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Original Article

Effect of Ptosis Surgery on Tear Oxidative Stress Levels in Patients with Blepharoptosis and Pseudoptosis

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Abstract

Objectives: Investigation of the surgical correction effect on tear film functions and tear oxidative stress levels in patients with blepharoptosis and pseudoptosis.

Methods: Sixty patients with blepharoptosis or pseudoptosis due to dermatochalasis and 30 healthy controls were included in the study. Forty eyes underwent upper blepharoplasty and 20 eyes underwent levator surgery. The tear oxidative stress levels (8-hydroxydeoxyguanosine [8-OHdG] and 4-hydroxy-2-nonenal [4-HNE]) were determined by enzyme-linked immunosorbent assay and tear film functions were evaluated pre-operatively and at the post-operative 1st and 6th months.

Results: 8-OHdG and 4-HNE levels in tears were found higher in patients with dermatochalasis (86.3±38.2 ng/mL; 29.8±11.4 ng/mL, respectively) and blepharoptosis (95.3±43.8 ng/mL; 40.8±3.8 ng/mL, respectively) compared to healthy controls (52.9±14.0 ng/mL; 27.8±6.6 ng/mL, respectively). Both levels decreased 1 month after blepharoplasty surgery. The 8-OHdG level in tears of patients who underwent levator surgery increased 1 month after the surgery (p=0.008). No change was detected in tear function tests findings between visits in any patient group.

Conclusion: Dermatochalasis and blepharoptosis may lead to an increase in the tear oxidative stress levels. Contrary to a decrease in these levels after blepharoplasty, they may increase in the early period after levator surgery followed by a return to normal levels at the 6th-month visit.

Keywords: Blepharoplasty, blepharoptosis, dermatochalasis, oxidative stress, pseudoptosis

Introduction

The eyelid contributes to the formation of the tear film layer and its dispersion on the ocular surface with its effective blinking function and protects the eye from drying (1). In a study comparing the blink dynamics of ptosis patients and controls using a high-speed camera, it was observed that the blink duration was longer although insignificant and the speed of initial opening phase was reduced in ptosis patients. This suggested that there was an intrinsic muscle function change in ptosis patients (2). Since effective blinking function is impaired in blepharoptosis patients, (2) some changes may be expected in the biochemical content of tears. Surgical treatment can also be expected to cause changes

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in objective tear function tests such as Schirmer, tear break up time (TBUT), and subjective tear function test such as ocular surface disease index (OSDI) and also oxidative stress markers in patients with dermatochalasis and blepharoptosis.

Oxidative stress results from an increase in the formation of reactive oxygen species (ROS) and/or a decrease in antioxidants (3). Among the oxidative markers, 8-hydroxydeoxyguanosine (8-OHdG) is produced by the damage of the hydroxyl radical (the strongest ROS) to the guanine base in DNA (4,5). 4-hydroxy-2-nonenal (4-HNE) is an alpha-beta-unsaturated aldehyde formed in the membrane lipid peroxidation process (6,7). ROS are present at many points of dry eye vicious cycle (8) and cause deterioration tear functions.

In this study, we investigated the effect of ptosis surgery on tear film functions and tear oxidative stress levels in patients with blepharoptosis and dermatochalasis by determining the levels of the products of damaged DNA and lipid.

Methods

Subjects

This prospective study included 60 patients with dermatochalasis or blepharoptosis who were operated in Cerrahpaşa Medical Faculty Oculoplasty department between May 2018 and March 2020 by the same surgeon (CA) together with age – and gender-matched 30 healthy controls. The study was approved by the Cerrahpaşa Faculty of Medicine Ethics Committee (approval number: 83045809–604.01.02) and conducted in accordance with the tenets of the Declaration of Helsinki. Written informed consent was obtained from all the participants.

Forty eyes underwent upper blepharoplasty and 20 eyes underwent levator surgery. Either the operated eye or the random eye of bilateral operations were included in the statistical analysis. There was no accompanying blepharoptosis in patients in the blepharoplasty group (upper margin reflex distance [uMRD] ≥3 mm). uMRD was <3 mm in all eyes operated for blepharoptosis. The etiological factor was involutional in all patients in the blepharoptosis group. Healthy controls were selected from those who did not meet the exclusion criteria, had µMRD ≥3 mm, had normal tear function tests, and with the two patient groups in terms of age and gender.

