

Clinical characterisation and cytological study of dry eye in patients with autoimmune disease

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Abstract

Objective: To assess the clinical characteristics and changes in ocular surface cytology of dry eye in patients with systemic autoimmune disease.

Method: The case-control study was conducted in the Second Hospital of Tianjin Medical University, Tianjin, China, from February 2016 to January 2017, and comprised systemic autoimmune disease patients and healthy controls. Schirmer's I test, tear breakup time test, and fluorescein staining were performed on all subjects. Both groups were evaluated for dry eye with the current diagnostic criteria. Conjunctival impression cytology and the morphology of epithelial cells were observed in both groups of subjects. Flow cytometry was used to identify the amount of apoptosis. SPSS 15 was used to analyse the data.

Results: Each of the two groups had 60(50%) subjects each. The morbidity of dry eye in the control group was 17(28.3%), while it was 31(51.7%) in the patients ($p<0.01$). Among the patients with dry eye, the severity level of cells obtained by conjunctival impression sampling was significantly higher in patients than in controls ($p<0.01$). The percentage of conjunctival epithelial cells undergoing apoptosis was higher in patients with dry eye than in patients without dry eye in each group, and among patients with dry eye, the percentage of conjunctival epithelial cells undergoing apoptosis was higher in the patients than in controls ($p<0.01$ each).

Conclusion: The cell injury on the ocular surface was more serious in subjects with dry eye in systemic autoimmune disease than in subjects with dry eye in healthy controls.

Keywords: Autoimmune disease, Dry eye, Impression cell, Apoptosis. (JPMA 68: 353; 2018)

Introduction

Dry eye, known as dry keratoconjunctivitis, is a multifactorial disease of the tear and ocular surface that results in symptom of discomfort, visual disturbance, and tear film instability by increased osmolarity of the tear film and inflammation of the ocular surface.¹ The prevalence of dry eye ranges from 5% to 50%.¹ There is a wide array of causes for dry eye that can induce an alteration of the ocular surface system and determine the chronicity of the disease, systemic and topical drugs, contact lens wear, et al.²

Autoimmune disease is due to the loss of the body's immune system against its own tissue antigen immune tolerance, a pathological immune response, leading to tissue organ damage type of disease. Systemic autoimmune disease included systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), systemic sclerosis, idiopathic Sjögren syndrome (SS) and so on. Many studies have pointed out that patients with autoimmune diseases not only have the systemic effects

but also have ocular manifestations. Dry eye is a common manifestation in autoimmune diseases.^{3,4}

In recent study autoimmunity, inflammation, and apoptosis are hot spots of the pathogenesis of dry eye. Many studies have shown that dry eye is an ophthalmic autoimmune disease caused by an imbalanced regulatory mechanism of protective immunity on the ocular surface.⁵ Those results indicate that autoimmunity plays an important role in dry eye.⁶ The animal experiments have shown that the role of apoptosis in the pathogenesis of dry eye mainly involves the lacrimal glands and conjunctival epithelial cells.⁷ Apoptosis of ocular surface epithelial cells increased in the patients with severe dry eye.⁸

Most published studies focus on immune factor mechanisms and apoptosis associated with dry eye in animal experiments and basic research,⁹ while not so many in clinical research. Based on our clinical experience we found that patients with systemic autoimmune disease have an abnormal immune state which leads to ocular surface disorder. We wanted to investigate that whether the dry eye pathogenesis in patients with systemic autoimmune disease is similar to that of patients without it, or not? The current study was planned to

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evaluate the systemic autoimmune patients and their conditions of dry eye, to collect cells via conjunctival impression sampling and detect apoptosis with flow cytometry, and to observe the changes in ocular surface cytology in these patients.

Patients and Methods

The case-control study was conducted in the Second Hospital of Tianjin Medical University, Tianjin, China, from February 2016 to January 2017, and comprised patients with systemic autoimmune disease and healthy controls. The study was approved by the institutional review board. Before examination, each subject gave written informed consent. Subjects with systemic autoimmune disease were classified as the research group and included SLE, RA, SS, and systemic sclerosis. They were diagnosed by the American Rheumatism Association corresponding classification diagnostic criteria.¹⁰⁻¹³ The control group samples were collected randomly from among healthy persons without autoimmune and other systemic diseases. All included subjects were aged at least 18 years. Subject with any history of ophthalmic problems, such as inflammation, trauma, contact lens wearing, or ophthalmic surgeries within 3 months were excluded.

