Review Article

A Systematic Review on the Role of Antioxidants in Thyroid Eye Disease

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Abstract

Purpose: To systematically review the role of antioxidants in management of patients with thyroid eye disease (TED).

Methods: A literature search of the electronic databases was performed without restrictions on the date of publication till the end of March 2021, using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines. Clinical trials, case–control studies, cohorts, case series, case reports, and experimental (including *in vitro*) studies in the English language were included. The primary outcome in human studies was improvement in severity, activity scores, and/or quality of life scores. There was a decrease in the level of H_2O_2-dependent oxidative stress, Hyaluronic acid release, reactive oxygen species, cell proliferation, or antifibrotic/antiproliferative actions in the *in vitro* studies.

Results: Out of 374 initially screened articles, 157 studies were selected, the full texts of 82 were reviewed, and 14 papers were finally included. There were 4 clinical and 10 *in vitro* studies from 1993 to 2018. While β-carotene, retinol, Vitamin E, Vitamin C, melatonin, resveratrol, N-acetyl-l-cysteine, and quercetin showed some efficacy in *in vitro* studies; allopurinol, nicotinamide, pentoxifylline, and selenium (Se) were effective in both clinical and experimental reports. Se was the only recommended antioxidant based on one high-level randomized controlled trial.

Conclusion: While different antioxidants could potentially be effective in the management of TED, no strong recommendation for any or combination of antioxidants could be made to be implemented in the daily practice.

Keywords: Antioxidants, Selenium, Systematic review, Thyroid eye disease

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Introduction

Thyroid eye disease (TED) is an autoimmune inflammatory disease known as the most common cause of orbital disease worldwide.[1] Although it is mostly associated with hyperthyroidism (Graves' disease [GD]), hypothyroidism and euthyroidism are present in some patients.[2,3] TED is clinically present in one-third of the patients with different underlying thyroid diseases.[3] Clinical presentation of the TED is the same for different underlying thyroid diseases[2] and between patients with unilateral versus bilateral orbital involvement.[4] While the quality of life (QoL) is impaired in patients with TED,[5] medical and surgical treatments lead to improvement of both visual and psychosocial QoL.[6,7] To prevent the progression of TED to sight threatening stages with detrimental effect on QoL of patients,[8] early diagnosis and treatment of TED among patients with thyroid dysfunction has received added emphasis.[9]

Oxidative stress is a process which is normally controlled under physiological conditions.[10,11] It is believed that any alteration of cell oxidative stability leads to cell damage.[12] A rise in several parameters of oxidative stress seems to be involved in the development of some autoimmune and endocrine diseases namely hyperthyroidism and TED.[12,13] Therefore, antioxidants

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have been studied to find out how they might change the effect of oxidative stress in different diseases. Consequently, a few studies[13-17] have attempted to investigate the role of antioxidants in autoimmune hyperthyroidism/thyroiditis and TED treatment with inconsistent conclusions on the effect and type of antioxidants in this regard. This study was designed to investigate the efficacy and safety of reported antioxidants in patients with TED.

Methods

A comprehensive literature search of the EMBASE, PubMed, MEDLINE, Google Scholar, and Scopus electronic databases was performed without restrictions on the date of publication till the end of March 2021, using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines.[18] The research keywords were "Thyroid eye disease OR Thyroid-associated ophthalmopathy OR Thyroid-associated orbitopathy OR Graves' ophthalmopathy OR Graves' orbitopathy," AND "Antioxidants OR Selenium OR Pentoxifylline OR Vitamin C OR Vitamin E OR Carotenoids OR Glutathione OR Uric acid."

Included articles were the clinical trials, case–control studies, cohorts, case series, case reports, and experimental (including *in vitro*) studies published in the English language. Review articles, patents, book chapters, commentaries, editorials, animal studies, and articles with irrelevant content or insufficient information were excluded. Two authors (S.C. and S.A.) independently followed the search strategy, screened the abstracts, then the full-text, and also the quality of evidence of the selected articles, and finally included the articles for the systematic review. Disagreement was resolved by the involved senior author (M.B.K.). The reference list of included papers was checked for further reports and citations of published or unpublished researches, and simultaneously registered trials were screened on three different websites including https://clinicaltrials.gov, https://who.int and https://www.cochranelibrary. com.

