17.86±3.08 | 0.290 |
| Hertel exophthalmometry, mm | 20.22±2.77 | 21.00±1.78 | 0.368 |

*Statistically significant. BCVA: Best corrected visual acuity; IOP: Intraocular pressure.

**Table 2.** Clinical and immunological parameters in Graves' ophthalmopathy patients

| | Normal range | Group 1 (inactive GO) n=26 | Group 2 (active GO) n=14 | p |
|---|---|---|---|---|
| Restricted eye movements, present/absent | | 7/19 | 9/5 | 0.041* |
| Diplopia, present/absent | | 2/24 | 6/8 | 0.008* |
| CAS | | 0.88±0.65 | 3.57±0.85 | <0.001* |
| Aqueous flare, ph/ms | | 6.59±2.20 | 7.07±6.40 | 0.726 |
| TSH, mIU/L | 0.4–4.0 | 2.16±2.87 | 2.37±4.96 | 0.871 |
| fT4, ng/dL | 0.6–1.0 | 11.28±6.61 | 9.10±8.24 | 0.372 |
| fT3, pg/mL | 2.5–5.0 | 4.11±2.51 | 5.05±2.99 | 0.345 |
| TRAb, U/L | 0–9 | 10.77±12.56 | 336.10±857.70 | 0.354 |
| TPOAb, IU/mL | <34 | 50.47±74.22 | 289.68±257.53 | 0.006* |
| TGAb, IU/mL | <20 | 200.0±510.62 | 910.60±1462.50 | 0.116 |
| Smoking, present/absent | | 10/16 | 5/9 | 1.000 |

*Statistically significant. CAS: Clinical activity score; TGAb: Antithyroglobulin antibody; TPOAb: Anti thyroperoxidase antibody; TRAb: Thyroid stimulating hormone receptor antibody; TSH: Thyroid stimulating hormone.

**Table 3.** Correlation analysis of the data

| | CAS | IOP | Hertel exophthalmometry | TRAb | TGAb | TPOAb | TSH | fT3 | fT4 |
|---|---|---|---|---|---|---|---|---|---|
| Aqueous flare | r=0.091 | r=0.039 | r=0.016 | r=0.121 | r=-0.050 | r=-0.274 | r=0.014 | r=0.321 | r=0.045 |
| | P=0.578 | P=0.733 | P=0.895 | P=0.602 | P=0.797 | P=0.135 | P=0.933 | P=0.073 | P=0.787 |
| CAS | | r=0.076 | r=0.234 | r=0.491* | r=0.387* | r=0.391* | r=0.172 | r=0.019 | r=0.138 |
| | | P=0.642 | P=0.169 | P=0.024 | P=0.038 | P=0.029 | P=0.288 | P=0.917 | P=0.401 |

*Statistically significant. CAS: Clinical activity score; IOP: Intraocular pressure; TGAb: Antithyroglobulin antibody; TPOAb: Antithyroperoxidase antibody; TRAb: Thyroid stimulating hormone receptor antibody; TSH: Thyroid stimulating hormone.

the orbital tissues, there are intraocular complications such as increased IOP, choroidal folds, and optic disc edema (11). To the best of our knowledge, no other study has investigated the relationship between the aqueous flare and the GO severity. In this study, we investigated intraocular inflammation in active and inactive GO patients and its relationship between CAS, thyroid hormone, and antibody levels.

GO is more common in women. However, it has been shown that the F/M ratio gradually decreases as the severity of the disease increases (12,13). Consistent with previous studies, there was female gender predominance in all patients (F/M: 29/11). While this tendency was also observed in Group 1, the number of male patients was equal to the number of female patients in Group 2 (p=0.019).

The protein concentration in the aqueous humor is determined by the aqueous humor turnover and the filtration of plasma proteins into the anterior chamber (14). The normal value for the aqueous flare is between 1.3 and 7.6 ph/ms (8,15). Possible mechanisms affecting the BAB in GO have been proposed due to its inflammatory etiopathogenesis. These mechanisms include reduced aqueous outflow due to increased episcleral venous pressure, and damage to the BAB as a result of damage to the tight junctions of the non-pigmented ciliary epithelium due to an increase in inflammatory mediators and reactive oxygen species (16).