All research subjects received a routine examination for dry eye. Inquiries were conducted regarding ocular medical history, including symptoms such as dry, foreign body sensation, burning, pinkeye. The results were recorded about tear film breakup time (BUT), fluorescein staining (FL) grading and Schirmer's I test (SIT).

For conjunctival impression cytology (IC), after topical anaesthesia with oxybuprocaine, strips of supor-type microfiltration membrane (Pall, AM; 4 mm×5 mm) were applied on the temporal bulbar conjunctiva to get conjunctival epithelial cells. The specimen was fixed in 95% ethyl alcohol for more than 10 min and stained with Periodic Acid-Schiff (PAS). Five random areas of each sample were photographed using an optical microscope. Finally, the outcomes for a single sample score were averaged. The degree of squamous metaplasia was graded from 0 to 3 as described by Nelson.¹⁴ Using

13×6.5mm semi-circular filter membrane to get single-cell suspension by IC, they were subjected to propidium iodide (PI, Sigma Company, United States) staining. And the percentage of apoptotic cells was detected by deoxyribonucleic acid (DNA) hypodiploid measurement with flow cytometry.

The sample size was calculated by keeping the power of study equal to 90% and level of significance equal to 5%. Based on sample size calculation formula in case-control study,^{15,16} all parameters were assumed as follows: $p_1=0.31$; $p_0=0.24$; $\alpha=0.05$; $\beta=0.10$, by table look-up way $Z\alpha=1.96$, $Z\beta=1.28$.

SPSS 15 was used for data analysis. Continuous variables were expressed as mean $\pm$ standard deviation (SD), whereas categorical variables in the form of frequency and percentage. T-test was used to compare age in research group and controls. Chi-squared test was used to compare gender and the morbidity of dry eye in research group and controls. Rank sum test was used to compare the BUT, SIT and FL results, because they are not conforming to the Gaussian distribution and homogeneity of variance.¹⁷ As the IC grading data is ranked data and does not conform to the Gaussian distribution and homogeneity of variance, we used rank sum test of the ranked data to compare. T-test was used to determine the percentage of apoptotic cells, because test variables were continuous and grouping variable was categorical. $P<0.05$ was set as the standard for statistical significance.

Results

Each of the two groups had 60(50%) subjects. The research group included SLE 16(26.7%), RA 25(41.7%), SS 12(20%), and systemic sclerosis 7(11.7%). There were 13(21.7%) males and 47(78.3%) females in the research group compared to 18(30.0%) males and 42(70.0%) females in the control group ($p=0.312$), whereas, the mean age was 42.21 ± 11.30 years and 43.69 ± 12.60 years, respectively ($p=0.689$). In the control group 17(28.3%) were diagnosed as dry eye. In the research group, 31(51.67%) patients were diagnosed as dry eye ($p<0.01$) (Table-1).

Table-1: Subjects' characteristics and dry eye morbidity.

	Research group (n=60)	Control group (n=60)	Tested value	P value
Age (years)	45.24 $\pm$ 15.32	43.82 $\pm$ 14.63	t =0.519	0.689*
Gender (Male/Female)	13/47	18/42	$\chi^2=1.087$	0.312**
Dry eye/No dry eye	31/29	17/43	$\chi^2=6.806$	P<0.01**

* t-test for continuous variables

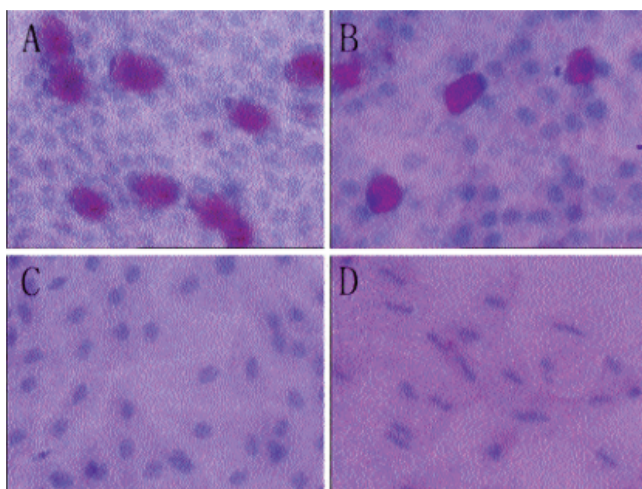
**chi-square test for categorical variables where applied

p values relate to group differences. $P<0.05$ as the standard for statistical significance.