Extracted and documented data were the authors' name and year of publication, the design of the study, average age, stage of TED, thyroid function status, type of antioxidants, results, follow-up duration, and side effects.

The primary outcome in human studies was improvement in severity, activity scores, and/or QoL scores. Reduced lid retraction, proptosis, and/or diplopia were the outcome measures in the studies where the primary outcomes had not been reported.

The primary outcome of *in vitro* studies was a decrease in the level of H_2O_2-dependent oxidative stress, hyaluronic acid (HA) release, reactive oxygen species (ROS), cell proliferation or antifibrotic/antiproliferative actions.

The risk of bias was evaluated using a rating scheme [Table 1].[19] Briefly, the level of evidence was assessed using "hierarchy of evidence pyramid."

Results

Out of 386 initially screened articles, 177 studies were selected for more assessment. Reviewing the abstracts led to exclusion of 81 articles. Full-text review was performed on the remaining 96 articles, from which 76 did not have a defined outcome. Finally, 20 papers met the inclusion criteria and were considered in this review [Figure 1]. There were four human studies[20-23] and sixteen *in vitro* studies[24-39] from 1993 to 2021 [Table 2]. Fifteen studies were from Taiwan (2), Canada (1), Greece (1), Brazil (2), Poland (1), the USA (1), Japan (1), Italy (5), South Korea (3), Germany (1), China (1), and one was supervised by the international multicentral European Group on Graves' Orbitopathy (EUGOGO) association.

Chang *et al*.[28] investigated the role of PTX (0.1–1,000 mg/L) on cultured fibroblasts in 1993. The cultures were collected from biopsies of extraocular muscles (EOM) from two severe TED patients during orbital decompression surgery and two normal EOM tissues of individuals with strabismus during resection surgery and from the skin of two patients affected with pretibial myxedema. Dose-dependent inhibition of orbital fibroblast proliferation and glycosaminoglycan (GAG) release was reported.

In 1997, the human orbital fibroblast was obtained from two severe TED patients and two normal controls to investigate the effect of allopurinol (1.0 mM) and nicotinamide (10 μM) on cell proliferation induced by xanthine oxidase/hypoxanthine system.[29] The proliferation inhibition was significant after preincubation with allopurinol, nicotinamide, and methimazole (0–25 μM), but not with propylthiouracil (10 μM).

The role of nicotinamide was exclusively evaluated on orbital fibroblast to explore cell surface expression of human leukocyte antigen (HLA)-A, B, C antigen, HLA-DR antigen, intercellular adhesion molecule 1 (ICAM-1), CD44, and Fas expression induced by cytokines (interferon-γ [IFNγ] and tumor necrosis factor-α [TNFα]). Tissue harvest was from four TED and three glaucomatous patients. It was observed that nicotinamide did not interfere with induction of HLA-A, B, C, or CD44 expression but demonstrated an inhibitory effect on ICAM-1 and HLA-DR expression as well as the proliferation of orbital fibroblasts.[30]

To investigate the effect of quercetin on orbital fibroblasts, tissue samples from 5 TED patients and 5 control subjects were exposed to quercetin or its glycosides rutin and quercitrin, and subsequently, apoptosis, necrosis, cell proliferation, HA production, and cell cycle were measured.[31] Quercetin inhibited cell proliferation and HA production in both TED patients and control subjects.

Another study assessed the effect of quercetin on fibrotic markers and matrix metalloproteinases (MMP) of cultures from 13 TED patients and 3 normal females aged between 51 and 63.[32] Quercetin decreased the secretion of MMP-2 and MMP-9

Table 1: Rating of the level of evidence[19] in the studies included in this systematic review