The exclusion criteria were as follows: The history of eye surgery, ocular trauma, any eyelid diseases other than ptosis, systemic or topical medication that may impair tear production or cause dry eyes, smoking, and alcohol consumption.

Blepharoplasty for Dermatochalasis Repair

"Skin pinch" technique was performed as a methods of marking. After performing the skin incisions, the redundant skin was excised using a radiofrequency cautery. The preseptal orbicularis was not excised. The skin was sutured with 6/0 propylene continuously.

Levator Surgery for Blepharoptosis Repair

Surgery was performed with the traditional anterior approach (9). After marking, eyelid crease skin incision was performed using radiofrequency cautery. Dissection was applied under the orbicularis oculi muscle. An incision was made in the orbital septum. Preaponeurotic fat tissue was dissected. Levator aponeurosis was reapproximated to the superior border of the upper tarsus. The eyelid crease was formed by placing the suture on the skin-aponeurosis-skin. The skin incision was closed with 6-0 polypropylene suture continuously.

Visit Calendar

Pre-operative evaluation was accepted as the evaluation on day 0. Control examinations were scheduled at 1 and 6 months after the operation.

Clinical Examinations during Each Visit

Tear functions of the patients were evaluated with Schirmer's test, TBUT, and OSDI questionnaire. Schirmer's test was performed with Schirmer tear test without anesthesia. The strip was placed in the lower fornix and the patient was requested to close their eyes. The wetted length of the strip was recorded. For TBUT test, 5 µL of non-preserved 2% liquid sodium fluorescein was instilled into the inferior cul-de-sac. TBUT determined by the number of seconds that elapse between the last blink and the appearance of the first breaking. OSDI questionnaire was used to estimate the effect of dry eye symptoms on daily visual function.

Tear Collection and Detection of 8-OHdG and 4-HNE

Tear samples were collected using the eye flush method as described previously (10). 60 µL drop of non-preserved saline was instilled into the inferior cul-de-sac using an Eppendorf pipette. Then, the participants were asked to close their eyes and to rotate them twice. Fluid was harvested with a microcapillary tube. Tear samples were kept at −80°C until further examination. A commercially available 8-OHdG and 4-HNE enzyme-linked absorbent assay (ELISA; Bioassay Technology Laboratory) was used according to the manufacturer's instructions to determine their concentration.

Statistical Analysis

SPSS version 20.0 (SPSS, Chicago, IL) was employed for all statistical analyses. For statistical comparisons, differences with p≤0.05 were considered statistically significant.

Results

Patient Characteristics

Demographic characteristics of controls and patients are presented in Table 1. The three groups were homogenous in terms of gender and age (p=0.32, p=0.62).

The mean uMRD was significantly increased 1 month after blepharoptosis surgery (1.1±0.8 mm vs. 3.6±0.8 mm, p<0.001) and maintained at 6 months (3.5±0.7, p=1.0).

Tear Function Tests Findings and Changes during Each Visit

The change of Schirmer, TBUT, OSDI scores among different visits in all groups are summarized in Table 2, and between-group analysis is presented in Table 3. Among the controls, blepharoplasty and the levator surgery groups, in the pre-operative period, no difference was detected in terms of the Schirmer's test and TBUT scores (p=0.84 and p=0.729, respectively).

Table 1. Demographic characteristics of controls and patients

	Age (mean)	Gender, n (%)		Total
		Female	Male	
Controls	53.2±7.1	18 (60)	12 (40)	30
Blepharoplasty group	55.3±7.3	22 (55)	18 (45)	40
Levator surgery group	53.8±15.2	13 (65)	7 (35)	20

