

Topical citicoline and vitamin B12 versus placebo in the treatment of diabetes-related corneal nerve damage: a randomized double-blind controlled trial.

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Abstract

Purpose To evaluate the effects of topical citicoline and vitamin B12 (OMK2, Omikron Italia srl, Italy) on the corneal sub-basal plexus density (SBP) of patients with diabetes.

Methods This prospective, randomized, double blind, placebo-controlled study included thirty patients with diabetic neuropathy randomised with 2:1 ratio to OMK2 or placebo 3 times daily for 18 months. At baseline and at months 4,8,12,18 patients underwent the Ocular Surface Disease Index questionnaire (OSDI), tear break-up time, evaluation of corneal and conjunctival staining, Schirmer I test, Cochet-Bonnet esthesiometry, and confocal biomicroscopy of SBP. SBP density (mm/mm²) was calculated using NeuronJ. Raw data and differences from baseline were analysed in the two groups.

Results 29/30 patients concluded the study. The two groups had similar SBP at baseline; it progressively improved up to month 18 in both groups (OMK2, $p < 0.0001$; controls, $< 0.0001-0.03$); improvement at month 18 vs baseline was higher in OMK2 than placebo (33% vs 15%, $p = 0.04$). A progressive amelioration of corneal sensitivity was also shown just on OMK2 group (baseline, 28 ± 18 mm; month 18, 52 ± 10 mm, $p < 0.0001$). In OMK2 patients, also conjunctival staining and OSDI questionnaire improved during the study ($P = 0.04$ and 0.05). Both treatments were well tolerated and adherence during the study was high.

Conclusions OMK2 ameliorated both morphology and function of corneal nerves in patients with diabetes, thus suggesting a neuroprotective effect.

Background

Cornea innervation plays a key role in the homeostasis of the cornea and the ocular surface. Nerve modifications of the subbasal nerve plexus (SBP) occur in several conditions such as keratoconus, infectious keratitis, corneal dystrophies, neurotrophic keratopathy, long-standing contact lens wear, eye surgeries, and diabetes (1). SBP can be clinically evaluated using confocal microscopy, a non-invasive technique whose clinical relevance is well-recognized in the diagnosis of ocular surface pathologies (2).

In patients with diabetes, corneal SBP loss correlates with the severity of systemic neuropathy (3) and, more generally, with the severity of diabetes (4); also, SBP density improves after pancreas transplantation (4,5). These findings suggest that corneal confocal microscopy may be a valid tool for monitoring diabetic neuropathy.

Recently, eyedrops and surgical strategies that specifically target axonal regeneration of corneal nerves have started to become available; these include nerve growth factor (6, 7), coenzyme Q10 (8), and corneal neurotization (9, 10). Among possible treatments, citicoline (associated with vitamin B12) facilitates the recovery of cell membrane damage. Several experimental studies *in vitro* and *in vivo* have suggested the neuroprotective effects of citicoline and vitamin B12. Systemic administration of citicoline has been found to counteract neuronal cell damage in animal models of cerebral ischemia (11); vitamin B12

supplementation ameliorated the peripheral nerve lesions in experimental diabetic neuropathy (12,13). In human studies, recent data suggest that administration of systemic citicoline may slow down neurodegenerative diseases such as glaucoma (14). Topical administration of citicoline was effective in reducing neurodegeneration in models of diabetic and non-diabetic retinal degeneration, both in-vitro (15) and in-vivo (16, 17). Topical citicoline also proved to be a viable neuroprotective strategy in glaucoma patients (18). Currently no clinical data on the effects of the treatment with both citicoline and vitamin B12 eye drops on corneal nerves.

Aim of this study was to test the hypothesis that an eyedrop containing citicoline and vitamin B12 may stimulate SBP axonal regrowth in patients with diabetes.