Level of evidence	Included studies	Description
Level I	None	Evidence from a systematic review or meta-analysis of all relevant RCTs or evidence-based clinical practice guidelines based on systematic reviews of RCTs or three or more RCTs of good quality that have similar results
Level II	Finamor *et al*.[20], Marcocci *et al*.[21]	Evidence obtained from at least one well-designed RCT (e.g. large multi-site RCT)
Level III	Balazs *et al*.[22], Bouzas *et al*.[23]	Evidence obtained from well-designed controlled trials without randomization (i.e. quasi-experimental)
Level IV	Dehina *et al*.[24], Federige *et al*.[25], Liu *et al*.[26], Olesik *et al*.[27]	Evidence from well-designed case-control or cohort studies
Level V	None	Evidence from systematic reviews of descriptive and qualitative studies (meta-synthesis)
Level VI	None	Evidence from a single descriptive or qualitative study
Level VII	None	Evidence from the opinion of authorities and/or reports of expert committees
Level VIII (foundational evidence)	Chang *et al*.[28], Burch *et al*.[29] Hiromatsu *et al*.[3], Lisi *et al*.[31], Yoon *et al*.[32], Tsai *et al*.[33], Kim *et al*.[34], Dottore *et al*.[35], Dottore *et al*.[36], Dottore *et al*.[37], Dottore *et al*.[38], Kim *et al*.[39]	Evidence from animal/laboratory research and in-vitro studies

RCT: Randomized controlled trial

proteins and inhibited fibrotic markers in the TED group. Its antifibrotic effects occurred through a noncytotoxic process.

In 2013, Tsai *et al*.[33] examined the biphasic effect of H_2O_2 on cellular proliferation of Graves' orbitopathy (GO) orbital fibroblasts and also protective effect of N-acetyl-l-cysteine (NAC) and Vitamin C against it. Cultures from seven GO patients and five age-matched normal subjects were exposed to various concentrations of H_2O_2. The peak cellular proliferation was observed at 6.25 μM of H_2O_2 in GO fibroblasts. Protective effects were reported when GO cells pretreated with NAC (200 μM) and Vitamin C (500 μM) for 1 h and followed by the addition of 6.25 μM H_2O_2 for 24 h, by reversing enhanced proliferation and increased levels of transforming growth factor, beta 1 (TGF-β1), interleukin-1 β (IL1 β), and superoxide anion.

Kim *et al*.[34] exposed orbital connective tissue of 6 TED patients and 4 controls to assess the effect of resveratrol (30–50 μM) treatment on intracellular ROS levels and the expression of heme oxygenase-1 (HO-1), superoxide dismutase (SOD), catalase, and thioredoxin. It decreased ROS production, adipogenesis, and HO-1 level induced by oxidative stress. Treatment with 50 μM resveratrol also reduced ROS levels during adipogenesis.

Selenium (Se), in the form of selenium-(Methyl) selenocysteine (SeMCys), was examined on primary cultures of orbital fibroblasts from 6 TED patients and 6 control subjects in 2017.[35] While SeMCys inhibited proliferation and HA secretion just in TED patients, its effect on various concentrations of H_2O_2-induced oxidative stress (glutathione disulfide [GSSG]) and cytokines were similar in both groups.

SeMCys was also investigated in another experiment.[36] Tissue samples from six GO patients and six normal ones were collected and preincubated for 2 days at 37°C with a medium containing a 10 μM concentration of SeMCys hydrochloride, then treated with a concentration of 50 μM H_2O_2. Increased GSSG, apoptosis, and lactate dehydrogenase as a measure of necrosis were counteracted by SeMCys with no differences between GO and control fibroblasts.

Tissue samples from 6 TED patients and 6 patients with other conditions lacking fibroadipose tissue were treated with retinol, β-carotene, and Vitamin E at various concentrations.[37] β-carotene significantly decreased the raised H_2O_2-induced proliferation in TED but not the control fibroblasts. Retinol and Vitamin E had no effect. None had a significant effect on HA. Among TNFα, IL1 β, IFNγ, and endogenous cytokines which are involved in the pathogenesis of TED, IL1 β was the only responder to all the three antioxidant substances. Its H_2O_2-dependent rise significantly reduced in both TED and control fibroblasts.

Antioxidant effects of Vitamin C, NAC, and melatonin on primary cultures of 6 TED patients and 6 normal individuals were evaluated.[38] Vitamin C and NAC reduced H_2O_2-induced proliferation in TED fibroblasts. Melatonin and NAC decreased IFNγ in the TED fibroblasts.

Obtained orbital adipose/connective tissue samples from seven GO patients and five individuals with noninflammatory problems were studied to determine the effect of Se in the form of sodium selenite at various concentrations.[39] Hyaluronan, ROS production, and inflammatory cytokines including IL1α, IL1 β, IL6, IL8, and TNFα concentrations were measured and showed that serum Se suppressed hyaluronan production, IL1α, and TNFα in cultured orbital fibroblasts of patients with GO in a dose-dependent manner while suppression intracellular ROS generation and IL8 were not dose-dependent. IL1 β and IL6 were not suppressed by sodium selenite in cultured GO orbital fibroblasts.

