MICROBIOLOGY SYNERGISM BETWEEN TEAR SUBSTITUTES AND SYMBIOTIC TREATMENT OF PATIENTS WITH IRRITABLE BOWEL SYNDROME

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ABSTRACT

The tear film consists of a set of heterogeneous substances (lipids, proteins, mucin and water) combined in order to form a highly organic tropism structure specialized in the defense of the ocular surface. Dry Eye Disease (DED) is characterized by discomfort, visual disturbance, constant irritation, foreign body sensation, and blurred vision. We evaluate in this preliminary report the effect of supplementation with symbiotic Maxiflor on tear film in DED patients. We recruited 40 patients (27 females and 13 males, age 51.5 ± 11. 1) showing signs of discomfort and / or dry eyes (burning, foreign body sensation, dryness or itching). Following the run-in period subjects were randomized in two groups: group A (N°20 subjects) and group B (N°20 subjects). Group A (control) treated only with substitute tear and group B treated with substitute tear + mixture (symbiotic). The data obtained in the two study groups A and B were, respectively the following: Schirmer 10.1±0.2 vs 12.7±0.4 (p< 0.001); Schirmer II 3.6±0.1 VS 4.6±0.2 (p<0.001); Fluorescein in break-up time (TF BUT) 4.1±0.3 vs 6.5±0.2 (p<0.001). Culture test showed initial bacterial growth in 18 out of 40 samples tested in group "A" (control), corresponding to 45.0%; whereas bacterial culture was found positive after treatment in 14 out of 40 tests, equal to 35.0%, in group "B" (symbiotic). A reduction of 16 to 12 strains of aerobic and anaerobic isolates from 10 to 6 has been found. The present study shows that the administration of Bifidobacteria and Lactobacillus may represent a successful treatment in ameliorating DED.

***Key words**: Dry eye, tear, ecosystem eye, Lactobacillus and Bifidobacterium.*

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Introduction

Dry Eye Disease (DED) is characterized by symptoms of discomfort, visual disturbance, and tear film instability with damage to the ocular surface. The symptoms of DED include constant irritation, foreign body sensation, and blurred vision which compromise with the ability to work and carry out daily functions[1-3]. Epidemiological studies have demonstrated that DED has a prevalence of 5-35% depending on the criteria applied, the population studied and geographic location. Despite its high prevalence, DED is frequently under-recognized, owing to its negative influence on visual function and quality of life of patients. DED represents a big burden in public healthcare[4-5].

These estimated suggest that DED is more prevalent than diabetes, cancer[6-7], heart disease, Parkinson disease[8], and systemic erythematous lupus[9-10]. Despite attempts to find better diagnostic

approaches and appropriate treatment, only a handful of therapies are available for DED patients and are used according to the disease severity[11]. Artificial tears provide palliative relief to eye irritation in patients with aqueous tear deficiency, but do not prevent the underlying inflammation and restore normal tear film in patients with mild-to moderate disease. Combination of artificial tears with steroids, hormonal therapy, antibiotics cyclosporine A and supplementary treatments with essential fatty acids are used to combat underlying inflammation. Recent data demonstrate that intestinal microorganism could influence lipid metabolism and act as environmental factors triggering development of metabolic and cardiovascular diseases[12-15].

In addition other data have revealed a close relationship between inflammatory and metabolic pathways. Traditionally colonic bacteria were considered as agents activating inflammatory mechanisms. This is supported by multiple data showing the link between microbiota, inflammation and autoimmunity[16-18].

Materials and methods

Study design

This clinical trial was a pilot study to evaluate the effects of consumption of symbiotic (Maxiflor) on the tear film. The study was approved by the Institutional Review Board of Science of Senescence of Catania University and was conducted in accordance with Declaration of Helsinki. Eligible subject included males and females aged 29-73 years.

Interventions

Subjects began a 1-week run-in period during which the normal diet was consumed without taking any study product. During this period subjects were asked to discontinue the consumption of dietary fiber supplements and probiotics.

Following the run-in period subjects were randomized in two groups: group A (N°20 subjects) and group B (N°20 subjects). Group A (control) treated only with substitute tear (Lacrisek 1 drop 2 times / in both eyes / day, for 28 days) and group B treated with substitute tear (Lacrisek 1 drop 2 times / in both eyes / day, for 28 days) + symbiotic (1/die maxiflor/day, for 28 days).

The mixture (maxiflor) is composed of lactobacillus acidophilus 1010, Streptococcus thermofhilus 109, Lactobacillus plantarum 12.108, Lactobacillus rhamnosus 8.108, Bifidobacterium lactis 4.109 associated to Zn mg 6, Vitamin B1 mg 0.7, vitamin B2 mg 0.8, vitamin B6 mg 1 and niacin mg 9. Participants were instructed to not change their routine diet or other lifestyle habits, and no restrictions were placed on use of routine taken drugs. In particular, the subjects belonging to the group B have consumed one capsule (Maxiflor) per day for 28 days.