Table 2. Comparison of the three groups using Kruskal Wallis test

	Control (n=30)		Blepharoplasty (n=40)		Levator surgery (n=20)		
	Mean	SD	Mean	SD	Mean	SD	p
Age	53.2	7.1	55.3	7.3	53.8	15.2	0.62
Schirmer (mm)							
Baseline	14.7	4.7	14.4	5.3	14.5	3.3	0.84
1st month	15.2	4.7	14.0	4.2	12.7	4.5	0.2
6th month	15.0	4.8	14.7	5.6	13.8	4.7	0.7
TBUT (sec)							
Baseline	11.9	2.4	11.6	2.6	11.9	2.7	0.729
1st month	11.5	2.2	11.0	1.9	10.5	2.7	0.429
6th month	11.7	2.2	10.9	2.1	11.1	2.9	0.384
OSDI							
Baseline	3.7	5.9	11.7	15.5	11.5	12.5	0.01*
1st month	4.1	5.3	9.2	10.3	15.6	11.7	0.001*
6th month	4.2	5.3	10.0	13.7	12.2	12.5	0.062
8-OHdG (ng/ml)							
Baseline	52.9	14.0	86.3	38.2	95.3	43.8	<0.001*
1st month	50.8	29.9	54.8	31.3	165.2	111.9	<0.001*
6th month	56.4	30.6	66.6	30.2	109.4	29.9	<0.001*
4-HNE (ng/ml)							
Baseline	27.8	6.6	29.8	11.4	40.8	3.8	<0.001*
1st month	29.2	8.0	15.3	4.6	44.1	4.7	<0.001*
6th month	29.7	6.5	14.9	4.1	38.8	4.1	<0.001*

*Statistically significant (p<0.05); OSDI: Ocular Surface Disease Index; SD: Standard Deviation; TBUT: Tear Break Up Time; 4-HNE: 4-hydroxy-2-nonenal; 8-OHdG: 8-hydroxydeoxyguanosine.

Table 3. Paired comparison tests of the three groups using the Mann Whitney U test with Bonferroni correction - p values

	Control vs Blepharoplasty Group	Control vs Levator Surgery Group	Blepharoplasty vs Levator Surgery Group
OSDI-0	0.03*	0.021*	1
OSDI-1st month	0.069	<0.001*	0.069
8-OHdG-0	<0.001*	<0.001*	1
8-OHdG-1st month	1	<0.001*	<0.001*
8-OHdG-6th month	0.564	<0.001*	<0.001*
4-HNE-0	1	<0.001*	<0.001*
4-HNE-1st month	<0.001*	<0.001*	<0.001*
4-HNE-6th month	<0.001*	<0.001*	<0.001*

*Statistically significant (p<0.05); OSDI: Ocular Surface Disease Index; SD: Standard Deviation; TBUT: Tear Break Up Time; 4-HNE: 4-hydroxy-2-nonenal; 8-OHdG: 8-hydroxydeoxyguanosine.

Among the controls, blepharoplasty, and levator surgery groups, no significant change was detected at the 1st and 6th months after surgery compared to the baseline levels in terms of the Schirmer's test (p=0.888, p=0.417, p=0.142, respectively), TBUT (p=0.793, p=0.054, p=0.115, respectively), and OSDI (p=0.922, p=0.573, p=0.333, respectively) scores.

The Change of Tear Oxidative Stress Levels at Each Visit

In the pre-operative period, the 8-OHdG and 4-HNE levels in the tear film in all groups are depicted in Figure 1 and the changes among the visits are shown in Figure 2a and 2b, respectively.

In the pre-operative period, the 8-OHdG levels in tear film were lower in the control group (52.9±14.0 ng/mL), compared to the blepharoplasty (86.3±38.2 ng/mL) and levator surgery groups (95.3±43.8 ng/mL) (p<0.001). While the tear 8-OHdG levels showed no significant difference between visits in the control group (p=0.079), decreased in the blepharoplasty group (p=0.002), and increased in the levator surgery group (p=0.008) between the baseline visit and the 1st month.