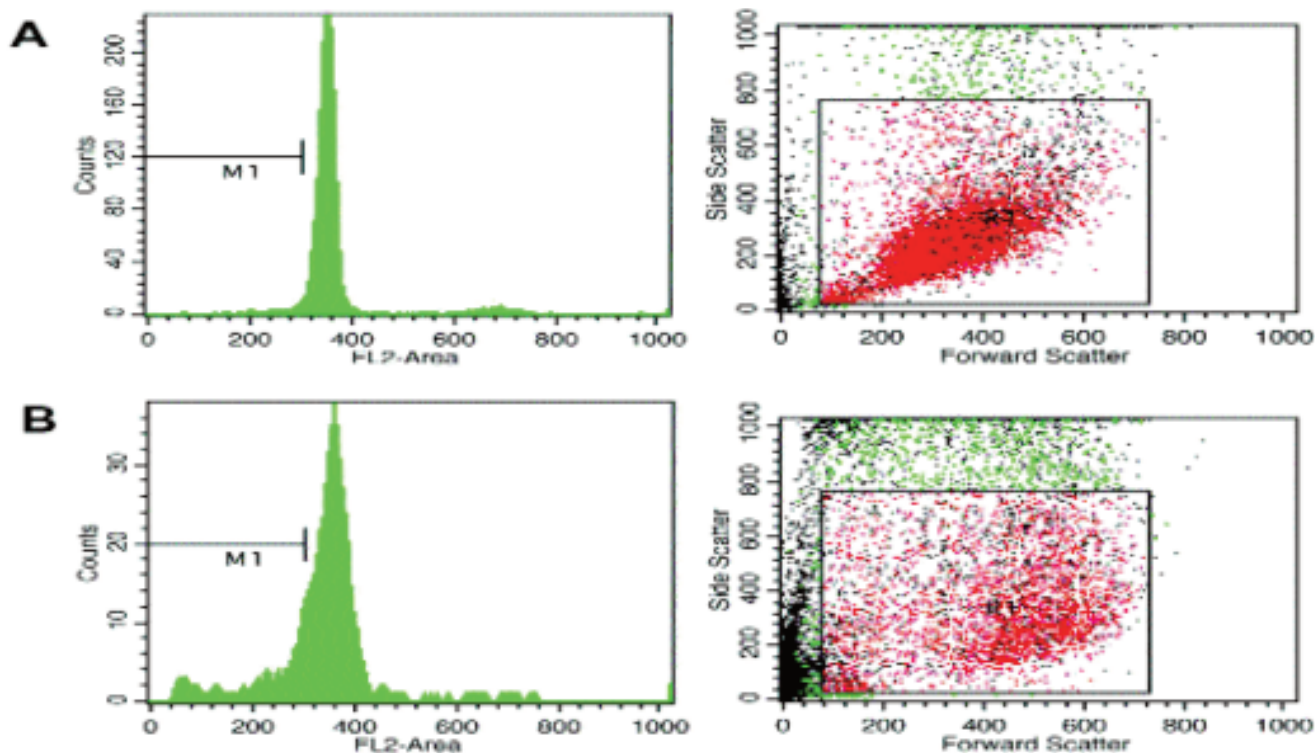
Table-2: Result of routine examinations and IC examinations.

Group	n(eyes)	routine examinations				IC Grade(eyes)			
		BUT (s)	S-I-T (mm)	FL (points)	0	I	II	III	
Control group	60	10.27±4.66	12.55±5.49	0.43±0.93	41	14	5	0	
Research group	60	7.38±6.08*	8.49±7.29*	2.50±3.09*	20	15	13	12	

*P < 0.05, compared with the control group.

**Figure-1:** Morphological examination of IC (PAS, ×400). A: Control group without dry eye. B: Research group without dry eye. C: Control group with dry eye. D: Research group with dry eye.

When the results of routine examinations for dry eye were compared between the groups, the BUT and SIT values were significantly lower in research group than in the controls ($p < 0.01$). FL staining was significantly higher in the research group ($p < 0.01$) (Table-2). When examining the conjunctival IC in all research subjects, the conjunctival epithelial cells were impressed on a filter membrane, and the goblet cells were stained red due to their glycogen granules (Figure-1 A-B). Fewer goblet cells were noted in patients with dry eye than in those without dry eye. The goblet cells were noticeably decreased or absent in patients with dry eye (Figure-1 C-D). Pyknotic nuclei and anucleated cells were only seen in the most severe dry eye. The morphological examination of the IC in subjects in both groups showed that the IC level was significantly higher in the research group than in the control group ($p < 0.01$) (Table-2).