Methods

The study was a single-centre, randomized, double-blind, placebo-controlled, prospective study on 30 patients with diabetes. It was conducted at the Eye Clinic of San Paolo Hospital, Università degli Studi, Milan, Italy and conducted according to the tenets of the Declaration of Helsinki. It was funded by Omikron Italia s.r.l., Rome, Italy and registered (clinicaltrials.gov, ID NCT03906513, retrospectively registered on 08/04/2019); it adhered to CONSORT guidelines. Informed consent was obtained from all patients prior to enrollment.

Inclusion criteria were: age > 18 years and patients with type 1 or type 2 diabetes who received Argon Laser Photocoagulation. Exclusion criteria were: neuropathy of any other cause than diabetes; history of conditions known to affect corneal sensitivity; coexisting other corneal diseases; autoimmune diseases; Sjogren syndrome; history of corneal trauma; contact lenses use; patients needing eye surgery or who received eye surgery at least 180 days before inclusion; contraindications to the use of any active substances and/or excipients; pregnant and lactating women.

Patients were randomized (list of random number) with a 2:1 ratio to two treatment arms: 20 patients were treated with active treatment (2% citicoline, 0.2% hyaluronic acid and 0.02% cyanocobalamin, OMK2, Omikron Italia srl, Italy) and 10 with placebo (0.3% hypromellose, SoftDrops, Farmigea S.p.A., Italy) given three times daily (8 am, 2 pm, 8 pm) for the duration of the study. Weighted randomization was used in order to balance in the two groups the following characteristics, known to affect SBP: duration of the disease, number of cases of insulin-dependent diabetes, and baseline SBP density.

The study consisted on 5 visits: baseline, month 4, month 8, month 12, month 18.

At each visit, the following examinations were performed in the following order: questionnaire of symptoms using the Ocular Surface Disease Index (OSDI, Allergan, Irvine, USA); anterior segment ophthalmoscopy; tear film fluorescein break-up time (TBUT), defined as the number of seconds that elapse between the last blink and the appearing of the first dry spot in tear film (TBUT was the mean of 2 consecutive measures); grading of corneal staining with fluorescein using Oxford scale; grading of conjunctival staining with fluorescein using Van Bijelsterveld scale; Schirmer I test; measure of central

corneal sensitivity by Cochet-Bonnet esthesiometer; confocal biomicroscopy of the central SBP of the cornea. This examination was performed using in-vivo confocal microscopy (HRT II Cornea Module, Heidelberg Engineering GmbH, Heidelberg, Germany); 9 central images not overlapping by more than 20% were used according to the strategy suggested by Vagenas et al. (19).

Blinding between operators was strictly maintained during the study. Three operators per patient were involved: confocal operator, clinical operator, and adherence operator (assessing at each visit adherence to treatment, stability of diabetes and presence of side effects by inspecting patients' diary). The study was designed to exclude patients who missed more than 3 consecutive days of treatment (a fact which did not occur).

Statistical analysis

The primary outcomes of the study were the change in corneal SBP density and sensitivity occurring at each visit vs baseline. The secondary outcomes were the changes in clinical signs (TBUT, Schirmer I, corneal and conjunctival epithelial staining) and symptoms (OSDI) of ocular surface damage.

Sample size was calculated using previous data on nerve density at confocal microscopy (which was the main outcome of the study). There is high span of literature data on corneal nerve density in diabetes, due to differences on confocal microscope, heterogeneity of diabetic populations, differences on data collection; there is also paucity of data on the efficacy of treatments to improve corneal axonal growth. We used two studies showing increase of SBP density after pancreas cell transplantation (4,5); we assumed a mean baseline value of 11.8 no/mm² and a 18-month value of 14.2 no/mm². Using alpha = 0.05, beta = 0.9, a worth-detecting difference of 20% from baseline and a two tailed test, about 15 patients on active treatment were; assuming a 20% drop-out per year, 20 patients were enrolled. The control group was not relevant for sample size calculation and therefore was not the object of sample size calculation; 10 patients were arbitrarily enrolled.