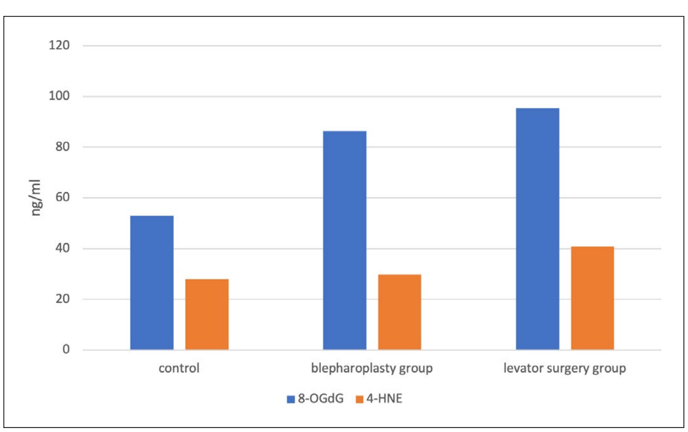

Figure 1. Baseline tear 8-hydroxydeoxyguanosine and 4-hydroxy-2-nonenal levels in the three groups.

In the pre-operative period, the 4-HNE levels in tear film were higher in the levator surgery group (40.8±3.8 ng/mL), compared to the control (27.8±6.6 ng/mL) and blepharoplasty groups (29.8±11.4 ng/mL) (p<0.001). While tear 4-HNE levels showed no significant difference in the control group

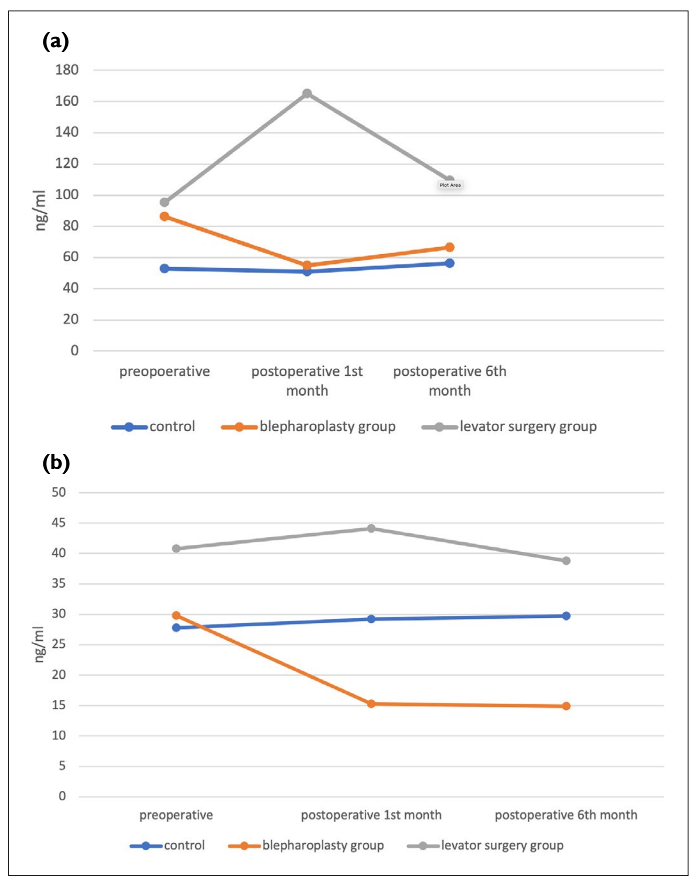

Figure 2. (a) Alteration of 8-hydroxydeoxyguanosine among the consecutive visits in the three groups. **(b)** Alteration of 4-hydroxy-2-nonenal among the consecutive visits in the three groups.

among different visits (p=0.061), it showed a decrease between the baseline visit and 1st month and between the baseline visit and 6th month in the blepharoplasty group (p<0.001) and showed a decrease between the baseline visit and 6th month and between the 1st and 6th months in the levator surgery group (p=0.034, p=0.001, respectively).

Correlation of the Tear Oxidative Stress Levels with Tear Function Tests Results

The correlation analysis among all patients at the baseline visit resulted in a positive correlation between the tear 8-OHdG and 4-HNE levels (p<0.001, r=0.333). 4-HNE was positively correlated only with the Schirmer's test (p=0.044; r=0.192).

At the 1st month after surgery, a positive correlation was observed between the tear 8-OHdG and the 4-HNE levels (p<0.001; r=0.538). 8-OhdG showed a negative correlation with the Schirmer's test and TBUT scores (p=0.04; r=−0.196, p=0.042; r=−0.194, respectively) and a positive correlation with the OSDI score (p=0.02; r=0.29). 4-HNE showed a negative correlation with the TBUT test (p=0.018; r=−0.225).

