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Salivary oxidative stress enzymes in patients with Graves orbitopathy: associations with clinical activity and treatment status

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Introduction

Graves orbitopathy (GO) is an autoimmune inflammatory disorder of the orbit associated with Graves disease (GD) and characterized by lymphocytic infiltration, cytokine release, adipogenesis, and tissue remodeling within the extraocular muscles and orbital connective tissue [1,2]. Although the precise mechanisms underlying GO are not fully understood, growing evidence suggests that oxidative stress plays a central role in its pathogenesis and progression.

Increased production of reactive oxygen species (ROS) has been demonstrated in orbital fibroblasts and peripheral blood of GO patients, contributing to inflammation, glycosaminoglycan accumulation, and tissue expansion [3-5]. Moreover, both in vivo and in vitro evidence indicate that oxidative stress interacts with pro-inflammatory signaling cascades, amplifying cytokine expression and extracellular matrix deposition in orbital tissues, thereby linking oxidative imbalance with clinical measures of disease activity [6].

Antioxidant defense systems, including enzymatic components such as superoxide dismutase (SOD), catalase (CAT), and myeloperoxidase (MPO), are critical regulators of oxidative balance. Disturbances in these systems may reflect disease activity and inflammatory status [7,8]. While systemic oxidative stress markers have been investigated in GD and GO, data on salivary antioxidant enzymes in GO are limited [9]. Saliva represents a noninvasive and easily accessible biological material that may reflect both local and systemic redox alterations [10-12]. Salivary antioxidant enzymes are known to respond dynamically to systemic inflammatory and oxidative conditions, suggesting that their activity may change in parallel with immune activation in autoimmune diseases [13]. Systematic analyses of salivary biomarkers in autoimmune thyroid diseases have identified alterations in oxidative status and antioxidant capacity, including changes in protein, cytokine and oxidative marker profiles, although specific data for GO are still lacking [14].

Therefore, we aimed to explore whether salivary activities of SOD, CAT, and MPO differ according to GO activity status and whether these markers are associated with clinical activity score (CAS) and treatment-related characteristics.

Patients and methods

This observational study employed consecutive enrolment. All adult patients of both genders diagnosed with GO who were treated at the Department of Endocrinology, Metabolism, and

Internal Medicine at Poznan University of Medical Sciences between October 2024 and September 2025 were included. GO was diagnosed in accordance with the European Group on Graves' orbitopathy (EUGOGO) guidelines [15]. Exclusion criteria comprised the presence of other autoimmune disorders, active malignancy, active inflammatory lesions within the oral cavity, antioxidant supplementation, and the use of medications known to affect salivary secretion.

Clinical and laboratory assessment

All participants underwent a comprehensive physical examination, including a detailed dental evaluation. Dentition status, oral hygiene, and periodontal condition were assessed using single-use sterile diagnostic kits consisting of a dental mirror and probe, as well as a sterile World Health Organization 621 Hu-Friedy periodontal probe (11.5-mm scale).

Patients received a full ophthalmological assessment as part of routine clinical care. Disease activity was evaluated using the CAS in accordance with the EUGOGO recommendations [15]. A CAS of three or more points was considered indicative of active orbitopathy. In addition, all patients underwent orbital magnetic resonance imaging to support differential diagnosis and assess disease activity.

Venous blood samples were collected after overnight fasting. Laboratory measurements included thyroid-stimulating hormone, thyroid hormone levels, and antithyroid autoantibodies. Unstimulated whole mixed saliva served as the study material. Samples were collected in the morning at a fixed time point. Participants were fasting and rinsed their oral cavity with water before collection. Saliva was collected for 10 minutes (minimum volume 2.5 mL) into sterile Falcon tubes. Following collection, samples were centrifuged at 4 °C for 20 minutes at 3000 × g and stored at −80 °C until analysis. Salivary activities of selected enzymes (SOD, CAT, MPO) were quantified using commercial colorimetric or fluorometric kits according to the manufacturer's protocols (Merck KGaA).

Ethics

The study protocol received approval from the Bioethics Committee of Poznan University of Medical Sciences (677/23) and was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants.

Statistical analysis

Quantitative data were presented as medians with interquartile ranges, and compared using the Mann–Whitney test due to non-conformity with normal data distribution. Formal correction for multiple comparisons was not applied because of the preliminary character and limited sample size of this exploratory study. To assess the relationship between the variables, Spearman rank correlation coefficients were calculated. Receiver operating characteristic (ROC) analysis was conducted to evaluate the diagnostic performance of selected markers, with cut-off values determined using the Youden index. Statistical analyses were performed using Statistica 13.3 software (TIBCO Software Inc.), with a significance level set at $\alpha = 0.05$. Selected results were visualized using violin plots generated with MedCalc 23.4.0.

