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Comparison of conjunctival impression cytology in primary open angle glaucoma, ocular hypertension and normal subjects

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原发性开角型青光眼和高眼压患者及正常人群结膜印迹细胞学检查的比较

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摘要

目的:比较原发性开角型青光眼和高眼压患者及拥有健康眼球表面的正常人群的泪膜功能和印象细胞学检查的数值。

方法:此前瞻性研究中纳入了原发性开角型青光眼患者11例11眼(平均年龄:62.7±6.1岁),高眼压患者12例12眼(平均年龄:62.8±6.4岁)及健康人12例12眼(平均年龄:62.9±6.03岁)。这些患者均是最近被诊断出患有原发性开角型青光眼及高眼压,且之前未接受过抗青光眼方面的治疗。均行结膜印迹细胞学检查、泪膜破裂时间和基础泪液分泌试验。每组印迹细胞学检查的样本根据Nelson分级法分为0~3级。应用Kruskal-Wallis检验和Dunn多重比较检验进行统计分析。

结果:原发性开角型青光眼患者,高眼压患者及正常人群平均基础泪液分泌值分别为10.4±1.3,10.9±1.2和11.1±1.1 mm/5min,其差距没有统计学意义(P=0.33);三组的泪膜破裂时间分别为11.2±1.1,11.3±1.1和11.8±1.2s,其差距没有统计学意义(P=0.35)。原发性开角型青光眼患者中6眼(54.5%)为0级,5眼(45.5%)为1级。高眼压患者中6眼(50%)为0级,6眼(50%)为1级,健康人中6眼(50%)为0级,6只眼(50%)为1级(P=0.97)。

结论:氧化应激可能会导致青光眼,眼表疾病,泪腺功能障碍及机体杯状细胞所分泌的黏液减少。原发性开角型青光眼患者,高眼压症患者及健康人群间的印象细胞学检查数值并无显著差异。

关键词:结膜印迹细胞学;原发性开角型青光眼;高眼压

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Abstract

• AIM: To compare the tear functions and the impression cytology scores of the patients with primary open angle glaucoma (POAG), ocular hypertension (OHT) and normal subjects with healthy ocular surface both functionally and clinically.

• METHODS: Eleven eyes of 11 patients with POAG (mean age: 62.7±6.1y), 12 eyes of 12 patients (mean age: 62.8±6.4y) with OHT and 12 eyes of 12 normal subjects (mean age: 62.9±6.03y) were included to this prospective study. The patients with POAG and OHT had been recently diagnosed with these diseases and none of them had taken anti-glaucoma treatment before. In addition to conjunctival impression cytology, tear break-up time (TBUT) and basal Schirmer's tests (BST) were performed. Impression cytology specimens of each group were graded and scored in the range of 0-3 according to Nelson's method. Kruskal-Wallis analysis and Dunn's multiple comparison tests were used for statistical analysis.

• RESULTS: The mean BST values were 10.4±1.3, 10.9±1.2 and 11.1±1.1 mm/5min of POAG, OHT and control groups respectively. The differences among the BST values of the POAG, OHT and control group were not statistically significant (P=0.33). The mean TBUT values were 11.2±1.1, 11.3±1.1 and 11.8±1.2s in POAG, OHT and normal subjects respectively. The differences among the BUT values of the POAG, OHT and control group were not statistically significant (P=0.35). Six eyes (54.5%) revealed grade 0 and 5 eyes (45.5%) revealed grade 1 impression cytology scores in POAG group. Six eyes (50%) revealed grade 0 and 6 eyes (50%) revealed grade 1 impression cytology scores in OHT group and 6 eyes

(50%) revealed grade 0 and 6 eyes (50%) revealed grade 1 impression cytology scores in normal subjects (P = 0. 97).