Patients

All patients with clinical or pathologic evidence of DED in our department were invited to participate in the study. 45 patients were enrolled and 40 completed the study. We collected all demographic data, date of first symptoms, date of diagnosis. None of the patients had infections of the ocular surface and appendages or allergic diseases of the ocular surface in the last 30 days. We excluded patients with previous eye surgery, lachrymal disorders, and medical therapy with systemic or topical medications that alter the tearing and / or topical steroids during the 4 weeks preceding the start of the study. Other exclusion criteria were use of any probiotic product intended to improve gastrointestinal function within the 2 weeks preceding study entry; major chronic and uncontrolled systemic medical conditions; lactose intolerance; chronic diarrhea; gastric bypass surgery or lap band insertion for weight loss; regular laxative use; pregnant or breast -feeding women.

Patients n°	Sex		Age	Range
	M	F	(mean)	
40	13	27	51.5	28-73

Table 1: Demographic characteristics of patients who completed the study.

In all patients, the subjective symptoms and objective signs at the time of enrollment visit and after 30 days of treatment (probiotic) were considered. Five days after cessation of treatment (washout) clinical parameters were reassessed. At the end of treatment, five days after washing, all of the patients belonging to the two groups A (control) and B (symbiotic) received Schirmer test I, Schirmer test II, Break-Up Time (BUT) Test and bacteriological research. All patients with DED were recruited and were admitted to the study (27 females and 13 males, age 51.5 ± 11. 1, Table 1) showing signs of discomfort and / or dry eyes (burn-

ing, foreign body sensation, dryness or itching).

Methods

Evaluation of the clinical signs of dry eye considers three features of the tears film and ocular surface tears functions, tear composition, and ocular surface alterations. Tests of tear function were performed by direct observation of all patients. Ocular Surface Disease Index questionnaire (OSDI) had been administered to all patients. Tear film instability is a valuable sign of dry eye and can be produced by either aqueous - deficient dry eye or evaporative dry eye or a combination of both mechanisms. The method for determining tear film stability is the tear flourescein in break-up time (TF BUT) that is performed by instilling a small amount of fluorescein into the tear film and having the patient blink while being observed through the slit-lamp with incident cobalt blue filtered light. The uniform greenish hue of the fluorescein across the cornea is observed for early breakup as identified by a dark spot forming in the tear film. Normal TF BUT range is 10-15 seconds. Rapid tear film breakup is an indicator of tear instability that can be due to dry eye or ocular surface irregularities. Determination of tear secretion rate differentiates aqueous - deficient dry eye from evaporative dry eye, and is most frequently done clinically by use of the Schirmer tear test strip.

The Schirmer test is performed placing a small strip of filter paper of known dimension (5x35 mm) on the margin of the lower eyelid at the junction of the lateral and middle third of the lid and leaving it in place for 5 minutes, then measuring the length of the strip that is wet with tears. In this test is done without prior instillation of topical anesthetic, it is a measure of reflex secretion of tear (Schirmer I test); if the test is done following instillation of a topical anesthetic, it is a measure of baseline tear secretion (basal tear secretion test). The normal value of the Schirmer I test is greater than 10 mm of wetting, but cutoff reference values for dry eye have been recommended as 5 mm of wetting. Some clinicians use a value of 7 mm with the Schirmer I test and 3 mm for the Schirmer with anesthetic.

Bacteriological analysis

It was carried out testing of conjunctiva swab Hess, to search for aerobic and anaerobic bacteria. Samples from patients were seeded in the appropriate culture medium and incubated in aerobic and anaerobic atmosphere for the isolation and identification of bacteria, with separate counts for aerobic and anaerobic bacteria. Bacteria identification has been confirmed through Vitek (Biomerieux, Mercy I'Etoile, Francia) for aerobic bacteria and through API 20A (Biomerieux) for anaerobic bacteria.

Statistical analysis

The results are expressed as mean +/- standard deviation. Statistical significance in contingency Tables was evaluated using the chi square and Fisher exact test. Student's test for unpaired data, one-way ANOVA, and Mann-Whitney rank sum test were used for comparisons of continuous variables. Statistical analysis was performed using tests for repeated measures as well by controls for multiple comparisons with correction by Duncan Procedure.

Results

Baseline data

Baseline subjects' characteristics were similar among the two treatment groups. Frequency of consumption of food categories was, in general, similar across the treatment groups.

Effect of the Mixture of symbiotic on the DED

The data obtained in the two study groups A and B were, respectively the following: Schirmer I 10.3±0.2 vs 12.9±0.4 ($p< 0.001$); Schirmer II 3.9±0.1 VS 4.5±0.2 ($p<0.001$); BUT 4.3±0.3 vs 6.2±0.2 ($p<0.001$). (Figure 1)

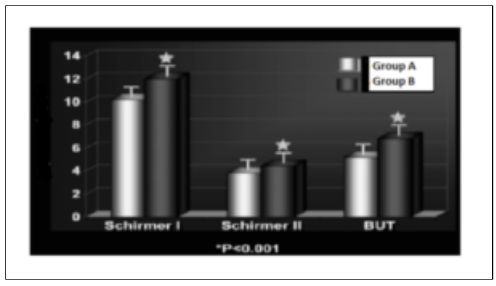

Figure 1: Statistical analysis of clinical parameters in subjects of the group "A" and "B".