Results

The study group included 32 individuals with GO (16 females and 16 males). Clinical and laboratory characteristics of the study population are presented in Supplementary material, *Table S1*.

Salivary activities of MPO, CAT and SOD were analyzed according to clinical activity assessed by CAS, and different therapeutic modalities in patients with GO.

When patients were stratified by clinical activity, no statistically significant differences in salivary enzyme activities were observed between active (n = 16) and inactive (n = 16) GO. Median MPO activity was 5.215 (Q1–Q3: 1.904–8.817) in active patients and 11.088 (1.707–20.000) in inactive patients ($P = 0.33$). CAT activity was similar between groups – 64.754

(27.224–100.000) vs 65.299 (44.795–87.959), respectively (P >0.99). SOD activity tended to be higher in active GO compared with inactive GO – 2.081 (1.386–4.001) vs 1.328 (1.060–1.898) – but this difference did not reach statistical significance (P = 0.07).

In contrast, treatment with antithyroid medications was associated with significantly higher salivary MPO and SOD activities. Patients receiving antithyroid drugs (n = 16) had a median MPO activity of 9.965 (3.788–20.000), compared with 2.435 (0.419–8.792) in untreated patients (n = 16; P = 0.03). Likewise, SOD activity was significantly higher in treated patients – 2.029 (1.476–4.231) vs 1.215 (0.990–1.767; P = 0.01). CAT activity did not differ between groups (P = 0.98).

No significant associations were observed between enzyme activities and a history of thyroidectomy. MPO activity was 6.848 (2.094–11.088) in patients after thyroidectomy (n = 14) and 3.980 (1.707–18.809) in those without surgery (n = 18; P = 0.77). CAT activity was 72.795 (44.795–100.000) vs 53.784 (30.438–98.850) (P = 0.19), and SOD activity was 1.601 (1.066–2.128) vs 1.730 (1.060–3.475; P = 0.65), respectively.

Regarding iodine-131 (I-131) therapy, no statistically significant differences in salivary enzyme activities were detected. Nevertheless, MPO activity tended to be lower in patients treated with radioiodine (n = 10) compared with those without I-131 therapy (n = 22): 1.707 (1.372–3.980) vs 8.592 (3.595–18.809), approaching but not reaching significance (P = 0.07). CAT and SOD activities were comparable between groups (P = 0.16 and P = 0.62, respectively).

Finally, qualification for systemic steroid therapy was not associated with significant differences in enzyme activities. Patients qualified for steroids (n = 10) had a median MPO activity of 3.788 (1.372–8.391) compared with 8.792 (2.094–20.000) in those not qualified for steroids (n = 22; P = 0.14). CAT activity was 40.024 (11.487–100.000) vs 71.464 (48.739–100.000) (P = 0.2). SOD activity tended to be higher in steroid-qualified patients – 2.698

(1.730–5.219) vs 1.435 (1.060–2.024) – but this difference did not reach statistical significance ($P = 0.06$).

In patients with active GO, salivary SOD activity showed a moderate positive correlation with CAS (Rs = 0.513, $P = 0.05$), indicating higher SOD levels with increasing inflammatory activity. In addition, SOD activity correlated negatively with GO duration (Rs = −0.574, $P = 0.02$), suggesting higher SOD activity in earlier stages of active disease. CAT activity demonstrated a significant negative correlation with CAS (Rs = −0.565, $P = 0.03$), indicating lower CAT activity with increasing clinical activity.

In patients with inactive GO, SOD activity was strongly and inversely correlated with disease duration (Rs = −0.719, $P = 0.003$), further supporting an association between higher antioxidant enzyme activity and shorter disease course. Moreover, salivary MPO activity correlated positively with thyroid autoimmunity markers: MPO showed significant positive correlations with thyrotropin receptor antibody levels (Rs = 0.564, $P = 0.045$) and anti–thyroid peroxidase (TPO) antibodies (Rs = 0.574, $P = 0.03$).

ROC analysis was used to evaluate the ability of salivary enzymes to discriminate between active and inactive GO based on CAS. Salivary SOD demonstrated a statistically significant and moderate discriminatory performance, with an area under the curve (AUC) of 0.704 (SE = 0.097, $P = 0.04$). The estimated optimal cut-off value was 1.73 U/L, indicating that higher SOD activity was associated with active disease (stimulant marker). In contrast, MPO showed a lower and non-significant discriminative ability, with an AUC of 0.665 (SE = 0.103, $P = 0.13$). The estimated optimal cut-off for MPO was 8.84 U/L, with lower values favoring active GO, consistent with a destimulant profile.