The analysis was performed on the worse eye of each patient (the one with lower SBP density). For the 9 images collected at each visit, axons were manually traced and axon length per field was calculated using NeuronJ software by ImageJ (<http://imagej.nih.gov/ij/>; provided in the public domain by the National Institutes of Health, Bethesda, MD, USA). Density was defined as the mean of the 9 confocal fields and expressed in mm/mm² (19).

OSDI questionnaire was classified as normal (values ranging between 0 and 12%), mild (13-22%), moderate (23-32%).

Missing data (7 patients missed one visit each) were managed using the mean value of the previous visit(s) for the given patient. Dataset for primary and secondary outcomes were analysed by means of analysis of covariance, using treatment as covariate. Raw data and percentage change from baseline were studied. In the case of positive results, inter-visit differences were inspected by means of t-test and X² (respectively for continuous and categorical variables).

Results

29/30 patients concluded the study; one patient withdrawn from the trial after month 4 for personal reasons; his data were not considered in the analysis. No differences were shown for age (66 ± 8 years in OMK2 vs 64 ± 10 in controls, $P=0.87$), sex (60% females in both groups), ethnicity (90% Caucasian in both groups). Duration of the disease was 7.8 ± 4.4 in OMK2 and 6.5 ± 6.2 years in controls ($P=0.24$); the percentage of insulin-dependent diabetes was 30% in both groups; during the study, diabetes was compensated in all patients.

Analysis of variance was statistically significant for change in SBP vs baseline ($P=0.012$), esthesiometry ($P=0.003$), change in esthesiometry vs baseline ($P=0.01$); a borderline p-value of 0.078 was obtained for SBP density.

Findings at confocal microscopy and corneal sensitivity were then compared in the two groups. Tables 1 and 2 respectively show SBP density in the two groups and percentage of change. The two groups had similar SBP density at baseline; both groups showed a progressive amelioration of data compared with baseline ($p<0.0001$ for OMK2, P ranging from <0.0001 and 0.03 for control group), and between month 4 and month 8 ($p=0.03$ for both OMK2 and control group). Considering percentage change, at the end of follow-up OMK2 showed a statistically significant improvement of corneal SBP compared with placebo: 33% vs 15% ($p=0.04$).

Tables 3 and 4 respectively show the results of esthesiometry and percentage change. OMK2 showed a progressive amelioration of corneal sensitivity, which was statistically significant at each visit compared with the previous one (month 4 vs baseline, $p<0.0001$; month 8 vs month 4, $p=0.002$; month 12 vs month 18, $p=0.03$, month 18 vs month 12, $p=0.05$; all visits compared with baseline, $p<0.0001$). In control group, the change of sensitivity was not statistically significant comparing any timepoint ($p>0.06$). Considering percentage change, OMK2 obtained better improvements than control at each timepoint ($p<0.01$).

Table 5 shows data of ocular surface signs and symptoms in the two groups at each visit. No statistically significant changes occurred during the study, apart from the reduction of OSDI and conjunctival staining occurring on OMK2 group at month 18 vs baseline (Figures 1 and 2).

According to OSDI classification, the prevalence of dry eye was 36% at the beginning of the study (40% in OMK2 group: 7 mild, 1 moderate; 30% in control group: 0 mild, 3 moderate), and 17% at the end of the study (10% in OMK2 group: 1 mild, 1 moderate; 30% in control group: 3 mild, 0 moderate, Figure 1). The change occurring between the beginning and the end of the study was statistically significant for OMK2 group ($P=0.05$).

Throughout the study period, a significantly reduction of conjunctival staining was also observed in OMK2 group ($P=0.04$).

Comparing patients with normal and abnormal OSDI at baseline, we found out an association between abnormal OSDI and esthesiometry. Cornea sensitivity was 21 ± 12 mm in patients with abnormal OSDI,

and 39 ± 18 mm in those with normal OSDI ($p=0.003$). The difference in cornea sensitivity between these two groups was still present at the second visit (at month 4, 28 ± 11 mm in abnormal OSDI; 48 ± 14 in normal OSDI, $p=0.0001$; at month 8, 45 ± 9 in abnormal OSDI; 51 ± 11 in normal OSDI, $p=0.08$). No other significant differences were found in the study.

