The Effect of Smoking on Mitochondrial Biogenesis in Patients With Graves Ophthalmopathy

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Purpose: To evaluate the effects of cigarette smoking on oxidative stress (OS) and mitochondrial biogenesis related parameters in patients Graves Ophthalmopathy (GO).

Methods: Patients with moderate-to-severe GO according to the European Group on Graves Orbitopathy (EUGOGO) criteria were prospectively enrolled in this study. Age- and sex-matched healthy volunteers who applied to outpatient clinic due to refractive problems consisted the control group. Participants were divided into 4 groups based on their diagnosis and smoking status: group 1 (n = 30) smoker GO patients, group 2 (n = 30) nonsmoker GO patients, group 3 (n = 30) smoker healthy controls, and group 4 (n = 30) nonsmoker healthy controls. In the sera, total antioxidant status, total oxidant status and OS index values, peroxisome proliferator-activated receptor-γ coactivator 1-α, mitochondrial transcriptional factor A levels, and paraoxonase-1 enzyme activity were evaluated.

Results: Total oxidant status and OS index values were the highest in group 1 compared to other groups ($p = 0.031$, $p = 0.042$; respectively). There was no statistically significant difference in total antioxidant status and peroxisome proliferator-activated receptor-γ coactivator 1α levels among the groups ($p = 0.521$, $p = 0.388$; respectively). Paraoxonase-1 enzyme activity was the lowest in group 1 and highest in group 4 ($p = 0.024$). The levels of mitochondrial transcriptional factor A was the lowest in group 1 compared to other groups ($p = 0.012$).

Conclusions: Cigarette smoking in GO patients seems to be a risk factor that increases OS, and therefore, it may have an unfavorable impact on the mitochondrial biogenesis.

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Graves' ophthalmopathy (GO) is an orbital inflammatory disease which is mostly seen with autoimmune thyroid dysfunction.[1,2] Oxidative stress (OS) which is known as decreased ability of the organism in detoxifying of reactive oxygen species (ROS) has been held responsible for many diseases, including autoimmune diseases.[3,4] To date, numerous reports have revealed the relationship between OS and GO.[5–11] The deterioration of clinical signs of GO with smoking is well known and this has been associated with the increased amount of ROS.[12]

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As the energy-producing organelle of the cell; mitochondria is in close relation with ROS because of its major role in oxidative phosphorylation. Therefore, while mitochondria is the main source of OS, it is also one of the most affected structure of the cell from OS. In the previous studies, particular antibodies against mitochondrial enzymes have been shown in GO patients.[13,14]

Peroxisome proliferator-activated receptor-γ coactivator 1-α (PGC-1α) and mitochondrial transcriptional factor A (MTFA) are 2 proteins which are responsible from proper mitochondrial functions, namely mitochondrial biogenesis.[15] In the presence of OS, their synthesis increase and they ensure the continuity of mitochondrial functions.

Paraoxonases (PON) are mitochondrial enzymes that help to maintain mitochondrial functions by controlling superoxide production and preventing apoptosis.[16]

In this study, we aimed to investigate the possible action mechanism of the cigarette smoking which is known to cause deterioration of the prognosis in GO, through the mitochondria that seems to be both the source and the victim of OS. Thus, we evaluated the serum levels of total oxidant status (TOS), total antioxidant status (TAS), PON-1, PGC-1α, and MTFA in smoker and nonsmoker GO patients.

METHODS

The study was performed according to the tenets of the Declaration of Helsinki for research involving human subjects. The protocol was approved by the ethics committee of Gazi University Medical Faculty and written consent was obtained from all participants.

The study was conducted at Ankara Ataturk Training ve Research Hospital, Ophthalmic and Plastic Reconstructive Surgery Division of Ophthalmology Department and Gazi University Medical Faculty, Medical Biochemistry Department between February and December 2018. Patients with moderate-to-severe GO according to the European Group on Graves' Orbitopathy (EUGOGO)[17] criteria were prospectively enrolled in this study.

Age- and sex-matched healthy volunteers who applied to outpatient clinic due to refractive problems were prospectively enrolled for this study as the control group. The disease activity was evaluated with the clinical activity score.[18] Graves ophthalmopathy patients with clinical activity score ≥ 4/10 were considered as active status.