• CONCLUSION: Oxidative stress may cause glaucoma, ocular surface diseases, lacrimal gland malfunction and a decrease in mucus secretion ofgoblet cells in all of the body. There were no significant differences between the impression cytology scores of patients with POAG, OHT and normal subjects.

• KEYWORDS: conjunctival impression cytology; primary open angle glaucoma; ocular hypertension
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INTRODUCTION

Glaucoma is a multifactorial optic neuropathycharacterized by visual field defects, retinal ganglion cell death, and progressive degeneration of the optic nerve and is the second leading cause of irreversible blindness worldwide[1-3]. Primary open angle glaucoma (POAG) is the most common form, with reported prevalence rates ranging from 1. 1% to 3. 8%[4,5]. Ocular hypertension (OHT) is the major risk factor for the development POAG, with a high intraocular pressure (IOP) but normal optic nerve and visual field findings[6-9]. Although increased IOP is a major risk factor for POAG, some other factors like oxidative stress, which is an imbalance between reactive oxygen species (ROS) and antioxidants, may also play an important role in the pathogenesis of the disease [1,10-12].

Oxidative stress has also been reported to be associated with ocular surface diseases, lacrimal gland malfunction and a decrease in mucus secretion of Goblet cellsthroughout the body[13,14]. Conjunctival impression cytology is a safe, relatively simple, minimally invasive biopsy method of obtaining specimens from the conjunctival surface[15-17]. This technique allows the investigator to asses' epithelial cell morphology, examine cytoplasmic and nuclear characteristics and quantify the goblet cell population in the conjunctiva.

In the present study, our aim was to compare the tear functions and the impression cytology of the patients who had been recently diagnosed with POAG and OHT with normal subjects who had clinically and functionally normal ocular surface, in order to investigate the effect of glaucoma on ocular surface.

SUBJECTS AND METHODS

We prospectively evaluated 11 eyes of 11 patients with POAG and 12 eyes 12 patients with OHT and 12 eyes of 12 normal subjects, who had been examined at Glaucoma clinic of Ankara Ulucanlar Eye Research Hospital. All of the study procedures were conducted in accordance with the Declaration of Helsinki, and informed consents were taken from all of the participants. This study was approved by The Ethical Committee of Ankara Training and Research Hospital. All patients were Turkish Caucasians.

The complete ophthalmologic examination was performed including best-corrected visual acuities with Snellen charts, anterior and posterior segment examinations, tear break-up time (TBUT) and basal Schirmer's tests (BST), gonioscopy with Goldmann's three-mirror lens, IOP measurements with Goldmann applanation tonometer, central corneal thickness (CCT) measurements by ultrasonic pachymeter, visual field examinations with Humphrey automated perimeter and confocal scanning laser ophthalmoscopy with HRT II at the time of the diagnosis of POAG and OHT.

They had been recently diagnosed with POAG and OHT and none of them had taken anti-glaucoma treatment before. The presence of glaucomatous visual field defects like nasal step, seidel or arcuate scotome with an IOP ≥21mmHg, grade 3-4 open angle according to Shaffer angle grading system and optic nerve head changes like cup to disc ratio ≥0. 3, localized neuroretinal rim defects, peripapillary choroidal atrophy or splitter hemorrhage revealed POAG. OHT was defined as IOP ≥21mmHg with normal optic nerve and visual field findings and grade 3-4 open angle. Age-matched control group consisted of normal subjects who had no ocular diseases other than refractive error.

We excluded the cases who reported topical or systemic cyclosporine or ophthalmic steroids use within the previous 6mo. Other reasons for exclusion included aged less than ≤ 40 years old, subjects who had previous histories of diabetes mellitus, connective tissue diseases, antihistaminic, antidepressant, retinoic acid use; eyes with previous histories of dry eye, keratitis, uveitis, contact lens use, chronic topical medication use including anti-glaucomatous drops, any ocular surgery, chemical, thermal or radiation injury and eyes which have active blepharitis or conjunctivitis and pseudoexfoliative material in the anterior segment. In all the groups, the right eyes were enrolled.