Both study treatments had high tolerability, as no patients discontinued the study due to side effects; a mean of 9 ± 15 administrations were missed between 2 consecutive visits, with no statistically significant differences in the two groups ($P>0.60$).

Discussion

This is the first paper to test the effects of topical citicoline and vitamin B12 as neuroprotective agents for the ocular surface, using diabetes as a model of axonal damage. Our results support this neuroprotective action, as we showed the superiority of OMK2 vs placebo in restoring both corneal nerve morphology and function.

In the group receiving topical citicoline and vitamin B12 over a 18-month period, a progressive improvement of axonal density and a corresponding amelioration of corneal sensitivity were shown. At the beginning of the study, OMK2 group had low nerve densities and sensitivities (although the study was designed not to have statistically significant SBP differences); at the end of follow up, these parameters were overlapping in the two groups. Percentage improvement of nerve density at the end of the study vs baseline was 33% for OMK2 and 15% for placebo ($p=0.04$). This corresponded to an amelioration of corneal sensitivity of +150% for OMK2 compared with +44% for placebo ($p=0.007$). Of note, in the OMK2 group mean sensitivity shifted from 28 ± 18 mm at the beginning of the study (which corresponds to corneal hyposensitivity) to 52 ± 10 mm (ie normal sensitivity) at the end of the study.

Some effects on nerve density and corneal sensitivity (although not statistically significant) were also shown on control group (0.3% hypromellose, a typical treatment for dry eye disease (DED) in many settings). It is impossible to have a "true" placebo on studies on DED as any eyedrop (also saline solution alone) modifies the ocular surface increasing tear volume and diluting inflammatory molecules. This, in the long term, may also ameliorate ocular surface homeostasis and potentially have a beneficial effect on nerve morphology and function.

It is also possible that high variability of confocal data may have played a role in our study; yet the concomitant increase of both morphology and function is more likely a descriptor of a real neuroprotective effect and not of an artifact.

The changes in ocular surface (both signs and symptoms) were secondary outcomes of the study, and the population was not selected on the basis of these findings. When inspecting mean data no significant changes on ocular surface were shown during the study (Table 5). Yet, studying OSDI questionnaire, we showed that both treatments are useful to ameliorate the symptoms related to dry eye. In fact, according to OSDI classification, the prevalence of dry eye reduced from 36% at baseline to 17% at the end of the

study. The change occurring OMK2 group (from 40% to 10%, Figure 1) was statistically significant. Throughout the study period, OMK2 also significantly reduced conjunctival staining ($P=0.04$).

These results are in agreement with a large part of literature, bringing evidence that nerve trophism plays a key role on the ocular surface homeostasis, and loss of neural network integrity or correct function is a frequent cause of dry eye disease (20), because of reduced corneal sensitivity and lacrimal gland dysfunction with reduced tear production and tear stability (21). Our population was not selected on the basis of OS findings, and we found out that at baseline patients with abnormal OSDI had significantly lower sensitivity (21 ± 12 mm) than those with normal OSDI (39 ± 18 mm). These results are in contrast with Lyu et al. (22), who suggested that patients with longstanding diabetes may be less symptomatic due to corneal denervation. Another recent paper by Ferdousi et al. (21) found out that DED prevalence in 42 patients with type-1 diabetes was not related with corneal nerve structure and density. Probably, these controversial findings reflect the well-known different effects due to type of diabetes (23), populations (DED on patients with diabetes ranges from to 17.5% (24) up to 76.5% (25)), and stage of the disease (19).