Se and selenoprotein P (SePP) concentrations were assessed against clinical activity score (CAS) and severity status (based

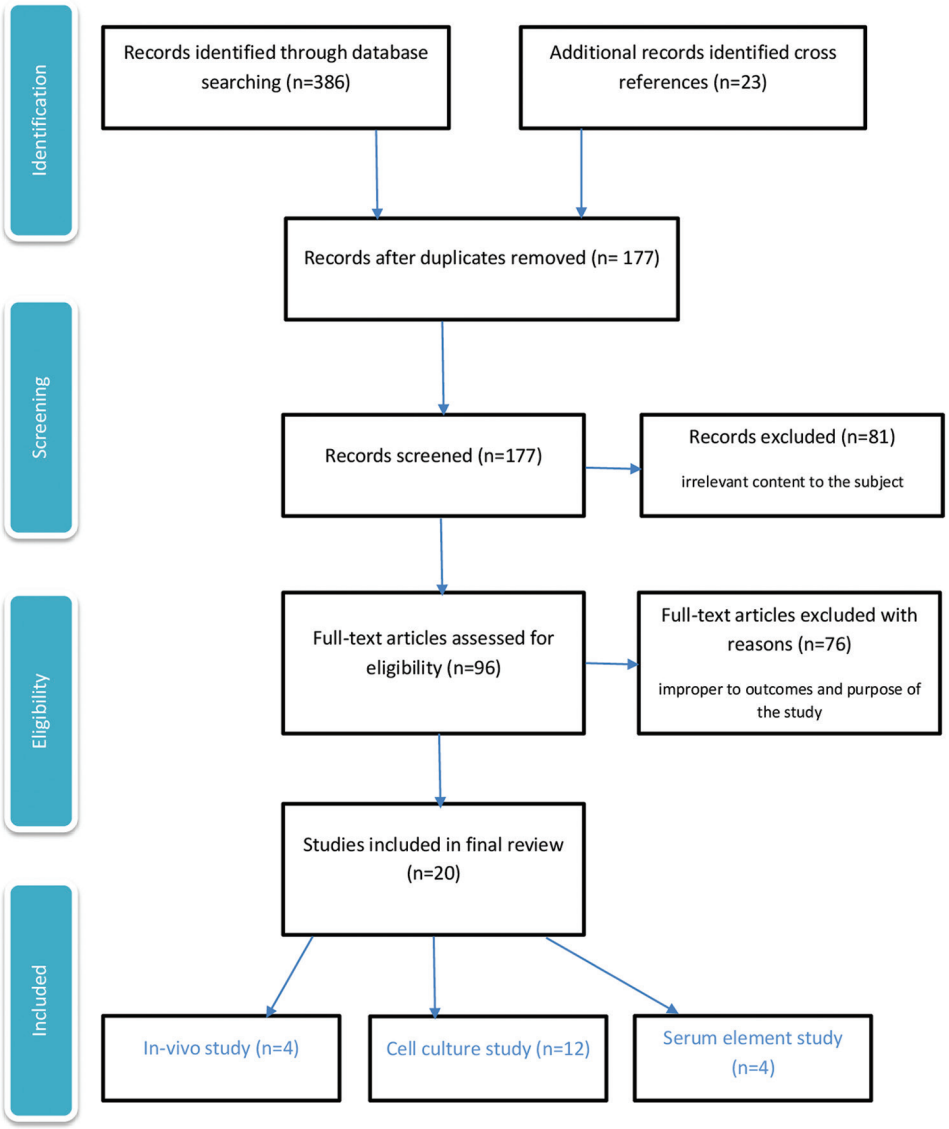

Figure 1: Screening and selection process of the articles on the role of antioxidants in thyroid eye disease

on NOSPECS [No sign or symptoms, only signs, soft tissue involvement with symptoms and signs, proptosis, extraocular muscle involvement, corneal involvement, sight loss]) as well as concentrations of thyroid stimulating hormone (TSH) receptor autoantibody (TRAB) or IGF1 receptor (IGF1R-aAB) autoantibodies in 31 mild and 53 moderately severe TED patients.[24] Se level did not significantly correlate with thyroid hormone concentrations, activity, and the severity of TED. Se or SePP concentrations were significantly different among the patients with mild versus moderately severe and active versus inactive TED. A significant inverse correlation was reported between serum Se and TRAB. This was not significant for the SePP. There were also no connections between IGF1R-aAB levels and serum SePP/serum Se concentrations.