The patients with any ocular diseases other than GO, using of any antioxidant drug or other than thyroid metabolism regulators, pregnancy, acute or chronic systemic diseases, such as diabetes mellitus, liver or kidney diseases, cancer, other endocrine dysfunction, immunological, or inflammatory disorders were excluded from the study. Because the abnormal levels of thyroid hormones may affect the OS status, only the GO patients euthyroidism (free T3 and free T4 were within normal range, thyroid-stimulating hormone [TSH] was low or within normal range) for at least 6 months were included in this study.

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None of the patients had previously systemic steroids, radiotherapy, or undergone orbital decompression, strabismus, or eyelid surgery for GO.

Participants were divided into 4 groups based on their diagnosis and smoking status:

Group 1 consisted of 30 moderate-to-severe GO patients who smoke ≥ 5 per day cigarettes at least for 1 year. Group 2 consisted of 30 moderate-to-severe GO patients who does not smoke at least 1 year. Group 3 consisted of 30 healthy volunteers who smoke ≥ 5 per day cigarettes at least for 1 year. Group 4 consisted of 30 healthy volunteers who does not smoke at least 1 year.

Biochemical Analysis. Venous blood samples (5 ml) for testing serum TAS, TOS, PON-1, MTFA, and PGC-1α were collected after 12 hours of fasting. Serum specimens were obtained after the samples were centrifugated at 3000 rpm for 10 minutes. All samples were immediately frozen to −80°C prior to analysis. Free T3, free T4, TSH, and thyroid-stimulating hormone receptor antibody (TSHR-Ab) levels were measured from whole blood samples on same day.

Serum TAS and TOS Analysis. In the serum samples, TAS and TOS were measured by using commercially available kits (Rel Assay Diagnostic, Gaziantep, Turkey). OS index (OSI) was calculated by TOS/TAS formula.

Serum PON-1 Enzyme Activity Analysis. Serum PON-1 enzyme activity was measured by using commercially available kits (Rel Assay Diagnostic, Gaziantep, Turkey).

Serum MTFA Analysis. Mitochondrial transcriptional factor A levels in serum were determined using the quantitative sandwich an enzyme-linked immunosorbent assay in accordance with the manufacturer's instructions (Catalog Number: YLA4057HU; Shanghai YL Biotech Co., Ltd.).

Serum PGC-1α Analysis. Peroxisome proliferator-activated receptor-γ coactivator 1α levels in serum were determined using the quantitative sandwich an enzyme-linked immunosorbent assay in accordance with the manufacturer's instructions (Catalog Number: YLA1036HU; Shanghai YL Biotech Co., Ltd.).

Serum-free T3, free T4, and TSH were determined using Cobas E601 auto-analyzer (Roche Diagnostics, Penzberg, Germany). Thyroid-stimulating hormone receptor antibody levels were measured with Stratec gamma counter analyzer (Stratec Biomedical Systems Birkenfeld, Germany).

Statistical Analysis. All statistical tests were performed using SPSS version 20.0 (Statistical Package for the Social Sciences, SPSS Inc., Chicago, IL, U.S.A.). The normality of the data was confirmed using the Shapiro-Wilk test. Chi-square test was used for the comparison of categorical variables. The differences in measured parameters between 2 groups were analyzed by 1-way ANOVA and Tukey posthoc test. The correlation between variables was evaluated by using Pearson correlation analysis. The differences were considered significant when the $p < 0.05$.

RESULTS

Demographic characteristics of the study groups were presented in Table 1. There was no statistically significant difference in sex and age between the groups ($p = 0.807$, $p = 0.720$; respectively).

Smoking characteristics in terms of number of cigarettes smoked per day and pack-year were similar between smoker GO and smoker controls ($p = 0.246$ vs. $p = 0.872$; respectively).

There was no significant difference in duration of hyperthyroidism and GO, number of active/inactive patients, thyroid function tests (free T3, free T4, TSH, TSHR-Ab levels) between smoker and nonsmoker GO patients (Table 2).

Serum levels of TAS, TOS, OSI, MTFA, PGC-1α, and PON-1 enzyme activity were summarized in Table 3.

There was no statistically significant difference in serum TAS levels between 4 groups ($p = 0.521$).

There was statistically significant difference in serum TOS levels between 4 groups ($p = 0.031$). The binary comparisons revealed significant differences among all the groups.

There was statistically significant difference in OSI levels between 4 groups ($p = 0.042$). The binary comparisons revealed significant differences among all the groups.

There was statistically significant difference in PON-1 enzyme activity between the groups ($p = 0.024$). When GO patients were evaluated within themselves (group 1 vs. group 2) no statistically significant difference was found ($p > 0.05$). Similarly, there was no statistically significant difference between control subjects (group 3 vs. group 4) ($p > 0.05$). The differences between group 1 and group 3 − group 4 and group 2 and group 3 − group 4 were statistically significant ($p < 0.05$).