TBUT and BST were performedby the same investigator (Sen E). In TBUT test, fluorescein strips (Fluorescein paper, Weck, USA) were inserted into the lower fornix. After several blinks, the patient was asked to stop. The tear film was examined with a broad beam and a cobalt blue filter. The time taken for black spots or lines to appear was recorded. BST was performed after a topical anesthetic (Alcain, 0. 5% proparacaine hydrochloride, Alcon, USA). The filter paper [Schirmer's tear test strips, (sno * strips® Chauvin)] was folded 5 mm from one end and inserted at the junction of the middle and outer one-third of the lower lid. After 5min, the amount of the wet area of the paper was measured. The period between TBUT and BST was at least 30min. Tear fluid

secretion ≤9 mm/5min in Schirmer's test and break up time <10s were defined as abnormal test results related with dry eye findings and these patients were also excluded from the study.

Impression cytology of the conjunctiva of the topically anaesthetized eye was performed according to the technique described by Nelson[18] by the same investigator (Elgin U) who was blinded to the results of TBUT and BST. Small disks of cellulose acetate filter paper (MFS, Advantec MFS, Pleasanton, USA, pore size 0.2μm) were cut into pieces approximately 4×5-mm^2 in size, placed on the superior nasal interpalpebral conjunctiva 5 mm from the limbus, gently pressed for 5s, and then removed. The specimens were placed in a fixative solution and stained with Papanicolau's modification of Gill's technique[7]. The specimens were examined with light microscopy by a pathologist who was blinded to the history of each specimen. The examination employed the Nelson's method, and the appearance of the conjunctival epithelial cells and goblet cells (if present) was recorded[6]. Two pathologists similarly blinded, prepared and examined all the slides (preparation by Haksever H, examination by Ustun H). All specimens were graded according to the following four-levels. Grade 0; the epithelial cells are small and round with eosinophilic - staining cytoplasm. The nuclei are large with a nucleocytoplasmic ratio of 1:2. The goblet cells are abundant, plump and oval with strongly Periodic Acid Schiff (PAS) - positive cytoplasm. Grade Ⅰ; the epithelial cells are slightly larger than those in grade 0 and more polygonal, with eosinophilic - staining cytoplasm. The nuclei are smaller with a nucleocytoplasmic ratio of 1:3. The goblet cells are fewer in number; however, they still maintain their plump, oval shape with strongly PAS-positive cytoplasm. Grade Ⅱ; the epithelial cells are larger than those in grade Ⅰ and polygonal, occasionally multinucleated, with staining cytoplasm. They have a nucleocytoplasmic ratio of 1:4 to 1:5. The goblet cells are markedly fewer in number and are smaller, less strongly PAS-positive and poorly defined. Grade Ⅲ; the epithelial cells are larger than those in grade Ⅱ and polygonal with basophilic-staining cytoplasm. The nuclei are small, pycnotic, or in many cells, completely absent. The goblet cells are completely absent. These changes also called metaplasia of conjunctiva.

Statistical differences among the two groups were evaluated by using Kruskal-Wallis analysis andpost-hoc Dunn's multiple comparison tests. The level of significance was set at $P<0.05$. All statistical analyses of the study were performed by using SPSS for Windows (SPSS Inc., Chicago, IL, USA) software.

RESULTS

The mean age of the 7 (63.6%) female and 4 (36.4%) male patients was 62.7±6.1 (54-70y) in POAG group, the mean age of the 7 (58.3%) female and 5 (41.7%) male patients was 62.8±6.4 (52-71y) in OHT group and the mean age of the 6 (50%) female and 6 (50%) male subjects was 62.9±6.03 (51-72y) in the control group. The differences between the ages of the POAG, OHT and control group were not statistically significant (Kruskal-Wallis analysis, $P=0.10$).