Confocal microscopy is a fascinating technique because it allows the in-vivo analysis of the ocular surface at cellular level. Still, several possible limitations may affect these studies: reproducibility may be low, as it is nearly impossible to evaluate exactly the same area of 400 x 400 microns at retest; also, nerve tracing is subjective and may be poorly reproducible. In this paper, we tried to protect our observations including 9 cornea fields at each visit - a fact that has been shown to reduce test-retest variability (19). Also, we traced the fibers using NeuronJ software, which automatically measures nerve length. Previous to the study, we tested test-retest variability of confocal microscopy in two consecutive sessions (it was good), and we tested both inter- and intra-evaluators readings with ImageJ (which were both excellent). In spite of our attempts to increase the validity of study measurements, confocal data had high variability; still the association between morphological and functional amelioration seems to corroborate the quality of our dataset despite confocal variability.

Another possible limitation of the study was the difference (though not statistically significant) of nerve density at the beginning of the study in the two groups. This difference was due to the necessity of balancing at baseline two other features which we considered as critical in the study (duration of the disease and number of cases of insulin-dependent diabetes). Weighted randomization on such a small population could not prevent the presence of a clinically relevant difference of nerve density at baseline. Such a difference may have supported a higher chance of amelioration in the group with lower baseline (OMK2). Yet, it should be noted that also esthesiometry data support the superiority of active treatment over placebo.

Finally, this pilot study evaluated a group of patients with conspicuous corneal nerve damage (mean density at baseline was two times lower than normal) (27). Future studies will need to confirm our findings and evaluate the possible efficacy of citicoline and vitamin B in different populations (low or absent nerve damage; moderate - severe DED; patients with neurotrophic corneal ulcers). Future

investigations may also explore other corneal features (ie the presence of neuromas), which were not considered in this study and may help in clarifying the course of diabetic neuropathy and the effects of potential treatments.

Conclusions

This pilot study suggests for the first time a positive effect of topical citicoline and vitamin B12 in ameliorating both corneal nerve density and sensitivity in patients with diabetes; such changes ameliorated the homeostasis of the ocular surface. Our findings raise a number of possible fields of study for topical citicoline, such as the effects on other conditions on which corneal nerves are acutely affected (refractive and corneal surgery) or infected (herpes infections), and the utility on patients complaining for eye pain or severe dry eye with abnormal nerve regulation.

Abbreviations

DED, dry eye disease

OMK2, topical 2% citicoline, 0.2% hyaluronic acid and 0.02% cyanocobalamin

OSDI, Ocular Surface Disease Index questionnaire

SBP, sub-basal plexus density

TBUT, tear film fluorescein break-up time

Declarations

Ethics approval and consent to participate: study protocol and consent form were approved by Comitato Etico Milano Area 1, ASST Santi Paolo e Carlo. Approval #607, 22/03/2017. Consent form was signed by all participants at the beginning of the study

Consent for publication: not applicable

Competing interests: The authors have no financial interest in the material or products discussed in the article. Paolo Fogagnolo and Luca Rossetti are Editors of BMC Ophthalmology

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Authors' contribution: PF study design, analysis and manuscript writing; EM data collection and analysis; LT data collection and analysis; LR data interpretation, manuscript revision. All authors have read and approved the manuscript.

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Availability of data and materials: The datasets during and/or analysed during the current study available from the corresponding author on reasonable request

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Tables

Table 1. Corneal SBP density (mm/mm²)

	OMK2	Placebo	p
Baseline	10.6 ± 2.9	12.7 ± 2.9	0.09
Month 4	13.4 ± 4.1*	15.8 ± 3.9*	0.07
Month 8	14.3 ± 4.1*	16.5 ± 2.9 *	0.08
Month 12	12.8 ± 4.1*	14.8 ± 3.0§	0.10
Month 18	14.2 ± 4.6*	14.4 ± 4.0^	0.43