In 2017, Federige *et al*.[25] studied and compared Se and SeP*P* values in patients with GD with and without GO, Hashimoto's thyroiditis (HT) patients and in control individuals. Although serum Se levels were similar among all groups, SePP serum was lower in GO and HT patients compared to the control group.

Serum trace elements were assessed in four groups of newly diagnosed GD patients, GD and GO patients in euthyroid status or subclinical thyroidism after treatment, and normal controls in a population in northeast China.[26] Among evaluated elements, serum Se levels in three first groups were significantly lower than those in normal individuals and serum copper levels were significantly low only in the GO group than those in normal ones.

The effect of thyroid hormone abnormalities on selected antioxidant parameters were investigated in GD patients with thyroid-associated orbitopathy (TAO) from both hyperthyroid and euthyroid patient categories.[27] The blood was collected, and the sera were obtained after centrifugation.

Table 2: Comparing different studies on the role of antioxidants in thyroid eye disease

	Writer/year	Study design	Population	Mean age (year)	TED grade	Thyroid status	Type of antioxidant treatment	Follow-up	Result	Side effects
1	Chang *et al.*, 1993[28]	Experimental, orbital fibroblast cell culture	Case: 2 Normal: 2 (2 PTM)	32	Moderately severe, activity is not specified/with OD	Not mentioned	PTX	NA	Inhibition of GAG release and fibroblast proliferation	NA
2	Balazs *et al.*, 1997[22]	Quasi-experimental, pilot study	Case: 10	45.2	Moderately severe, activity is not specified/with OD	Euthyroid	PTX	12 weeks	↓Serum GAG and TNFα, ↓Soft tissue involvement	Moderate and persistent nausea at the begging
3	Burch *et al.*, 1997[29]	Experimental, orbital fibroblast cell culture	Case: 2 Normal: 2	Not available	Moderately severe, activity is not specified/with OD	Not mentioned	Allopurinol nicotinamide	NA	↓Cell proliferation	NA
4	Hiromatsu *et al.*, 1998[30]	Experimental, orbital fibroblast cell culture	Case: 4 Normal: 3	Not available	Activity and severity are not specified/with OD	Not mentioned	Nicotinamide	NA	↓ICAM-1 and HLA-DR expression and ↓Cell proliferation	NA
5	Bouzas *et al.*, 2000[23]	Prospective nonrandomized comparative study	Case: 11 Normal: 11	Case: 36.7 Control: 34.8	Mild and moderately severe, active	Euthyroid	Allopurinol nicotinamide	3 months	↑Total eye score (NOSPECS) ↑Patient satisfaction	None
6	Finamor *et al.*, 2004[20]	Prospective randomized trial	Case: 9 Normal: 9	Case: 41.5 Control: 40	All 3 severity stages but inactive	Euthyroid	PTX	6 months	↓Proptosis and questionnaire scores	Nausea, abdominal pain
7	Marcocci *et al.* 2011[21]	Prospective randomized double-blind, placebo-controlled trial	Total: 159 Se: 55 PTX: 52 Placebo: 52	Se: 43 PTX: 43.7 Placebo: 44.6	Mild GO, active and inactive	Not mentioned	Se, PTX	12 months	↑QoL ↓CAS	Se (none) gastrointestinal with PTX
8	Lisi *et al.*, 2011[31]	Experimental, orbital fibroblast cell culture	Case: 5 Normal: 5	Case: 47.4 Control: NA	Moderately severe, inactive/with OD	Euthyroid	Quercetin	NA	↓Cell proliferation and HA release	NA
9	Yoon *et al.*, 2012[32]	Experimental, orbital fibroblast cell culture	Case: 13 Normal: 3	Case: 46 Control: 56	Moderately severe, inactive/with OD	Euthyroid	Quercetin	NA	↓MMP-2 and MMP-9, fibrotic markers and suppressive effects	NA
10	Tsai *et al.*, 2013[33]	Experimental, orbital fibroblast cell culture	Case: 7 Normal: 5	Case: 37.6 Control: 35.2	Severity is not specified inactive/ with OD	Euthyroid	Vitamin C N-acetyl-l-cysteine	NA	↓Cell proliferation and superoxide anion	NA
11	Kim *et al.*, 2015[34]	Experimental, orbital fibroblast cell culture	Case: 6 Normal: 4	Not available	Moderately severe, (CAS <4)/with OD	Euthyroid	Resveratrol	NA	↓Oxidative stress and ROS	NA
12	Dehina *et al.*, 2016[24]	Case-control, serum elements study	Total: 84 Mild: 31 Severe: 53 Active: 62 Inactive: 22	Not available (median: 46)	Used NOSPECS (mild, moderately severe, and sight threatening), active and inactive	Hyper: 51 Hypo: 10 Eu: 23	Serum Se, SePP	NA	No significant associations and changes	NA
13	Dottore *et al.* 2017[35]	Experimental, Orbital fibroblast Cell culture	Case: 6 Normal: 6	Case: 60.1 Control: 66.1	Moderately severe, inactive/with OD	Not mentioned	Se	NA	↓Oxidative stress and cell proliferation	NA