There was no statistically significant difference in serum PGC-1α levels between 4 groups ($p = 0.388$).

TABLE 1. Demographic characteristics of patients with GO and healthy controls.

	GO		Controls		
	Smoker (n = 30)	**Nonsmoker (n = 30)**	**Smoker (n = 30)**	**Nonsmoker (n = 30)**	***p***
Age (year)	37.36 ± 8.84 (22–50)	35.14 ± 8.39 (23–50)	35.43 ± 9.11 (20–50)	36.36 ± 8.55 (22–50)	0.720
Sex (F/M)	14/16	13/17	14/16	16/14	0.807
Number of cigarettes smoked per day	14.3 ± 8.04 (5–40)	—	16.3 ± 7.97 (5–30)	—	0.246
Number of pack-year	14.3 ± 8.04 (5–30)	—	13.2 ± 8.61 (5–30)	—	0.872

GO, Graves Ophthalmopathy.

TABLE 2. Clinical properties of smoker and nonsmoker GO patients

	GO smoker (n = 30)	**GO nonsmoker (n = 30)**	***p***
Duration of hyperthyroidism (year)	3.48 ± 2.43 (1–10)	4.01 ± 2.07 (1–8)	0.455
Duration of GO (year)	2.03 ± 1.03 (1–5)	2.7 ± 1.61 (1–6)	0.341
Active/inactive GO	11/19	9/21	0.367
CAS	3.18 ± 2.15 (0–7)	2.87 ± 2.18 (0–7)	0.711
Free T3 (pg/ml)	3.21 ± 1.07 (2.01–4.21)	2.91 ± 1.72 (2.21–3.98)	0.512
Free T4 (ng/dl)	1.1 ± 0.34 (0.98–1.56)	1.4 ± 1.1 (1.01–1.63)	0.423
TSH (μIU/ml)	0.56 ± 1.12 (0.32–3.98)	0.81 ± 0.98 (0.78-2.88)	0.366
TSHR-Ab U/L	7.12 ± 1.01 (2–14)	5.95 ± 1.34 (4-12)	0.721

CAS, clinical activity score; GO, Graves Ophthalmopathy; TSH, thyroid-stimulating hormone; TSHR, thyroid-stimulating hormone receptor.

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TABLE 3. Biochemical parameters in GO and healthy controls

	GO		Controls		
	Smoker; group 1 (n = 30)	Nonsmoker; group 2 (n = 30)	Smoker; group 3 (n = 30)	Nonsmoker; group 4 (n = 30)	*p*
TAS (mmol/L)	1.64 ± 0.16 (1.35–1.98)	1.65 ± 0.16 (1.32–2.07)	1.67 ± 0.16 (1.34–2.03)	1.68 ± 0.15 (1.38–1.95)	0.521
TOS (μmol/L)	12.16 ± 4.10*†‡ (4.28–21.55)	10.38 ± 3.52*§‖ (4.50–22.83)	8.83 ± 5.02*§¶ (4.39–17.36)	6.01 ± 2.40*‖¶ (3.89–15.61)	0.031
OSI (AU)	0.69 ± 0.42*†‡ (0.27–2.18)	0.61 ± 0.35*§‖ (0.28–1.66)	0.64 ± 0.34*§¶ (0.27–2.07)	0.52 ± 0.14*‖¶ (0.25–0.90)	0.042
PON-1 (U/L)	303.88 ± 204.47†‡ (108–775)	347.70 ± 224.31§‖ (127–962)	433.73 ± 182.54†§ (116–1006)	437.92 ± 255.90‡‖ (117–1090)	0.024
PGC-1α (ng/ml)	3.39 ± 1.22 (1.15–6.98)	3.48 ± 1.36 (1.92–6.97)	3.69 ± 1.31 (2.28–6.86)	4.05 ± 1.49 (2.40–7.62)	0.388
MTFA (pg/ml)	60.72 ± 26.56*†‡ (26.40–147.74)	82.78 ± 29.39*§‖ (41.78–148.21)	97.40 ± 24.72*§¶ (51.78–151.25)	104.06 ± 29.73*‖¶ (43.94–151.66)	0.012

*$p < 0.05$, Group 1–Group 2.
†$p < 0.05$, Group 1–Group 3.
‡$p < 0.05$, Group 1–Group 4.
§$p < 0.05$, Group 2–Group 3.
‖$p < 0.05$, Group 2–Group 4.
¶$p < 0.05$, Group 3–Group 4.
GO, Graves Ophthalmopathy; MTFA, mitochondrial transcriptional factor A; OSI, OS index; PGC-1α, peroxisome proliferator-activated receptor-γ coactivator 1-α; PON-1, paraoxonase-1; TAS, total antioxidant status; TOS, total oxidant status.

