

THE EFFECTS OF BLOOD GLUCOSE REGULATION ON THE MICROBIOTA OCULAR IN TYPE II DIABETIC SUBJECTS

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ABSTRACT

The authors studied the effects of blood glucose regulation on the microbiota ocular in type II diabetic subjects. 30 type II diabetic subjects were considered for at least 2 years and subdivided into three groups A, B and C based on the A1c (HbA1c) glycemic hemoglobin assay. The group "A" includes 10 subjects with HbA1c <7.5%, in the group called "B" there are 10 subjects with the same ocular but with HbA1c range between 7.5 and 9.5 and 10 were included in group C, 10 diabetic subjects with HbA1c > 9.5%. All patients in the three groups were considered the following parameters: Schirmer I test, Schirmer II Test, time to break the tear film (BUT, sec)

and conjunctival buffer for aerobic and anaerobic bacteria research. The cultivation examinations showed a positivity for the tests in group A of 5, group B of 7 and group C of 7 bacteriological examinations. In the group A we found a total of 4 mono microbial and 1 poly microbial finds, mono microbial group B 4 and 7 poly microbial, and mono microbial and 3 poly microbial C 3 groups. Our data show that there is a close relationship between the concentration of glucose in the blood and its modification of the ocular microbiota .

Keywords: type 2 diabetics, the eye surface, ocular microbiota.

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Introduction

Type II diabetes has been identified as a risk factor for the dry eye in many studies⁽¹⁻⁶⁾. Hyperglycemia is often a cause of alteration in the physiology of the tear film. Indeed, in our previous research, we observed in the type 2 diabetic patients an alteration of the tear film associated with ocular discomfort symptoms⁽⁷⁻⁹⁾.

Other studies have predicted the increase in some tear film components in such patients. The presence of squamous conjunctival metaplasia and its possible correlation with diabetic retinopathy has been confirmed by cytology. Ocular dryness in the diabetic subject manifests itself after some years of the onset of the disease. The age often advanced

by patients can be considered a classic disapproval of senile due to degenerative causes of the structures involved in maintaining a physiological functional balance of the tear film⁽¹⁰⁻¹²⁾. The integrity of the corneal nerve pathways is important because the pathways can induce variable-sized hypolarization. One thing can be an alteration of the ocular surface and become a specific dry eye disease known as diabetic keratopathy (diabetic keratopathy)⁽¹³⁻¹⁶⁾. The ocular surface, now regarded as a functional unit, whose individual anatomical components are coordinated by neurological control. This Diabetic Disease (DK) is not a mere discomfort, but a real tear film, which deserves to be recognized and treated prematurely, otherwise corneal lesions may be compromised that may affect vision.

Environmental factors, behavioral factors, but also the increase in mean life and the increase in some chronic diseases, are the basis of the growing progress of this disease in the last decades in the diabetic population. Surely the first event is made from low tear production, by a qualitative alteration and / or by an increased evaporation of the same⁽¹⁷⁻²³⁾. Presumably, the quantitative-quantum abnormalities of the tear film result in a change in the ocular microbiogram with a remarkable reduction in eye immune defenses, thus facilitating the emergence of a possible infectious process. of glucose (hyperglycaemia) in the blood was observed and altered by the ocular surface and therefore of the ocular microbial.

Materials and methods

Study design

30 type II diabetic subjects were considered for at least 2 years and subdivided into three groups A, B and C based on the A1c (HbA1c) glycemic hemoglobin assay. The group "A" includes 10 subjects with HbA1c <7.5%, in the group called "B" there are 10 subjects with the same ocular but with HbA1c range. between 7.5 and 9.5 and 10 were included in group C, 10 diabetic subjects with HbA1c > 9.5%. All patients in the three groups were considered the following parameters.

Inclusion criteria

Type II diabetic subjects for at least two years.
No corneal staining with fluorescein
Absence of ocular surface infections and out-buildings.
Absence of allergic ocular surface diseases.

Exclusion criteria

Previous eye surgery.
Alteration lacrimal.
Medical therapy with systemic or topical medications that alter the tearing and / or topical steroids during the four weeks preceding the start of the study⁽²⁴⁻²⁶⁾.

Parameters considered

• Examination of the front segment by slit lamp

- Schirmer I test (mm / 5')
- Schirmer II Test (mm / 3')
- Time to break the tear film (BUT, sec)

- conjunctival buffer for aerobic and anaerobic bacteria research.

Schirmer I test

A strip of 35 mm long absorbent graduated paper was applied to the outer third of the lower eyelid and the patient was asked to glance up. After 5 minutes, the strips were removed and the length of wet paper was estimated (normal values between 10 and 15 mm).

Schirmer II test

We administered one drop of anesthetic (novesina) every three minutes for three times and we proceed as Schirmer I test. After 3 minutes the strips were removed, and portion of paper soaked was estimated (normal >10 mm)^(20, 23, 28, 29).