The mean IOP was 24.2±1.6mmHg (21-27mmHg) in POAG, 22.9±1.6mmHg (21-26mmHg) in OHT group and 15.3±1.6mmHg (12-17mmHg) in normal subjects. The differences between the IOPs of the POAG, OHT and control group were statistically significant (Kruskal-Wallis analysis, $P<0.0001$). The mean BST values were 10.4±1.3, 10.9±1.2 and 11.1±1.1mm/5min POAG, OHT and control groups respectively. The differences between the BST values of the POAG, OHT and control group were not statistically significant (Kruskal-Wallis analysis, $P=0.33$). The mean TBUT values were 11.2±1.1, 11.3±1.1 and 11.8±1.2s in POAG, OHT and normal subjects respectively. The differences between the BUT values of the POAG, OHT and control group were not statistically significant (Kruskal-Wallis analysis, $P=0.35$).

The impression cytology scores of the groups are summarized in Table 1.

Table 1 Impression cytology grading scores for all groups

n(%)

Group	Control	POAG	OHT
Grade 0	6 eyes(50)	6 eyes(54.5)	6 eyes(50)
Grade 1	6 eyes(50)	5 eyes(45.5)	6 eyes(50)
Grade 2	0(0)	0(0)	0(0)
Grade 3	0(0)	0(0)	0(0)

Six eyes (54.5%) in POAG, 6 eyes (50%) in OHT group and 6 eyes (50%) in control group revealed grade 0 impression cytology scores which had normal amount of and well-formed goblet cells (Figure 1). Five eyes (45.5%) in POAG and 6 eyes (50%) in OHT and control groups revealed grade 1 impression cytology scores showed grade 1 which have lower in number but well-formed goblet cells (Figure 2). None of the eyes in the three groups showed grade 2 and 3 scores. There were no significant differences between the impression cytology scores of patients with POAG, OHT and normal subjects (Kruskal-Wallis analysis, $P=0.97$). Also, the differences of impression cytology scores, TBUT and BST values between POAG - OHT (Dunn's multiple comparison test, $P=0.87$, $P=0.72$, $P=0.67$ respectively), POAG-control (Dunn's multiple comparison test, $P=0.81$, $P=0.79$, $P=0.71$ respectively), and OHT - control groups (Dunn's multiple comparison test, $P=0.71$, $P=0.69$, $P=0.73$ respectively), were not statistically significant.

DISCUSSION

To our knowledge this prospective study is the first report which

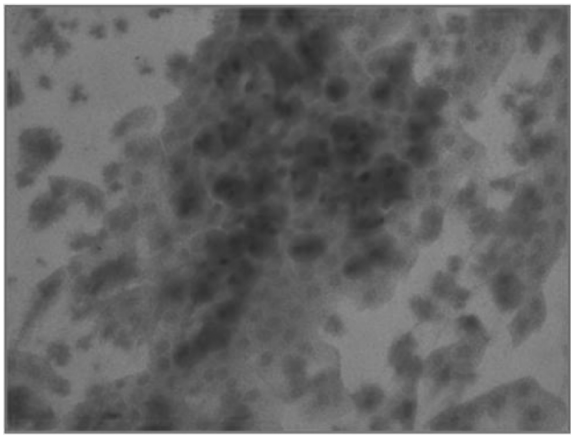

Figure 1 Impression cytology of a patient in control group with grade 0 score, who has normal in number and well-formed goblet cells(×100, periodic acid Schiff staining).