There was statistically significant difference in MTFA levels between groups ($p = 0.012$). The binary comparisons revealed significant differences among all the groups.

Evaluation of Biochemical Parameters According to Activity of GO Patients. The levels of biochemical parameters according to GO activity were summarized in Table 4.

Smoker (group 1) and nonsmoker GO (group 2) patients were further divided into 2 groups as active (group 1A and group 2A) and inactive (group 1B and group 2B) according to their clinical activity scores. There was significant difference in serum TOS, OSI, MTFA levels, and PON-1 enzyme activity between groups ($p = 0.027$, $p = 0.048$, $p = 0.022$, $p = 0.039$; respectively).

There was no significant difference between serum TAS and PGC-1α levels ($p = 0.092$, $p = 0.621$; respectively).

Serum TOS levels were the highest in active smoker GO patients (group 1A) and the difference was significant when compared to other 3 groups ($p = 0.00$).

OS index values were the highest in active smoker GO patients (group 1A) ($p = 0.048$), and there was no significant difference between the other 3 groups ($p > 0.05$).

Serum PON-1 enzyme activity was the lowest in active smoker GO patients (group 1A), and the difference was significant only between nonsmoker inactive GO patients (group 2B) ($p = 0.00$).

Serum MTFA levels was the lowest in active smoker GO patients (group 1A) ($p= 0.022$), and there was no significant difference between the other 3 groups ($p > 0.05$).

There were no significant correlation between the mean number of cigarettes smoked per day and any biochemical parameters in smoker GO patients.

TABLE 4. The comparison of the biochemical parameters according to clinical activity in smoker and nonsmoker GO patients

	GO smoker		GO nonsmoker		
	Active group 1A (n = 11)	Inactive group 1B (n = 19)	Active group 2A (n = 9)	Inactive group 2B (n = 21)	*p*
TAS (mmol/L)	1.62 ± 0.11 (1.35–1.92)	1.66 ± 0.06 (1.39–1.98)	1.60 ± 0.24 (1.34–2.07)	1.67 ± 0.11 (1.32–1.91)	0.092
TOS (μmol/L)	18.16 ± 4.51*†‡ (5.58–21.55)	6.99 ± 2.92*§ (4.28–16.21)	14.81 ± 4.22*§‖ (4.39–22.83)	7.12 ± 3.07*‖ (4.50–19.89)	0.027
OSI (AU)	0.74 ± 0.82*†‡ (0.41–2.18)	0.66 ± 0.47* (0.27–2.01)	0.65 ± 0.34† (0.28–1.66)	0.57 ± 0.19‡ (0.28–1.45)	0.048
PON-1 (U/L)	278.71 ± 217.71‡ (114–690)	328.11 ± 261.54 (108–775)	334.21 ± 145.14 (127–962)	360.82 ± 305.21‡ (177–878)	0.039
PGC-1α (ng/ml)	3.36 ± 1.81 (1.29–6.98)	3.42 ± 1.12 (1.15–6.01)	3.26 ± 1.91 (1.92–6.97)	3.90 ± 2.99 (2.28–6.62)	0.621
MTFA (pg/ml)	41.57 ± 20.17*†‡ (26.40–131.22)	80.56 ± 27.12* (38.91–147.74)	74.40 ± 31.62† (57.90–148.21)	96.12 ± 27.98‡ (51.78–139.74)	0.022

*$p < 0.05$, Group 1A–Group 1B.
†$p < 0.05$, Group 1A–Group 2A.
‡$p < 0.05$, Group 1A–Group 2B.
§$p < 0.05$, Group 1B–Group 2A.
‖$p < 0.05$, Group 2A–Group 2B.
GO, Graves Ophthalmopathy; MTFA, MTFA, mitochondrial transcriptional factor A; OSI, OS index; PGC-1α, peroxisome proliferator-activated receptor-γ coactivator 1-α; PON-1, paraoxonase-1; TAS, total antioxidant status; TOS, total oxidant status.

