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Goblet cell density association with tear function and ocular surface physiology

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ABSTRACT

Purpose: To determine the relationship of goblet cell density (GCD) with tear function and ocular surface physiology.
Methods: This was a cross-sectional study conducted in 35 asymptomatic subjects with mean age 23.8 ± 3.6 years. Tear film assessment, conjunctiva and cornea examination were done in each subject. Conjunctival impression cytology was performed by applying Nitrocellulose Millipore MF™-Membrane filter over the superior bulbar conjunctiva. The filter paper was than fixed with 96% ethanol and stained with Periodic Acid Schiff, Hematoxylin and Eosin. GCD was determined by optical microscopy. Relation between GCD and Schirmer score, tear break-up time (TBUT), bulbar redness, limbal redness and corneal staining was determined.
Results: The mean GCD was 151 ± 122 cells/mm^2. GCD was found higher in eyes with higher Schirmer score but it was not significant ($p = 0.75$). There was a significant relationship of GCD with TBUT ($p = 0.042$). GCD was not correlated with bulbar redness ($p = 0.126$), and limbal redness ($p = 0.054$) as well as corneal staining ($p = 0.079$). No relationship of GCD with age and gender of the subjects ($p > 0.05$) was observed.
Conclusion: GCD was found correlated with TBUT but no significant correlation was found with the aqueous portion of the tear, limbal as well as bulbar redness and corneal staining.

1. Introduction

Normal tear film maintains an optically uniform ocular surface, protects the eyes from infections and environmental hazards, washes out cellular debris and foreign bodies and sustains ocular comfort and a healthy corneal epithelium. Mucin, the innermost layer of the tear, changes the hydrophobic corneal surface into hydrophilic. It also makes the corneal surface smooth and helps to balance tear film on the anterior ocular surface. Conjunctival goblet cells produce mucin-MUC5AC and it is supposed to be one of the main gel forming substances in the tears [1]. Goblet cells are balloon shaped cells with eccentric nucleus found in the superficial layers of the conjunctiva.

Assessment of goblet cell on the ocular surface is important as it is a helpful diagnostic tool for many eye conditions. Goblet cell density (GCD) was found lower in eyes with ocular surface disease in comparison to normal eyes [2]. The number of goblet cells decreases with contact lens wear [3]. Torricelli et al. [4] found an increase in GCD due to air pollution. Doughty reviewed 24 different studies which reported the effects of contact lens wear on the goblet cells of the human conjunctiva and found that there has been limited consistency in the technique or the method of reporting the results across the various studies justifying the differences obtained [3]. Additionally, GCD in conjunctival surface varies highly from one region to another and from one person to another with a range from 24 to 2226 cells/mm^2 for average values [2]. Torricelli et al. [4] found higher GCD in tarsal conjunctiva in comparison to bulbar conjunctiva. Connor et al. [5] reported that GCD is higher in male than in females and in female, it is higher in oral contraceptive users. However, Tomlinson et al. [6] found no relationship between tear physiology and oral contraceptives. Yeo et al. [7] conducted impression cytology in 40 healthy Chinese subjects. They did not find any correlation between GCD and Schirmer score, non-invasive break-up time (NIBUT) and tear break-up time (TBUT).

Conjunctival impression cytology (CIC) is a simple and less invasive method (over a conventional surgical biopsy) to assess the conjunctival health including conjunctival epithelial metaplasia and goblet cell density [8]. Goblet cells are easily visualized through light microscope by staining their mucus content with Periodic Acid-Schiff (PAS) [9]. During the last decade, CIC has been applied

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enormously as a useful diagnostic aid for a wide variety of ocular surface pathologies and has greatly contributed to the understanding of ocular surface conditions [8]. It implies the collection of cells from the conjunctival surface via a special type of filter paper (or sometime disk) by impression on the surface and examination of the superficial layer(s) with different types of staining. It can be done with or without anesthesia [9].

Two different approaches have been used to evaluate the goblet cells obtained in a CIC sample: a direct assessment of the number of goblet cells or an indirect evaluation (based on assignment of a grade) [3]. Estimation of GCD has been carried out by different ways. Although 'number of goblet cells per millimeter', 'number of goblet cells per high power field' and 'number of goblet cells per 100 basal cells' have been reported in different studies; 'number of goblet cells/mm^2' is the mostly used unit [2]. Generally, the numbers of cells are counted in unit field of view of the microscope and cells/mm^2 is calculated.

Limited numbers of studies have been conducted to determine the relationship between conjunctival GCD with ocular surface physiology and tear function tests. The aims of this study were to investigate GCD in eyes of asymptomatic subjects and to determine its correlation with limbal/bulbar redness, corneal staining, Schirmer score, NIBUT and TBUT.

2. Methods

This was a cross-sectional study conducted in University of Minho, Portugal. Each subject signed a consent form after the procedures, time duration, possible consequences and other details of the study were explained. This study was ethically approved by Ethical Committee of School of Science, University of Minho and tenets of declaration of Helsinki were followed.