Discussion

In this study, we explored salivary activities of selected antioxidant and pro-oxidant enzymes in patients with GO and their relationships with disease activity, duration, and treatment. Although salivary enzyme activities did not differ significantly between clinically active and inactive GO, distinct correlation patterns, especially for SOD and CAT, suggest a nuanced involvement of redox balance in the inflammatory course of the disease.

Salivary SOD activity showed consistent associations with disease activity measures. In patients with active GO, SOD correlated positively with CAS and negatively with disease duration, indicating higher antioxidant response in earlier and more inflammatory stages. Enhanced SOD activity in active inflammation might reflect compensatory up-regulation of antioxidant defense in response to increased oxidative burden. This phenomenon was previously demonstrated in GO orbital fibroblasts challenged with oxidative stress, where SOD activity increases alongside other ROS markers and redox imbalance [16].

CAT activity demonstrated an inverse relationship with clinical activity, showing lower CAT with increasing inflammation. This divergent behavior of antioxidant enzymes may indicate differential regulation and consumption of specific antioxidant pathways under sustained oxidative stress. In cultured GO orbital fibroblasts, CAT and glutathione peroxidase activities decrease under oxidative challenge even as SOD increases, reflecting an imbalance that could exacerbate tissue damage [16].

The broader involvement of oxidative stress in GO pathogenesis is well documented. Several clinical and in vitro studies have shown that oxidative stress markers are elevated in GO patients and correlate with clinical activity, such as CAS and smoking status, which is a known risk factor for GO severity [6,17]. These findings are consistent with the previous literature implicating oxidative stress in GO pathophysiology, although the present study does not establish mechanistic or causal relationships. Comprehensive reviews also highlight ROS as

key mediators in orbital fibroblast activation, immune signaling, and tissue remodeling, reinforcing the biological plausibility of our salivary redox findings [4].

Salivary MPO activity did not differ significantly by GO activity; however, its positive correlations with TRAb and anti-TPO in inactive GO suggest a link between neutrophil-related oxidative mechanisms and underlying autoimmune processes, even in clinically quiescent phases. This supports evidence that immunological activity and oxidative stress can persist beyond overt inflammatory signs, detectable using sensitive redox biomarkers [6].

Our analyses of treatment associations revealed higher salivary MPO and SOD in patients receiving antithyroid drugs. Although causal inference is limited in cross-sectional design, these differences may reflect treatment-related modulation of oxidative status or selection bias based on disease phenotype and activity prompting therapy. Previously, it was shown that antithyroid therapy and restoration of euthyroidism can modify oxidative stress parameters systemically, underscoring the complex interplay between metabolic, immunological, and oxidative pathways in GD [18].

ROC analysis suggested a modest discriminatory performance of salivary SOD between active and inactive GO. While not sufficient for standalone clinical use, this finding suggests that salivary antioxidant enzymes may serve as complementary, noninvasive biomarkers reflecting inflammatory dynamics. Given the established role of urinary and tear oxidative damage markers (eg, 8-hydroxy-2'-deoxyguanosine and malondialdehyde) correlating with clinical activity in GO, expanding biomarker research into saliva is a logical extension of systemic and local redox profiling [19].

Importantly, the clinical applicability of salivary oxidative stress enzymes in GO remains uncertain. In our exploratory analysis, differences between active and inactive GO were limited, and the discriminatory performance of SOD was only moderate. Future studies should evaluate whether salivary oxidative markers provide incremental diagnostic or prognostic value beyond

established parameters such as CAS, magnetic resonance imaging findings, thyroid hormone status, and thyroid autoantibody levels.

Limitations

This study has several limitations. The sample size was relatively small, particularly within treatment subgroups, limiting statistical power and increasing the likelihood of both type I and type II errors. The cross-sectional design precludes conclusions regarding causality or temporal changes in enzyme activity. No healthy control group was included, which limits interpretation of whether observed enzyme activities are specific to GO or reflect broader systemic or inflammatory processes. Also, longitudinal sampling was not performed, preventing assessment of intra-individual changes during disease progression or treatment. In addition, treatment-selection bias cannot be excluded because therapeutic decisions were made clinically rather than randomly assigned and may reflect underlying disease severity. Moreover, we assessed only selected enzymatic antioxidants without evaluating non-enzymatic antioxidant capacity. Further prospective studies with larger cohorts and parallel assessment of systemic and salivary oxidative parameters are warranted to clarify the clinical utility of salivary biomarkers in GO.