Break-up time Test (B.U.T.)

Small quantity of fluorescein was introduced into the conjunctiva sac and, by the use of a blue filter on a slit lamp biomicroscope, was evaluated the time necessary for the appearance of the first break or dry spot on pre corneal tear film (normal-values range 10-15 sec.) (dryspots)^(23,26).

Bacteriological analysis

Testing of conjunctiva swab Hess was carried out 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. In particular, each anaerobic strain, was identified chemically after the specified patterns from "Anaerobe Laboratory Manual", 4th ed., Virginia Polytechnic Institute^(26, 29).

Statistical analysis

The results are expressed as mean±standard deviation. Statistical significance in contingency tables was evaluated using the chi square and Fischer 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

The demographic characteristics of type II diabetic patients included in our study are listed in Table 1 below.

Charateristics	n.	Mean ± Standard Deviation	Range	
Group (patients)		A	B	C
HbA1c % (range)		<7.5%	7.5-9.5	>9.5
Sex (F/M)		4/6	6/4	5/5
Age (years)		69.5±4.9	71.5±5.1	73.5±3.8
BMI (kg/m2)		25.8±3.1	26.8±4.9	26.4±6.4
Heart Rate (b.p.m.)		84±12	84±12	85±16
Systolic blood pressure (mmHg)		135±12	137±14	137±14
Diastolic blood pressure(mmHg)		84±10	85±10	85±10
Smokers (yes/no)		3/7	1/9	1/9
Plasma glucose (mg/dl)		165±10	187±14	205±12
Cholesterol (mg/dl)		214±16	230±12	244±14
HDL cholesterol (mg/dl)		167±10	175±12	175±12
Triglycerides (mg/dl)		186±10	195±15	196±15

Table 1: Basal characteristics of subjects group A, B and C with Type II diabetes and with different values of HbA1c% (range).

These subjects were subdivided into three groups (A, B and C) according to the glycosylated hemoglobin range: “A” with HbA1c% (range) <7.5%, group “B” with range 7.5-9.5 and group “C” with HbA1c > 9.5%. The mean values of the lacrimal secretion expressed as a percentage obtained in the three study groups are reported respectively in Figures 1 and 3, for the group “A” Schirmer I = 9.2, Schirmer II 3.6, B.U.T. = 4.3 for the group B Schirmer I = 6.3, Schirmer II = 3.1, B.U.T. = 2.9 and for the group C Schirmer I = 6.2, Schirmer II = 3.1 and, B.U.T. = 2.7.

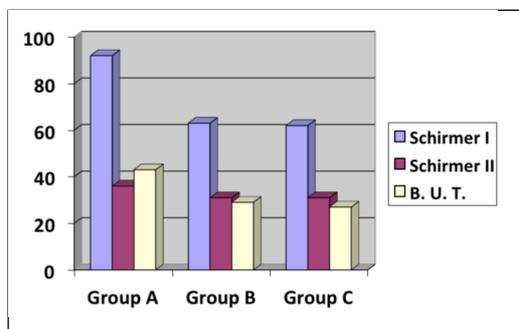


Fig 1: Mean of the clinical parameters of the ocular surface (Schirmer I test and Schirmer II and B.U.T test) expressed as percentages in the three study groups A, B and C.

The cultivation examinations are shown in Table N. 2 and Figure N. 2, showed a positivity for the tests in group A of 5, group B of 7 and group C of 7 bacteriological examinations. In the group A we found a total of 4 monomicrobial and 1 polymicrobial finds, monomicrobial group B 4 and 7 polymicrobials, and monomicrobial and 3 polymicrobial C 3 groups. In group A, we found a number of aerobic isolates equal to 5 strains, and a number of anaerobic strains equal to 1 (Table 3), in the “B” group we have obtained n. 7 aerobic and 3 anaerobic strains and in group C we isolated N°6 of aerobes and 5 of anaerobes.

Groups	Patients	Eye	Tests	Positive		
	N°	N°	N°	%	Mono microbial	Poly microbial
A	10	20	5	25	4	1
B	10	20	7	35	4	3
C	10	20	7	35	3	3

Table 2: Number and percentage of bacteriological positivity of ocular swab tests in the three study groups “A”, “B” and “C”.

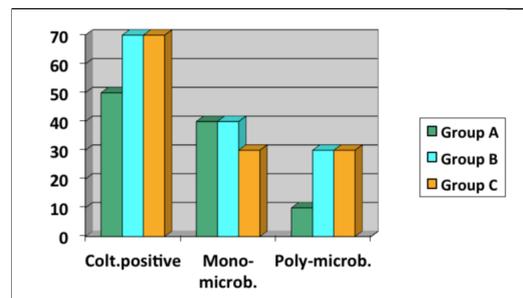


Fig 2: Specific incidence (%) of cultural positivity of bacteriological tests in study groups A, B and C.