Contd...

Table 2: Contd...

	Writer/year	Study design	Population	Mean age (year)	TED grade	Thyroid status	Type of antioxidant treatment	Follow-up	Result	Side effects
14	Dottore *et al.*, 2017[36]	Experimental, orbital fibroblast cell culture	Case: 6 Normal: 6	Case: 60.1 Control: 66.1	Severity is not specified inactive/ with OD	Not mentioned	Se	NA	↓Apoptosis, LDH (necrosis), inhibition of oxidative stress (↓GSSG)	NA
15	Federige *et al.*, 2017[25]	Case-control, serum elements study	GD without GO: 19 GD with GO: 21 HT: 14 HT+LT4: 19 Control: 21	Case: 52.5 Control: 51	GO defined as having proptosis and CAS >1, severity is not specified	Euthyroid	Serum Se, SePP	NA	↓SePP serum level in GO and HT patients	NA
16	Dottore *et al.*, 2018[37]	Experimental, orbital fibroblast cell culture	Case: 6 Normal: 6	Case: 49.1 Control: 62.6	Moderately severe, inactive/with OD	Not mentioned	Retinol, β-carotene, Vitamin E	NA	↓H_2O_2-dependent oxidative stress, antiproliferative action	NA
17	Dottore *et al.*, 2018[38]	Experimental, orbital fibroblast cell culture	Case: 6 Normal: 6	Case: 49.1 Control: 62.6	Moderately severe, inactive/with OD	Not mentioned	Vitamin C N-acetyl-l-cysteine, Melatonin	NA	↓H_2O_2-dependent oxidative stress	NA
18	Liu *et al.*, 2018[26]	Case-control, serum elements study	Newly diagnosed GD: 66 Euthyroid GD: 55 Euthyroid GO: 57 Control: 66	Case: 38.06 Control: 42.3	Mild-to-moderate GO according to EUGOGO classification, severity is not specified	Euthyroid/ subclinical hyper	Serum Se	NA	↓Se serum level in all cases than control group	NA
19	Olesik *et al.*, 2020[27]	Case-control, serum elements study	TAO: 56 Control: 20	Case: Hyper: 53 Eu: 48 Control: Not available	Active (CAS >3), moderate-to-severe according to EUGOGO classification	Hyper: 34 Eu: 22	Serum Vitamin C, Uric acid	NA	Lower Vitamin C, higher uric acid levels in active TAOs than controls	NA
20	Kim *et al.*, 2021[39]	Experimental, orbital fibroblast cell culture	Case: 7 Normal: 5	Case: 41.1 Control: Not available	Inactive (CAS <3), severity is not specified	Euthyroid	Se	NA	Suppression of hyaluronan production, IL1α, and TNFα (all in dose-dependent manner) Inhibition of ROS generation and IL8	NA