In our study, the mean aqueous flare value was 6.59±2.20 ph/ms in Group 1 and 7.07±6.40 ph/ms in Group 2 (p=0.726). These findings suggest that intraocular inflammation does not increase in GO patients and does not change with disease severity. Possible parameters that influence the aqueous flare value such as age, IOP, and mydriasis have been defined previously (17,18). In our study, all patients were measured by the same clinician 30 min after tropicamide instillation. The mean age and IOP were similar between the two groups. In addition, none of the patients had elevated IOP at the time of measurement. Therefore, we believe that we have excluded the possibility of misinterpretation.

The fibrotic phase following the inflammatory phase causes problems such as extraocular muscle movement limitation, intermittent or persistent diplopia, and lid retraction, requiring a series of surgical interventions. The only study on this subject in the literature was by Kłysik and Kozakiewicz who compared pre- and post-operative measurements of aqueous flare in patients with GO who had undergone orbital decompression surgery, strabismus surgery, and eyelid surgery (19). In their studies, baseline flare values were measured as 18.25, 13.05, and 10.14, respectively. Besides they found a significant correlation between the pre-operative basal aqueous flare and the maximum CAS values at any of the examinations during the follow-up for up who had undergone orbital surgery or strabismus surgery. They reported that there was no correlation between the maximum value of CAS and the flare value in patients who had undergone eyelid surgery. They reported that orbital and strabismus surgery caused an increase in the post-operative flare, whereas eyelid surgery had no effect on the flare on the 1st day and 1st-week post-operative controls. No difference between flare values and baseline values was observed in all groups at 3-month postoperatively. The high baseline flare values found by Kłysik and Kozakiewicz. are not compatible with our results. This may be due to the included patients having a more severe inflammatory and fibrotic stage. They obtained lower initial exacerbation scores in the group requiring eyelid surgery, which is considered to have a relatively milder course, compared to the group requiring orbital decompression. They also found no significant correlation between exacerbation scores and CAS in this group. Although we did not find a difference between the two groups, in more severe GO patients the BAB may be affected, and aqueous flare scores may increase. Studies with larger populations and more severe patients are needed.

Our study investigated the relationship between flare measurement and CAS on the same examination day. We believe that it would be more meaningful to simultaneously perform CAS, which indicates the severity of orbital inflammation, and aqueous flare, a marker of intraocular inflammation. In our study, the mean CAS value was 0.88±0.65 in Group 1 and 3.57±0.85 in Group 2 ($p<0.001$). Correlation analysis showed no significant relationship between aqueous flare value and concurrent CAS value ($p=0.578$). These results suggest that there is no significant intraocular inflammation in active and inactive GO patients.

The previous studies have reported that TRAb is an independent risk factor for GO and that it plays a crucial role in the severity of the disease and its prognosis (20-22). It has been shown that there is a correlation between CAS and TRAb elevation in GO patients, and it has been reported that TRAb levels gradually decrease after euthyroidization (23). Although not statistically significant, we found higher levels of TRAb and TGAb in Group 2 (each $p>0.05$) in our study. TPOAb was statistically significantly higher in Group 2 ($p=0.006$). There was also a significant positive correlation between CAS and thyroid autoantibodies in Group 2 (each $p<0.05$). However, we found no correlation between aqueous flare and thyroid hormone and antibody levels (each $p>0.05$).

Our study's most important limiting factor is that we did not include patients with optic nerve involvement, where intraocular inflammation is expected to predominate, or patients with an aggressive course requiring orbital decompression. The retrospective nature of the design and the small number of patients is other limiting factors.

## Conclusion

The aqueous flare values obtained in this study are close to the normal range in inactive GO patients. Aqueous flare values are not compatible with orbital inflammation scoring. These results suggest that BAB is not impaired in these patients and that the intraocular space is protected from orbital inflammation.

### Disclosures

**Ethics Committee Approval:** The study was approved by the Ethics Committee of Istanbul Training and Research Hospital (Approval date and number: 2023-113) and adhered to the tenets of the Declaration of Helsinki.

**Peer-review:** Externally peer-reviewed.

**Conflict of Interest:** None declared.

**Authorship Contributions:** Concept – H.T.; Design – H.T., B.K.A.; Supervision – K.S.C.; Resource – B.Y.O.; Materials – A.C.E., B.Y.O.; Data collection and/or processing – M.G.U., K.S.C.; Analysis and/or interpretation – H.T.; Literature search – H.T., B.K.A.; Writing – H.T., B.K.A.; Critical review – A.C.E., M.G.U.

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