*, p<0.0001; §, p=0.01, ^, p=0.03. All comparisons are vs baseline

Table 2. Corneal SBP: percentage improvement vs baseline

	OMK2	Placebo	p
Month 4	27 %± 23% (+63%; -14%)	26% ± 18% (+49%; -2%)	0.43
Month 8	37 %± 27% (+94%; -9%)	34% ± 30% (+98%; -2%)	0.39
Month 12	21 %± 29% (+66%; -44%)	19% ± 17% (+50%; -4%)	0.40
Month 18	33 %± 26% (+88%; -9%)	15% ± 25% (+42%; -39%)	0.04

Table 3. Corneal esthesiometry (mm)

	OMK2	Placebo	p
Baseline	28 ± 18	41 ± 16	0.07
Month 4	40 ± 16*	42 ± 16	0.79
Month 8	49 ± 10*	50 ± 11	0.85
Month 12	50 ± 12*	48 ± 12	0.70
Month 18	52 ± 10*	50 ± 13	0.63

*, p<0.0001 vs baseline

Table 4. Corneal esthesiometry: percentage improvement vs baseline

	OMK2	Placebo	p
Month 4	73 %± 79% (+317%; -17%)	5 %± 11% (+27%; -14%)	0.001
Month 8	143 %± 142% (+533%; -31%)	44%± 76% (+219%; -18%)	0.02
Month 12	135 %± 115% (+338%; -24%)	39 %± 82% (+244%; -27%)	0.01
Month 18	153 %± 121% (+362%; -27%)	44 %± 78% (+231%; -37%)	0.007

Table 5. Comparison of ocular surface parameters in the two groups at each timepoint of the study.

BUT	baseline	4 m	8 m	12 m	18 m
OMK2	10.1 ± 5.7	9.0 ± 5.9	9.7 ± 7.2	9.6 ± 4.7	9.8 ± 6.3
Placebo	8.6 ± 5.5	7.8 ± 5.0	7.4 ± 6.3	10.9 ± 6.5	9.4 ± 5.5

p	0.48	0.56	0.39	0.62	0.87
SCHIRMER	baseline	4 m	8 m	12 m	18 m
OMK2	15.9 ± 8.9	14.8 ± 7.1	14.7 ± 7.7	16.5 ± 9.0	14.4 ± 9.2
Placebo	14.1 ± 8.8	14.8 ± 9.2	15.5 ± 7.6	17.5 ± 11.1	16.4 ± 11.0
p	0.59	0.99	0.80	0.83	0.66

CONJUNCTIVAL STAINING

	baseline	4 m	8 m	12 m	18 m
OMK2	1.40 ± 0.60	1.60 ± 0.50	1.21 ± 0.42	1.11 ± 0.32	1.00 ± 0.47
Placebo	1.40 ± 0.70	1.40 ± 0.70	1.10 ± 0.57	1.13 ± 0.35	1.25 ± 0.46
p	0.99	0.43	0.60	0.89	0.22

CORNEAL STAINING

	baseline	4 m	8 m	12 m	18 m
OMK2	0.20 ± 0.41	0.40 ± 0.50	0.21 ± 0.42	0.05 ± 0.23	0.11 ± 0.32
Placebo	0.30 ± 0.48	0.20 ± 0.42	0.10 ± 0.32	0.01 ± 0.00	0.13 ± 0.35
p	0.58	0.26	0.43	0.33	0.89

OSDI	baseline	4 m	8 m	12 m	18 m
OMK2	9 ± 7	8 ± 7	7 ± 7	6 ± 5	8 ± 10
Placebo	13 ± 11	8 ± 6	7 ± 5	9 ± 7	8 ± 8
p	0.30	0.90	0.90	0.21	0.98

Figures

Figure 1.