TED: Thyroid eye disease, PTX: Pentoxifylline, GD: Graves' disease, GO: Graves' orbitopathy, HT: Hashimoto's thyroiditis, TAO: Thyroid-associated orbitopathy, Se: Selenium, NOSPECS: No sign or symptoms, only signs, soft tissue involvement with symptoms and signs, proptosis, extraocular muscle involvement, corneal involvement, sight loss, CAS: Clinical activity score, EUGOGO: European Group on Graves' Orbitopathy, SePP: Selenoprotein P , GAG: Glycoseaminoglycan, TNFα: Tumor necrosis factor-α, ICAM-1: Intercellular adhesion molecule 1, HLA: Human leukocyte antigen, QoL: Quality of life, MMP-2: Matrix metalloproteinase-2, ROS: Reactive oxygen species, LDH: Lactate dehydrogenase, GSSG: Glutathione disulfide, IL: Interleukin, NA: Not applicable, H_2O_2: Hydrogen peroxide, PTM: Pretibial myxedema, LT4: Levothyroxine, OD: Orbital decompression, HA: Hyaluronic acid, HLA-DR: Human leukocyte antigen-DR isotype, Eu: Euthyroid, ↓: Decreased, ↑: Increased

Enzymatic and nonenzymatic components of the antioxidant system were assessed, including the activity of glutathione peroxidase (GPx), SOD, and paraoxonase 1, as well as the total oxidant status expressed as the ferric reducing ability of plasma. The levels of Vitamin C, uric acid, and lipid peroxidation products (conjugated dienes [CD] and malondialdehyde [MDA]) were examined as well. While in hyperthyroid patients all values were significantly different from those in control ones, in euthyroid patients, only the activity of GPx was significantly higher than in controls, and other values nonsignificantly changed compared with the control group.

A pilot study of 10 patients described the beneficial effects of PTX therapy in euthyroid moderately-severe TED in 1997.[22] PTX (200 mg/day) was administered intravenously for 10 days and continued at 1800 mg/day orally for the first 4 weeks and then 1200 mg/day for the rest of treatment. Any improvement in total eye score of NOSPECS was defined as response. Serum GAG, TNFα, anti-TSH-receptor, anti-eye-muscle, anti-thyroglobulin, and anti-thyroid peroxidase antibodies were also documented sequentially. Soft tissue inflammation significantly improved in 80% of cases (8/10) with less significant improvement in proptosis and extraocular myopathy. Although moderate and persistent nausea was reported by two cases at the beginning of therapy, it did not interrupt treatment and responders continued oral PTX 1200 mg/day to maintain initial clinical benefit after 12 weeks.

Allopurinol and nicotinamide (300 mg/day, oral, 3 months) were compared with a placebo in a prospective nonrandomized study in 2000.[23] Treatment improved NOSPECS total eye score from 4.3 ± 1.9 to 2.0 ± 1.0 ($P = 0.0001$) and self-reported satisfaction in 22 newly diagnosed mild or moderately-severe TED patients.

Finamore *et al*.[20] compared an oral PTX (1200 mg/day) in 9 TED patients with placebo (9 TED patients) in a crossover (6-month) study. Exophthalmometry and health-related QoL questionnaire were the outcomes which were measured at baseline, 3, and 6 months after the treatment. Both showed significant improvement in the treatment group compared with placebo group. Gastrointestinal symptoms were, however, notable. The questionnaire scores were 5.5 and 5 ($P = 0.01$) at baseline and 6th months in the treatment group, respectively. A significant ($P < 0.05$) improvement of proptosis was also reported in the treatment group. Neither of the criteria changed significantly in the placebo group within two-time intervals of the crossover study.

In a randomized clinical trial, Se, PTX, and placebo were compared in 159 patients with mild TED.[21] They were assigned to three groups receiving sodium selenite (100 μg twice daily), PTX (600 mg twice daily), or placebo (twice daily) orally for 6 months and were followed for the next 6 months without treatment. Significant ($P < 0.001$) improvements in QoL (GO-QoL), CAS, soft tissue involvement and eyelid retraction with a less progression in TED severity ($P = 0.01$) were observed in the Se as compared with the placebo group. PTX group, however, did not show significant improvement as compared with the placebo group. While skin and gastrointestinal side effects were observed in the PTX group, no adverse effect was in the Se and placebo groups.

The result of hierarchical evaluation for included studies has been detailed in Table 1. Among all, the experiments of Finamor *et al*.[20] and Marcocci *et al*.[21] are in level 2, while the ones for Balazs *et al*.[22] and Bouzas *et al*.[23] are in level 3. According to defined criteria, the studies by Dehina *et al*.,[24] Federige *et al*.,[25] Liu *et al*.,[26] and Olesik *et al*.[27] stand at level 4, and the rest lie at the lowest part of the pyramid.