At the 6th month visit after surgery, a positive correlation was observed between the tear 8-OHdG and the 4-HNE levels (p<0.001; r=0.37).

Discussion

Although the effect of blepharoptosis repair and blepharoplasty operations on tear functions has been previously investigated; (11-17) to the best of our knowledge, the effect of these surgeries on oxidative stress markers in tears has not been studied.

Although not statistically significant, oxidative stress markers in tear in the control group were different between visits, this may be due to personal factors such as seasonal, hormonal changes, and changes in sleep patterns.

We found that the tear 8-OhdG levels were higher in the blepharoplasty group compared to the control group in the pre-operative period. This may be parallel to an increase of 8-OHdG in the skin tissue of the lid with age and leading to dermatochalasis. Oxidative stress damage may cause loosening and sagging of the eyelid skin, similar to the etiopathogenesis of conjunctivochalasis (18,19).

In the pre-operative period, the tear levels of 8-OHdG and 4-HNE were found higher in the levator surgery group compared to the control group. This may be explained with a possible 8-OHdG and 4-HNE increase in levator muscle and aponeurosis tissue, leading to their dysfunction. Although the patients in the study are Asian, consistent with our results, Kase et al. (20) investigated the oxidative stress in levator aponeurosis tissue immunohistochemically and found that 8-OHdG immunoreactivity was significantly higher in striated muscle cells of patients with involutional blepharoptosis compared to the control group, and lower in smooth muscle cells. It has been suggested that this oxidative stress in the striated muscle cells of the levator aponeurosis causes dysfunction in the aponeurosis together with the levator muscle.

Mak et al. (2) showed that the blink dynamics were impaired in patients with aponeurotic ptosis. In this study, it was showed that ptotic patients had insignificantly longer blink duration and significantly reduced speed of the initial opening phase compared to controls. This suggested that there was an intrinsic muscle function change in ptosis patients. The eyelid movement during blinking is very important in the release of lipid from the glands. The thickness of this layer decreases with insufficient blinking (21). Ineffective blinking causes a decrease in the thickness of the lipid layer, leading to the increased evaporation of the aqueous layer, a decrease in the tear film thickness, and deterioration of the stability of the tear film (22). Although inflammation caused by these changes in tear film did not make a significant change in the Schirmer and TBUT scores, it was revealed indirectly with the increase of oxidative stress markers in tears.

8-OHdG was found to be negatively correlated with Schirmer and TBUT scores and positively correlated with OSDI score and 4-HNE was found to be negatively correlated with TBUT scores in the post-operative 1st month. Since oxidative stress can be both the result and trigger of inflammation (19) and is involved in the dry eye vicious cycle, it is expected that oxidative stress markers are negatively correlated with Schirmer and TBUT scores, and OSDI scores positively (7).

In blepharoplasty group, the tear 8-OHdG and 4-HNE levels decreased in the post-operative 1st month. No change was detected in terms of the Schirmer, TBUT, and OSDI scores at the 1st and 6th months after blepharoplasty compared to baseline levels. This situation may be related to the absence of intervention to the orbicularis muscle, levator muscle, and aponeurosis in blepharoplasty surgery and the decrease of frontal and orbicular muscle movement after surgery. The absence of change in palpebral fissure height in the blepharoplasty group may have ensured that objective and subjective tests were not impaired in our study. Kase et al. (20) found that blepharoplasty was not related to a change in TBUT and Schirmer tests, but provided an improvement in the subjective symptoms and a decrease in an inflammatory reaction in impression cytology 3 months after blepharoplasty. In the previous studies, similar to our results, no significant difference was found 90 days after surgery in Schirmer, TBUT, (12,13) OSDI scores, (13) and no significant difference was found 6 weeks after surgery in TBUT values (14). The orbicularis muscle was preserved in the study of Zloto et al. (13) but not in the study of de Lima et al. (12) Furthermore, no change in the tear volume of the patients after blepharoplasty surgery has also been shown (16).