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DISCUSSION

Although the exact pathophysiology is not certain, GO is accepted as an inflammatory disease based on autoimmune background.[19] Chronic inflammation that was triggered by autoimmunity is characterized by increased ROS. Also, high levels of thyroid hormones lead to increased levels of ROS by speeding the basal and cellular metabolism and result in OS.[20] The antioxidant system and many OS parameters were evaluated in blood, urine, fibroadipose tissue in GO patients, and results of the most studies support the relationship between OS and GO.[5–11] In these studies, OS parameters were measured separately and independently of each other. Instead of measuring the parameters separately, a practical method was developed to evaluate the total OS in the body. With this method, the total amount of OS in the circulation is measured as TOS. This fully automated method is developed to overcome several major analytical problems and technical restrictions that other manual methods have before. Although many factors, such as stress, ischemia, hemorrhage, infections, radioactivity, medicines, ultraviolet, and aging process, may affect TOS, we tried to include patients with similar characteristics. In this study, TOS levels were higher in GO groups compared to control groups. When GO patients were evaluated within themselves, TOS values were higher in smoker GO patients. According to these results, it seems that smoking aggravates OS which has been already existed in GO. In control groups, TOS levels were also higher in smokers. Similarly, TOS levels were detected higher in a study that was conducted in smoker healthy men.[21]

The organism has an advanced antioxidant defense system that struggling with endogeneous and exogenous ROS to maintain homeostasis. It is not practicable to measure these numerous antioxidant molecules in terms of both cost, time, reliability, and sensitivity. Therefore, a fully automated TAS measurement method was developed to overcome these problems. In this study, no significant difference was found in TAS levels between 4 groups. Although TOS levels were higher in GO patients, especially in smoker GO patients, the similarity of antioxidant levels may be related to increased synthesis of antioxidant molecules to overcome OS. The similar TAS levels despite GO and smoking may be related to the relatively young age of participants (50 or less), no other accompanying systemic diseases and euthyroid status of GO patients.

The ratio of TOS to TAS is known as OSI and it is a marker of OS. In this study, the highest values were detected in smoker GO patients as consistent with the TOS results.

Today, studies are made to understand better the etiopathogenesis of the diseases are detailed into molecular level and they target the cell surface (receptors) or subcellular structures. As technology evolves and researches are elaborated, cellular organelles have been specifically associated with many ocular diseases.

Mitochondria is the only organelle that has its own DNA (mtDNA). It has transfer RNA (tRNA) and ribosomes that allow it to synthesize its own proteins. Because of its important role in ATP synthesis, healthy functioning of the all organelles is associated with healthy mitochondrial functions. In the process of oxidative phosphorylation, in which ATP is synthesized, a large amount of ROS are formed. Although it is closely related with ROS due to its function in the cell, many properties that protects nuclear DNA do not exist in mitochondria. This makes mtDNA more susceptible to ROS damage.[22] Therefore, mitochondria is both the main source of ROS in the cell and the mostly affected by OS.

The role of somatic mtDNA mutations has been shown in cancer, neurodegenerative diseases, cardiac, and some kind of autoimmune diseases.[23–25] Chen et al.[26] showed that mutational self-peptides resulted from somatic mtDNA mutations triggered the immune response and an immune response against healthy self-peptides by cross-reaction. Somatic mtDNA mutations were found higher in the synovia of the patients with rheumatoid arthritis compared to patients with osteoartritis.[27] Affected mitochondrial biogenesis was shown in systemic lupus erythematosus which is an another autoimmune disease.[28] Again, a relationship was found between disease activity index and mtDNA copy number and mtDNA D310 heteroplasmy.[29]

Because of the profound effects of thyroid hormones on the metabolism, the link between thyroid hormones and mitochondrial biogenesis is expected. In the literature, there are some studies that emphasizing the important role of thyroid hormones on mitochondrial biogenesis.[30,31] However, there is no study that is evaluating mitochondrial biogenesis in autoimmune thyroid disease and GO which are the most common autoimmune diseases.

Mitochondrial abnormalities were shown by electron microscopy in the muscle tissue of GO patients with extraocular muscle involvement.[32] The authors suggested that these mitochondrial abnormalities may reflect the autoimmune reaction in the eye muscles. Although that study shows the structural abnormalities of the mitochondria, it does not give information about the function of the mitochondria. Hosal et al. and Gunji et al. evaluated the antibody levels against the flavoprotein subunit of succinate dehydrogenase enzyme in sera of GO patients.[13,14] Both of these studies showed a relationship between antibody levels and extraocular muscle involvement. Cheng et al. reported increased levels of mitochondrial enzymes in terms of isocitrate dehydrogenase and ATP synthase in orbital fat of GO patients. In all of these researches, different mitochondrial enzymes were studied but evaluating of some individual enzymes would not give any idea for all of the mitochondrial functions.