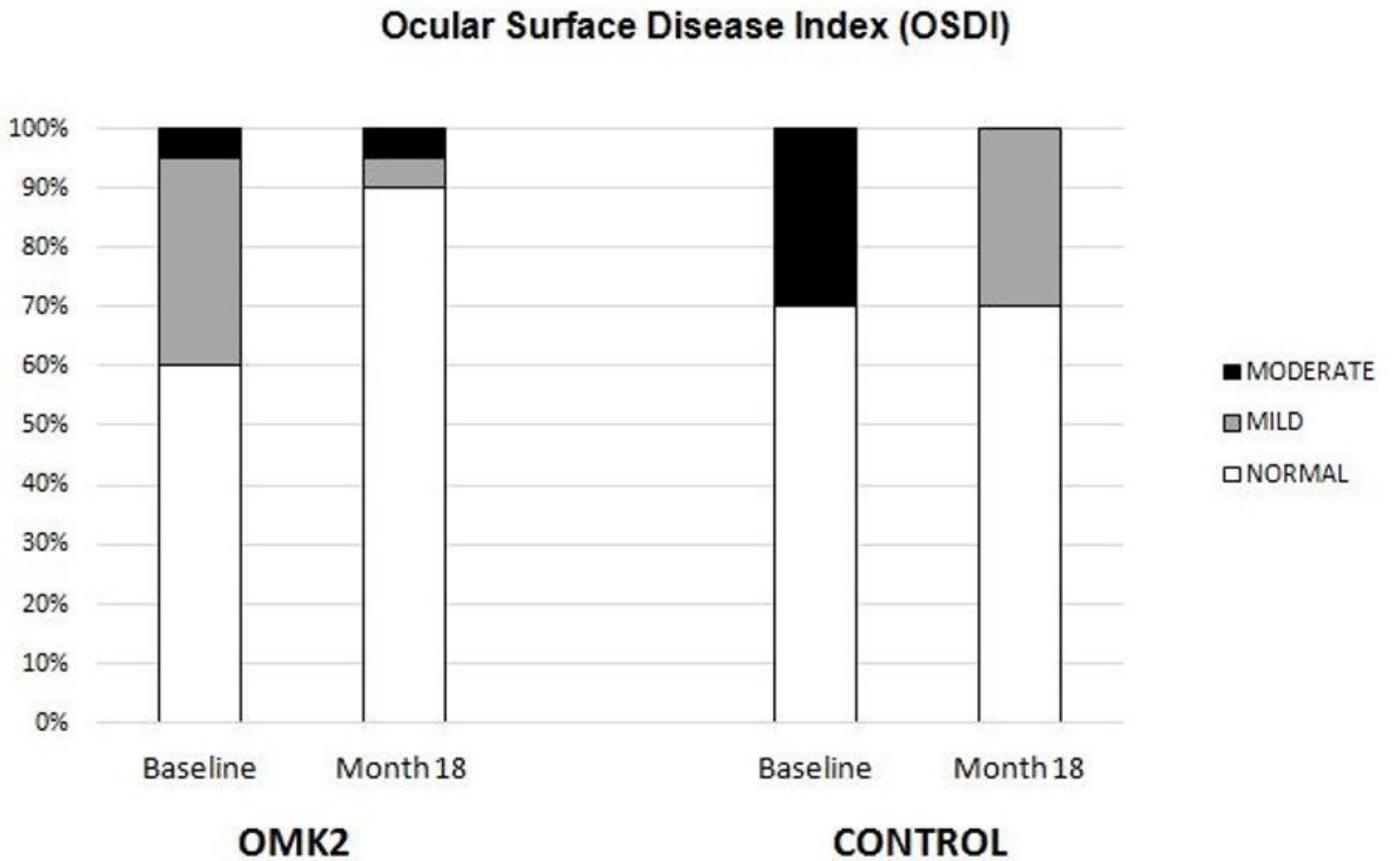


Figure 1

Changes of the frequency of stages of the Ocular Surface Disease Index at baseline vs month 18 in the two groups (OMK2, P=0.05). Normal, OSDI=12% or less; mild, OSDI between 13 and 22; moderate, OSDI between 23 and 32.

Figure 2.