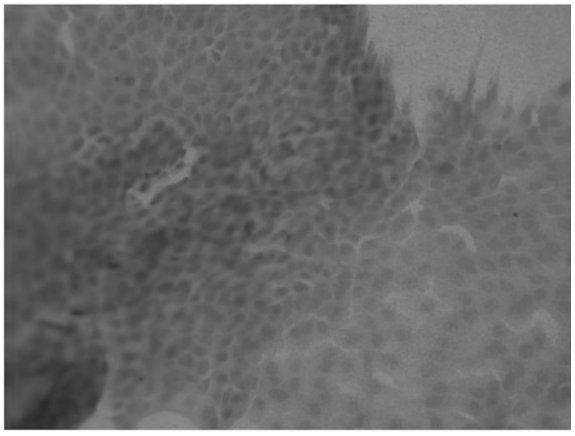

Figure 2 Impression cytology of a patient in POAG group with grade 1 score who has lower in number but well-formed goblet cells(×100, periodic acid Schiff staining).

compares the changes in ocular surface of POAG and OHT patients by impression cytology, which is an important, non-invasive, and easy-to perform diagnostic method. None of our patients used any anti-glaucoma treatment before. We observed no significant differences between the impression cytology scores of POAG and OHT patients and normal subjects.

Some ocular surface problems may occur in especially pseudoexfoliative glaucoma (PXG) and pseudoexfoliative syndrome (PXS). The accumulation of pseudoexfoliative material may alter tear secretion and tear film stability by changing goblet and epithelial cell morphology[19-21]. Öncel *et al*[19] found higher tear osmolarity in eyes with PXS compared with normal subjects and postulated that PXS could be more prone to the development of dry eye syndrome. Erdogan *et al* [20] compared conjunctival impression cytology, TBUT and BST of 45 eyes with PEG, 48 eyes with PXS and 50 eyes of 50 normal subjects. They excluded the patients who had histories of topical anti-glaucoma treatment and artificial tear drop use before, dry eye or other ocular surface disorders. They found significantly higher impression cytology scores in PXG and PXS patients than normal subjects but no significant difference between PXG and PXS. Like in our study, none of their cases had used any anti-glaucoma treatment before because; anti-glaucoma treatment might cause some ocular surface problems. Hong *et al*[22] found that, the patients with POAG under anti-glaucoma treatment had higher impression cytology scores than the ones followed-up without medication. Among the medication group, cytology scores were found to be significantly lower in the monotherapy group than the fixed-combination therapy group. Cennamo *et al*[23] evaluated conjunctival epithelium of glaucoma cases under treatment by impression cytology with light optic microscopy and scanning electron microscopy and found that reduced microvilli count was significantly associated with the duration of anti-glaucoma treatment. They postulated that loss of microvilli could be the first sign of cellular damage during chronic glaucoma therapy.

Different from Erdogan *et al*'s [20] study, we excluded the patients with pseudoexfoliation syndrome and glaucoma and the eyes with abnormal tear functions (tear fluid secretion ≤9 mm/5min in BST and <10s in TBUT). Our purpose was to exclude patients with dry eye or other ocular surface diseases in more objective way and include patients with both clinically and functionally normal ocular surfaces in order to investigate the effect of glaucoma itself on conjunctival cells.

Oxidative stress is known to be related with glaucoma, corneal and conjunctival inflammatory diseases, lacrimal gland malfunction and mucus secretion defects of goblet cells in all of the body[1,10,14]. Oxidative stress related conjunctival hypoxia may also affect the ocular surface. According to Kuppens *et al*'s [24] study, the patients with POAG who had never taken any anti-glaucoma treatment before had decreased the basal tear turnover compared with OHT once. The probability of more oxidative stress in POAG was the hypothesis of our study because it was known to be more advanced form of OHT. Our main purpose was to compare the conjunctival impression cytology scores of POAG and OHT but we observed no statistically significant differences.

Based on the fact that POAG may be associated with more oxidative stress than OHT, deteriorated impression cytology scores may be observed in POAG. Major limitation of this study is the inadequacy of the number of the cases. The other limitation was the absence of the comparison of the oxidative stress markers of our groups in our study. Since some associations between glaucoma and ocular surface disorders were found, further investigations with great number of cases with different types of glaucoma with the comparison of oxidative stress markers, should be encouraged.

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