Peroxisome proliferator-activated receptor-γ coactivator 1α is one of the important protein that organizes the mitochondrial biogenesis and so known the main organizator of the cellular energy metabolism. As a transcriptional factor, it is responsible for the gene expression of the important proteins which take roles in mitochondrial pathways.[34] The increased level of this protein induces mitochondrial biogenesis and leads to increase in number and capacity of mitochondria.[35] It has been also shown that it suppresses OS by inducing several antioxidant enzymes such as superoxide dismutase1, superoxide dismutase2 (MnSOD), Glutathione peroxidase 1.[36] In our study, there was no significant difference in PGC-1α levels between 4 groups.

In the process of mitochondrial biogenesis, after PGC-1α become activated, it first activates nuclear respiratory factor-1 and nuclear respiratory factor-2, then MTFA. The activation of PGC-1α—nuclear respiratory factor—MTFA pathway induces new mitochondria generation by increasing the synthesis of mtDNA and protein. Mitochondrial transcriptional factor A is a nuclear-encoded protein and it helps to normal functioning of mitochondria by maintaining the mtDNA in OS status.[37] In this study, MTFA levels were found lower in smoker GO patients who have the highest TOS levels. According to these results, GO and smoking together prevents the synthesis of MTFA. Thus, maintaining mitochondrial biogenesis may be compromised. In the presence of a healthy antioxidant defense system, PGC-1α and MTFA are expected to overcome the negative effects of OS. Although the PGC-1α levels were similar between the groups, the statistically significant difference in MTFA levels may be related to other yet unknown pathways.

Thyroid hormones may affect MTFA.[38] It has been shown that MTFA levels and mtDNA copy number decrease in skeletal

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muscle tissue in patients with Hashimoto hypothyroid myopathy.[39] These findings support the effects of thyroid hormones on mitochondrial functions. Although all patients with GO in this study are euthyroid, it is possible that the mitochondrial function will be damaged by this mechanism because of the underlying thyroid dysfunction.

Paraoxonases degrades intracellular lipid peroxides in the lipids thus they are accepted as an endogenous antioxidant enzyme. They control the mitochondria-associated O_2-production and prevent apoptosis.[16] Thus, they have important roles on preventing mitochondrial dysfunction. In this study, serum PON-1 enzyme activity of both smoker and nonsmoker GO patients were detected lower than both of smoker and nonsmoker healthy controls. When GO patients and controls were evaluated within themselves, PON-1 enzyme activity were found lower in smokers although the difference was not significant. Korkmaz et al.[40] reported decreased serum PON-1 enzyme activity in autoimmune thyroid disease (Hashimoto thyroiditis ve GH). Similarly, Raiszadeh et al.[41] reported decreased PON-1 enzyme activity in patients with hyperthyroidism. According to these results, it seems that both GO and smoking may decrease PON-1 enzyme activity. However, it should be kept in mind that several factors like genetic polymorphism, age, gender, drugs may affect PON-1 enzyme activity.[42]

We did not find a significant correlation between the number of cigarettes per day used by GO patients and the biochemical parameters. At this point, more patients are needed to make a conclusive comments.

This study also evaluated the relationship between biochemical parameters and clinical findings of GO patients. Active phase of GO is characterized with inflammatory findings, and the correlation between OS and level of inflammation is well known.[43] Steroid theraphy, that is recommended for active phase decreases both inflammation and OS.[9,44] In this study, we detected higher levels of serum TOS and OSI; and lower levels of MTFA and PON-1 enzyme activity in active smoker GO patients. In the light of these findings, it can be suggested that orbital and/or systemic inflammation in active GO patients triggers ROS formation, causes OS, and smoking aggravates these effects and deteriorates mitochondrial biogenesis related parameters.

In conclusion, as a result of OS or an exacerbating factor of OS, GO may have a negative effect on mitochondrial biogenesis. Smoking also may aggravate these negative effects. The investigation of the biogenesis of mitochondria as a causative and/or affected structure in the complex OS table will help to understand the role of mitochondria in the etiopathogenesis of GO. New treatment and preventive options can be developed to increase the levels of specific antioxidants molecules that support mitochondrial life and function.

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