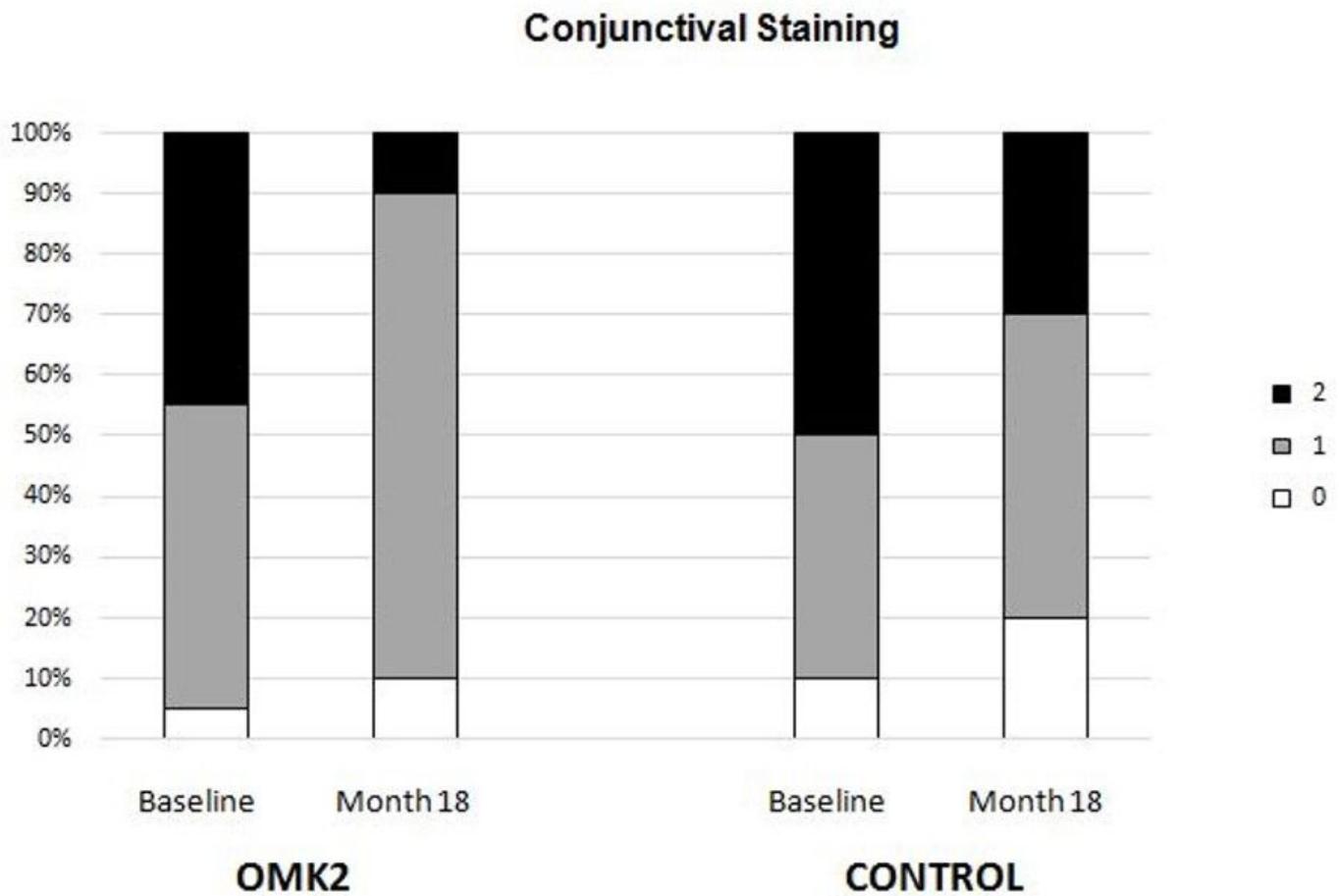


Figure 2

Changes of conjunctival staining according to Van Bijelsterveld scale at baseline vs month 18 in the two groups (OMK2, $P=0.04$).

Supplementary Files

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- [CONSORTCiticoline.docx](#)