Culture test showed initial bacterial growth in group "A" (control) in 18 out of 40 samples tested, corresponding to 45.0% whereas in group "B" after treatment (symbiotic) culture was found positive in 14 out of 40 tests, equal to 35.0%. (Table 2). The total numbers of isolations of aerobic and anaerobic bacteria found in group A and B after treatment are

shown in Table 3. A reduction of 16 to 12 strains of aerobic and anaerobic isolates from 10 to 6 has been found. Table 4 shows the species of aerobic and anaerobic bacteria found in patients A (placebo) and B after probiotic treatment on the fifth day after discontinuation of therapy.

N° pazients (40)	N°. Eyes (80)	Group A		Group B	
		N°.	%	N°.	%
Culture test	80	18	22.5	15	18.7

Table 2: Overall incidence of culture positivity of bacteriological tests "A" and "B".

Microrganisms	Group A	Group B
Aerobes	16	14
Anaerobes	11	7

Table 3: Total number of aerobic and anaerobic isolates of cultures group "A" and "B".

Microrganisms	Group A		Group B	
	N°	%	N°	%
S. epidermidis	8	29.6	10	47.7
S. aureus	4	14.9	2	9.6
S.pneumoniae	2	7.4	1	4.7
S.pyogens	1	3.7	4.7	
P. vulgaris	1	3.7	-	-
Peptococcus spp.	5	18.5	5	23.9
Peptostreptococcus spp.	3	11.1	1	4.7
Propionibacterium spp.	2	7.4	1	4.7
Bacteroides spp.	1	3.7	-	-
Total strains	27	100	21	100

Table 4: Overall incidence of culture positivity of bacteriological tests group "A"and "B".

Conclusions

The present study shows that the administration of Bifidobacteria and Lactobacillus may represent a success full treatment in ameliorating DED. Commensal bacteria or probiotics interact with the endogenous enteric microbiota and gut cells therein conferring health benefit to the host[19], in fact, the effects of imbalanced microbiota are not restricted by gastrointestinal abnormalities but could have systemic impact on immunity[20-22].

Although data are somewhat limited, it has been shown that lactobacilli and Bifidobacteria species display potential therapeutic proprieties[21-23]. Lactobacilli strains have also been shown to normalize steroid release. Bifidobacteria are used as beneficial food supplements in dairy products and play a protective role against pathogenic bacteria and allergies[24-26]. The gut microbiota appears to play role in chronic inflammatory disease, through several mechanisms. Colonic microbiota could stimulate infiltration of macrophages in the adipose tissue by providing inflammatory stimuli such as Lipopolysaccarides (LPS) and enhancing energy intake from the food that leads to adipocyte hypertrophy[25-27].

Free fatty acids and bacterial LPS act synergistically in stimulation of that inflammation. LPS represents an endotoxin whose production by gut microbes could lead to chronic low-grade inflammation, in addition LPS, the microbiota produces many proinflammatory molecules including flagellin and peptidoglycan. Although the gut flora contributes to a healthy environment, acute and chronic mucosal inflammation can arise as a result of both commensal and pathogenic bacteria that influence the innate and adaptive immune-systems. Intestinal microbes can alter host defense mechanisms, leading to the activation of cytokines and the stimulation of adaptive T-cell and B- cell response[26-31].

Several studies demonstrate that Bifidobacteria may have a range of health effects, including the regulation of intestinal microbial homeostasis, the inhibition of pathogens and harmful bacteria, the modulation of local and systemic immune reactions, the suppression of procarcinogenic substances and the making of vitamins[28]. Other studies investigate the effects of lactobacillus, as probiotic on the lipid profile in type 2 diabetes mellitus, showing a significant decrease of total Low-density Lipoprotein (LDL) and choles-

terol[29-32] Many intervention studies were performed for a shorter periods, thus limiting a correct evolution of effectiveness, tolerability and adverse effect. The good compliance in taking capsules demonstrated by all patient, represent another relevant point for connect study outcome[30-35].

In the present study no serious adverse events were detected. The intestinal symptoms described by two patients did not represent a limiting occurrence. They lasted for a short time, were mild, were not accompanied by an increase in stool frequency or a physical change in stool and did not interfere with normal activities. Because symptoms occurred at the start if both the probiotic and the placebo supplementation it is plausible to exclude any cause-effect relationship. The mechanisms involved in improvement of ocular surface are not known, but the probiota might contribute to immunomodulation in tandem with both the innate and adaptive immune systems.

In conclusion our paper confirms the benefit of this probiotic combination in reducing DED and in improving quality of life. The mechanism of the benefit observed with probiotic remains a matter of significant interest. With regard to the impact of the DED symptoms on the patients overall well-being, symptoms of discomfort visual disturbance exhibit higher psychological distress associated with decrease in quality of medications. Further studies are needed to investigate the relative contribution of treatment aiming to reduce discomfort symptoms and visual disturbance.

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