Conclusions

In our exploratory cohort, salivary oxidative stress enzyme activities showed limited differences between active and inactive GO. Among the analyzed salivary enzymes, SOD was the only marker with significant moderate accuracy for distinguishing active from inactive GO, whereas MPO showed only a nonsignificant trend. These findings support the need for longitudinal studies combining salivary, tear, and systemic redox markers to clarify their prognostic and monitoring value in clinical practice.

Article information

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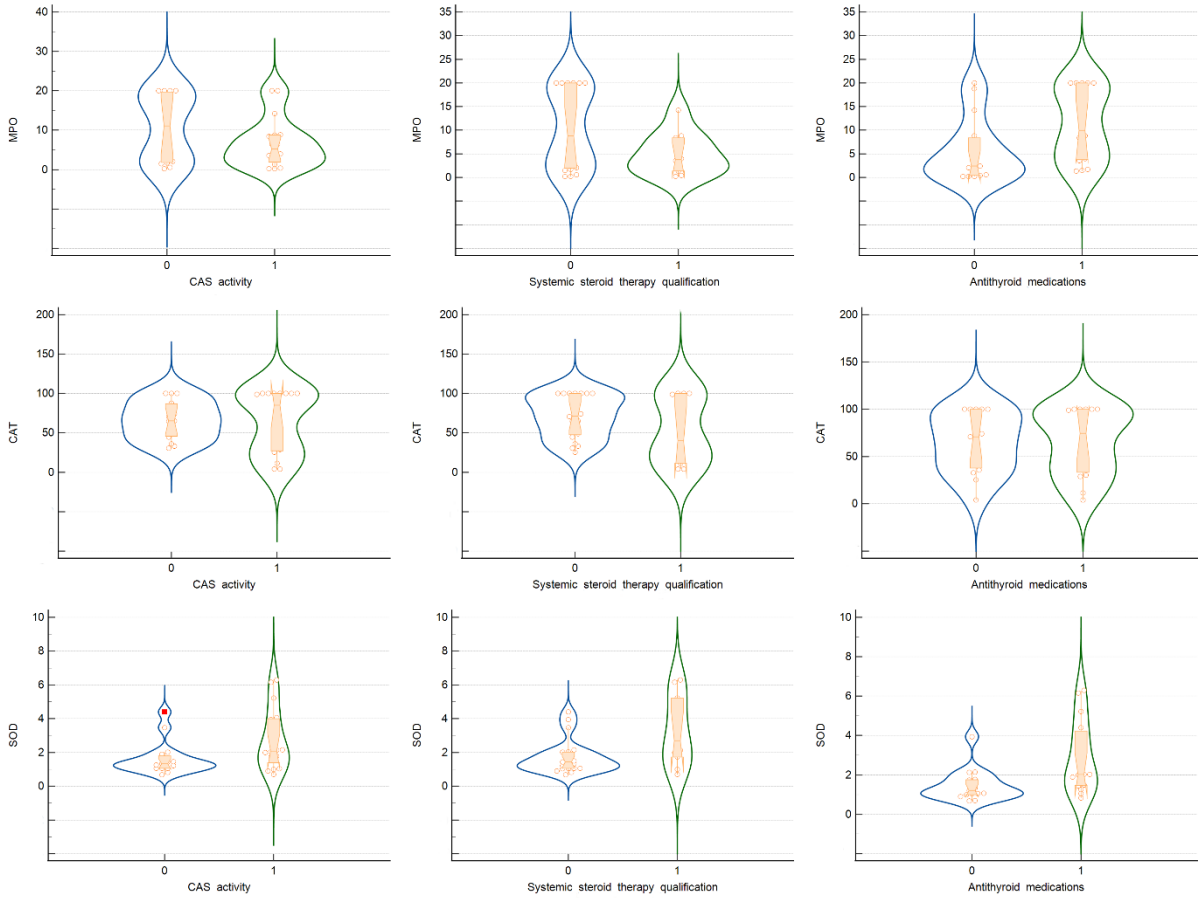

Figure 1 Violin plots presenting salivary activities of enzymes myeloperoxidase, catalase and superoxide dismutase [U/L] depending on CAS activity, systemic steroid therapy qualification and antithyroid medications. The box represents the interquartile range (Q1–Q3), with the horizontal line indicating the median, while the whiskers are error bars representing the 95% CI. The dots represent the raw data, and the red squares the outliers.