Discussion

Impaired balance between production of oxidative stress and consumption of antioxidant defenses (inactivation or excessive usage) leads to oxidative damage of biological membranes and molecules, which can be measured by either direct estimation of the ROS or indirect methods including detection of the resulting damage to biomolecules (DNA/RNA damage, lipid peroxidation, and protein oxidation/nitration) and antioxidant levels.[40] Increased oxidative stress and decreased scavenging ability of the cells have been recognized to be involved in the pathogenesis of autoimmune disorders particularly GD and TED.[12] Using a variety of techniques to induce oxidative stress while applying diverse measuring systems (ROS production, MDA, SOD activity, LPO, 8-OHdG, GSH, and GSH/GSSG ratio), certain studies have found a significant imbalance of prooxidant/antioxidants status in TED versus normal orbital fibroblasts.[29,41-45] This imbalance accounts for proliferation of orbital fibroblasts, synthesis of autoantibodies, breakdown of preadipocytes into adipocytes, secretion of endogenous cytokines (TNFα, IL1 β and IFNγ), and increased production and secretion of GAGs in TED patients which consequently lead to fibroadipose tissue expansion and infiltration of EOM.[14]

The aim of this systematic review was to provide the clinicians with essential information about potential role of antioxidant agents in the management of TED. Some of the studies have compared the efficacy of more than one type of antioxidants[21,23,27,29,33,37,38] in a single experiment.

β-carotene, retinol, Vitamin E, Vitamin C, melatonin, resveratrol, NAC, uric acid, and quercetin were solely evaluated in *in vitro* environment, and no experiments were found to discuss their clinical results. While quercetin,[31,32] Vitamin C,[27,33,38] and NAC[33,38] were found to be effective in more than one study, the efficacy of other antioxidants has been reported in just one *in vitro* study. Therefore, further *in vitro* and more importantly human studies is suggested before drawing any conclusion on their effect in TED management.

Allopurinol, nicotinamide, PTX, and Se, on the other hand, have been studied both *in vitro* and clinical. Allopurinol and nicotinamide have shown both proliferation inhibition in *in vitro* and improved patient satisfaction and total eye

score in clinical studies. Exposed orbital fibroblast cultures to PTX demonstrated inhibition of GAG release and fibroblast proliferation.[28] PTX treatment also led to a marked improvement in total eye score of 80% of TED patients (after 12 weeks),[22] and progressive improvement of proptosis and QoL questionnaire response.[20] However, no significant difference in overall ophthalmic outcome at 6-month was observed between PTX and placebo in another study.[21]

Decreased serum Se level was reported in all groups of patients regardless of TED presentation,[26,46] and SePP status was lower in GO and HT patients than in normal controls.[25] Serum Se and SePP concentrations were not different between mild versus moderately severe as well as active versus inactive TED patients.[24] However, this study was unable to draw a strong conclusion about Se supplementation as it was not a longitudinal study and did not include a control group of healthy subjects or GD patients without TED.

SeMCys suppressed fibroblast proliferation, HA secretion, apoptosis, and necrosis in TED orbital fibroblasts in the Dottore *et al*.'s *in vitro* studies.[35,36] While sodium selenite form of Se inhibited ROS production and inflammatory cytokines (except IL1 β and IL6) in a laboratory experiment,[39] it decreased eye involvement, improved QoL, and slowed TED progression in comparison to PTX and placebo in an randomized controlled trial (RCT).[21] Consequently, European Thyroid Association/EUGOGO released a guideline in 2016,[47] in which they recommended a 6-month use of antioxidants, mainly Se for mild TED in order to prevent its progression to advance stages and improve ocular manifestations and QoL.

Although there are some published reviews on the role of antioxidants in patients with TED, to the best of our knowledge, this is the first systematic review on this topic. While it is clear that antioxidants play an important role in the management of TED, no strong recommendation for any or combination of antioxidants could be made to be implemented in the daily practice on the grounds that all the reviewed studies had used different methods, stage of disease, patient selection, and randomization in this regard. Therefore, further well-designed RCTs are required to especially compare different single or combined antioxidants in different severity grades of TED.

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Conflicts of interest
There are no conflicts of interest.

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