Tear dysfunction following blepharoplasty surgery may occur in the presence of anatomical conditions such as exophthalmos, negative orbital vector, maxillary hypoplasia, scleral appearance, and lower eyelid hypotonia (15,23). In our study, patients with such anatomical conditions were not included in the blepharoplasty group. Complications of blepharoplasty include iatrogenic damage to the lacrimal gland, edema caused by surgical trauma in the early period, anesthesia applied during surgery, and a temporary lagophthalmos due to paralysis of the orbicular muscle caused by surgical trauma (24). In these cases, tear functions may be negatively affected. To reduce the risk of deterioration in tear function following blepharoplasty surgery, some authors have recommended conservatively excising the skin tissue and protecting the orbicularis muscle as much as possible (15,17,24,25). Conversely, some authors reported that preseptal orbicular muscle resection did not alter blink function (26,27). In our study, the orbicularis muscle was preserved in blepharoplasty surgery.

We observed that in the levator surgery group, the 8-OHdG levels showed an increase in the post-operative 1st month and remained similar in the 6th month. No change was detected in terms of the Schirmer, TBUT, and OSDI scores among successive visits. The increase of the tear 8-OhdG levels in the post-operative 1st month after levator surgery could be explained with a temporary ocular surface inflammation in the 1st month. Exposure of the ocular surface to more ultraviolet light with the widened palpebral fissure after the surgery may be responsible for the increased 8-OHdG in the 1st month after surgery. The previous studies have shown the association of photo-oxidation processes with increased ROS production in rabbit corneal epithelial cells (28) and photo-oxidative reactions due to ultraviolet light have been related to dry eye (8). The mechanisms for the deterioration of tear functions after blepharoptosis surgery performed with the anterior approach are the increase in evaporation due to the increased eyelid fissure, the changed sensitivity of the cornea and conjunctiva, the increase in the efficiency of the lacrimal pump with the improvement in the blink function, and especially the damage to the tear ducts originating from the palpebral lobe of the lacrimal gland (15). The tear volume of the patients has been shown to decrease 1.5 months after the levator surgery and persist until the 6th month. The authors suggested that this may be related to the increase in the speed of blinking after surgery, which results in the lacrimal pump working more effectively and increasing tear drainage (16). In our study, although a decreasing trend was observed in Schirmer and TBUT scores was observed after levator surgery, no significant difference was detected.

Our study has some limitations. First, the tear film functions were evaluated only with Schirmer's, TBUT, and OSDI tests. Another limitation of our study is that oxidative stress was evaluated with only 8-OHdG and 4-HNE. Studies examining the changes in other oxidative stress markers will provide a more detailed understanding of the effects of surgery on oxidative stress. However, to the best of our knowledge, the effect of blepharoplasty and levator surgery on tear oxidative stress markers has not been investigated before.

The increase in 8-OHdG levels in levator surgery in the 1st month postoperatively may suggest direct targeting of oxidative stress markers in the topical treatment of patients with dry eye symptoms after blepharoptosis repair surgeries.

Conclusion

This report demonstrated that patients with pseudoptosis due to dermatochalasis or blepharoptosis had higher levels of oxidative stress markers (4-HNE and 8-OHdG) in tear film compared to the healthy controls. The rise in baseline of 4-HNE in blepharoplasty group compare to control is not statistically significant. Contrary to a decrease of oxidative stress levels in tear after blepharoplasty, they may increase in the early period after levator surgery followed by a return to normal levels at the 6th-month visit. Our study also showed that there was no change in Schirmer, TBUT, and OSDI scores at 1 and 6 months after surgery in patients who underwent blepharoplasty or levator surgery compared to the pre-operative period. We found that oxidative stress markers were negatively correlated with Schirmer and TBUT scores and positively correlated with OSDI score in the post-operative 1st month. The obtained results may indicate that modulation of the oxidative stress markers could be considered as a future therapeutic approach for dry eye which may occur after blepharoptosis repairing.

Disclosures

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Ethics Committee Approval: The study was approved by the Cerrahpaşa Faculty of Medicine Ethics Committee (approval number: 83045809–604.01.02) and conducted in accordance with the tenets of the Declaration of Helsinki. Written informed consent was obtained from all the participants.

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

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