Microrganisms	A	B	C
Aerobes	5	7	6
Anaerobes	1	3	5
Total strains	6	10	11

Table 3: Total number of aerobic and anaerobic isolates from the cultures of the ocular surface of typo II diabetic subjects in the different groups.

The identification of aerobic and anaerobic bacterial strains isolated from type II diabetic patients in the three study groups is highlighted in Table no. 4 showing a high incidence of S. epidermidis and S. aureus compared to the rest of isolates with an increase in anaerobic isolates in groups B and C.

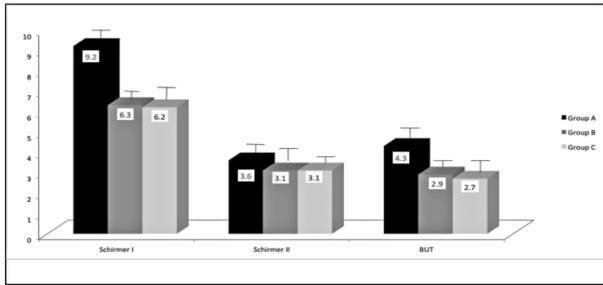


Fig 3: Statistical analysis of clinical parameters in subjects of the group “A” ,”B” and “C”.

Microorganisms	A		B		C	
	N°	%	N°	%	N°	%
Aerobes						
<i>S. epidermidis</i>	3	50.2	4	40.0	3	27.3
<i>S. aureus</i>	1	16.6	1	10.0	1	9.1
<i>S. pneumoniae</i>	1	16.6	1	10.0	1	9.1
<i>S. pyogens</i>	-	-	1	10.0	-	-
Anaerobes						
<i>Peptococcus spp.</i>	1	16.6	2	20.0	3	27.3
<i>Peptostreptococcus spp.</i>	-	-	1	10.0	2	18.1
<i>Propionibacterium spp.</i>	-	-	-	-	1	9.1
Total strains	6	100	10	100	11	100

Table 4: Bacterial strains (percentage) isolated from the ocular surface of typo II diabetic subjects in the three groups (group A, B and C).

Statistical analysis

Clinical parameter data (Schirmer I, Schirmer II and B. U.T.) obtained in the three

Study groups are expressed as mean \pm D.S. of the samples. Significance the statistics of the differences between group “A” and group “B” and group “C”^B have been calculated applying the one-ANOVA ($p < 0.001$) (statistical test Figure N. 3 and Table N. 5). How long with regard to the amount of germs obtained, no test could be applied statistical.

	Group A \rightarrow B		Group A \rightarrow C		Group B \rightarrow C	
	t	p	t	p	t	p
Schirmer I	12,834	<u><0,001</u>	15,411	<u><0,001</u>	0,497	0,622
Schirmer II	2,148	<u>0,038</u>	2,260	<u>0,030</u>	0	1
BUT	6,433	<u><0,001</u>	8,376	<u><0,001</u>	1,205	0,236

Table 5: Statistical analysis of clinical parameters in subjects of the group “A” ,”B” and “C”.

Discussion and conclusions

The data reported in our study clearly show that the increase in glycosylated hemoglobin results in a specific interference on the ocular surface of the type II diabetic. This is confirmed by the reduction of eye parameters (Schirmer I, II and B. U.T) and bacteriological isolates. We also observed that in the patient group called A with HbA1c $< 7.5\%$, moderate ocular dryness was observed with monomicrobial bacteriological findings. Whereas, in groups B (HbA1c range 7.5 - 9.5) and C, (HbA1c $> 9.5\%$) severe eye dryness was observed with Schirmer I and II and BUT quite reduced with its mixed bacteriological isolates. These bacteriological data, observed differently in the three study groups, confirm that type II diabetic subjects with a HbA1c range 7.5 - 9.5 and $> 9.5\%$, have a homogeneous but different microbial than the patients in Group A. The presence in the B and C groups especially of mixed bacteriological findings confirms a modification of the organic ecosystem of the ocular surface with an increase in the incidence of anaerobic bacteria.

The frequent isolation of anaerobic bacteria in these two groups of patients demonstrates a likely “role of opportunism” of these microorganisms. The close correlation with the worsening of tear tests (Schirmer I, Schirmer II and BUT) confirms in these patients an ocular dryness with an increased risk of infectious eye damage^(23,26,29). These data are also supported by the small number of *S. epidermidis* that exercise an adherent action on the ocular surface through the action of the glycocalyx. The increase in saprophytic bacteria allows these bacteria to integrate into the ocular epithelial cells of the glycocalyx, resulting in a barrier of stability and prevention of possible surface infections^(30,31).

These data confirm that the different glucose concentration in the blood determines a selective factor in normal bacterial flora with an increase in anaerobic isolates. In light of our first results we find that the quantitative-quantitative alterations of the tear film result in a consequent modification of the ocular microbial with a remarkable reduction in the eyepiece immune defenses, thus facilitating the emergence of a possible infectious process.

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