Thirty-five volunteers with age 18–35 years and minimum best corrected visual acuity of 6/6 in each eye, were included in the study. The sample size was calculated to warrant the 70% statistical power with 0.05 significance level which was estimated on the basis of our preliminary data. Subjects with past history of contact lens wear were excluded from the study. Each subject filled up McMonnies questionnaire and those with score more than 14 were excluded from the study [10,11].

Schirmer test was done without anesthesia with a commercially available paper strip (Sno strips, Laboratoire Chauvin, France). The strip was inserted into lower temporal conjunctival sac and the wet length of the paper was measured on millimeter after 5 min. Eyes were closed during the test to get more reliable data [12]. NIBUT was performed with Tearscope Plus (Keeler Instruments Ltd., Windsor). By applying fluorescein dye, TBUT was measured as the time interval between the subject blink and the first appearance of a black spot or line on the cornea observed by slit lamp with cobalt blue light and a Wratton-12 yellow filter. Schirmer score less than 10 mm [13,14] and TBUT score less than 10 s [14,15], were considered as low values.

Bulbar conjunctiva and limbus were divided into four regions: nasal, temporal, superior and inferior and cornea was divided into five regions: central, nasal, temporal, superior and inferior as described in the CLEK study [16]. Bulbar and limbal redness and corneal staining were graded applying Efron grading system with score 0–4 [17]. Each of the gradings was done on 0.1 step to optimize grading sensitivity [18]. Average score was used for data analysis.

Impression cytology was performed on the superior bulbar conjunctiva on both eyes of each selected subject [9]. Nitrocellulose Millipore^2 MF^{TM}-Membrane filter (MILLIPORE, Ireland) with pore size 0.45 μm was used without application of topical anesthesia [19,20]. Briefly, a circular paper of diameter 13 mm was cut into two equal semi-circular pieces. The semi-circular piece of filter was placed on the superior bulbar conjunctiva, 1–2 mm away from the limbus and removed in peeling motion. The paper was dried and fixed with 96% ethanol for 15 min in a 24 well plate. The paper was then stained with PAS, Haemotoxylin and Eosin and dried in ascending concentration of ethanol [21]. The slides were prepared by dissolving the paper with Xylene and mounted with Xylol. The slides were observed by light microscopy with total magnification 100× and 400×. Goblet cells were counted in the higher magnification with an area of 0.16 mm^2 (total magnification of 400×) and GCD was calculated as the number of cells per square millimeter. This procedure was repeated in three random fields of areas and the average was used in analysis.

Data were analyzed with Statistical Software (SPSS 22, IBM Corp., Armonk, NY). Kolmogorov–Simirnov test was done to find out the normality of the variables. Parametric tests were applied for normally distributed variables and non-parametric tests were applied for others. Correlation between two variables was found out by Spearman test, and the association with gender or oral medication was found out by one way ANOVA with post hoc. p value of less than 0.05 was considered as statistically significant. Data of the right eyes were used for the analysis.

Table 1
Descriptive statistics of the variables.

	Mean	SD	Range
GCD (cells per mm^2)	151	122	18–522
Schirmer score (mm)	22.7	11.6	3–45
NIBUT (s)	20.0	14.4	7–60
TBUT (s)	12.4	7.2	5–36
Bulbar redness	0.3	0.23	0–0.9
Limbal redness	0.4	0.26	0–1.0
Corneal staining	0.2	0.13	0–0.5

GCD – goblet cell density, NIBUT – non-invasive tear break up time, TBUT – tear break up time, and SD – standard deviation.

3. Results

Among the 35 asymptomatic subjects enrolled in this study, 23 (65.7%) were females. Mean age of the subjects was 23.8 ± 3.6 years. Majority (91.4%) of the subjects were Caucasians. None of the subjects had worn contact lens before the study.

After conjunctival impression cytology, the cells imprinted in the paper filter were stained and visualized by optical microscopy. The goblet cells were easily identified under the microscope due to their dark pink color after PAS staining (Fig. 1).

The conjunctival cytology was observed with 100× and 400× total magnification with light microscope (Fig. 1) and the goblet cells were counted. Descriptive statistics of the variables are shown in Table 1. A high variation in GCD was observed with a mean value of 151 cells/mm^2 and standard deviation of 122 cells/mm^2.

The correlation between each variable is shown in Table 2. As it can be seen, no correlation between GCD and Schirmer score ($r = 0.05$, $p = 0.75$) was found. A positively correlation with the NIBUT was observed, but not statistically significant ($r = 0.224$, $p = 0.195$). The GCD was found to be positively correlated with TBUT ($r = 0.338$, $p = 0.042$). Fig. 2 shows the scatter plot of GCD versus Schirmer score and TBUT. The inverse correlation of GCD with conjunctival bulbar as well as with limbal redness and corneal staining could not reach a statistically significant level ($p > 0.05$). Corneal staining was found inversely correlated with Schirmer score and positively correlated with bulbar/limbal redness ($p < 0.05$).

As shown in Table 3, eyes with low TBUT had statistically significant lower GCD ($p = 0.043$), however, the difference in GCD, in eyes with low and high Schirmer values, could not reach to statistically significant level ($p = 0.058$).