**Figure-2:** IC apoptosis A: Patients without dry eye (3.49%), B: Patients with dry eye (20.39%).

Conjunctival epithelial cells apoptosis was detected in both groups. The amount of apoptosis was $3.29 \pm 0.25\%$ in control group without dry eye, $11.12 \pm 4.19\%$ in control group with dry eye, $4.20 \pm 1.19\%$ and $18.14 \pm 7.12\%$ in research group with and without dry eye, respectively ($p=0.986$) (Figure-2). The percentage of conjunctival epithelial cells undergoing apoptosis was higher in patients with dry eye than in patients without dry eye in each group, and among patients with dry eye, the percentage of conjunctival epithelial cells undergoing apoptosis was higher in the research group than in control group ($p<0.01$ each).

Discussion

According to the report of the International Dry Eye Research Team, the prevalence of dry eye with high morbidity is common in the Asian population.¹⁵ Due to increases in video terminal syndrome and office eye disease syndrome, the prevalence of dry eye rises annually. The morbidity of dry eye in our control group was 28.3%, which is similar to the morbidity of dry eye in normal populations in previous studies. However, for patients with systemic autoimmune disease, the morbidity of dry eye was 51.7%, which is noticeably higher than that of the control group. Regarding routine examinations for dry eye, significant differences were found between the control group and the research group, indicating that the disease state of dry eye is more serious in patients with systemic autoimmune disease than in normal populations. Due to immune system abnormalities, these patients appear to have an autoantibody that causes an abnormal immune response to auto-antigens.¹⁸ These auto-antigens can attack various organs or systems, and the eyes can become the target organ, resulting in dry eye symptoms during an immuno-inflammatory response. Numerous studies have categorised dry eye disease as an autoimmune-related inflammatory disease.¹⁹ Accumulating evidence shows that regulatory T cells are critically involved in diverse autoimmune diseases.²⁰ In many systemic autoimmune diseases, such as SLE and idiopathic SS, abnormalities in regulatory T cells occur.²¹ An abnormality in regulatory T cells also exists in patients with dry eye.²⁰⁻²² This study has confirmed that the lack of regulatory T cells can not only result in SS, but it can also be an important pathology of dry eye.²³ Our result indicates that systemic autoimmune disease and dry eye have the same pathogenesis. Systemic autoimmune disease causes changes in tear gland permeability, lymph hyperplasia abnormalities, and chronic inflammation of the glands, which can contribute to the occurrence and development of dry eye in these patients.

Some subjectivity exists in the examination used to diagnose dry eye. Large discrepancies in the objectivity and repeatability of the BUT, SIT, and FL have been reported in the literature.²⁴ Therefore, this study included the cytological examination of conjunctival IC using minimally invasive surgery to directly collect epithelial cells from the ocular surface of living patients and investigated the pathological and physiological changes in these cells based on ocular surface cytology.²⁵ The IC examinations were very objective, very rare influenced by the operation process. The number of goblet cells can indicate a lack of mucoprotein, resulting in a relatively accurate evaluation of the degree of dry eye. Our study found that, in patients who did not have sufficient symptoms to be diagnosed with dry eye upon routine examination, the IC results differed by 1 or 2 points. This result further indicates that changes in ocular surface cytology occurred prior to symptoms and signs of dry eye. This result is beneficial for the early diagnosis of dry eye. In addition to being used for glycogen staining (as described in this study), IC can also be used for the analysis of chemically stained immune tissues and protein-encoding genes, which is convenient for further research.²⁶ As shown in the literature, many immuno-inflammatory cells are present in the ocular surface conjunctiva, which results in a corresponding expression of immuno-inflammatory reactions on damaged ocular surfaces.²⁷ Consistent with this result, our research found that inflammatory cells are present in the filter membranes of some Grade III conjunctival IC. Conjunctival impression provides an easy and quick identification of the lacrymal film alterations with high specificity and sensitivity, giving valuable information about the qualitative disorder.²⁸

Apoptosis is the initiation of programmed cell death. As shown in the literature, apoptosis may induce damage of the ocular surface,²⁹ tear gland apoptosis and epithelial apoptosis on the ocular surface are abnormally increased in patients with dry eye and diabetes, which destroys the structure and function of tissues.³⁰ As shown in other studies, Fas-Fas ligand, protein kinase C delta that is correlated with abnormal expression, is found in the tear gland and blood of SS patients.³¹ This study indicates that the apoptotic process for maintaining normal physiological function in many tissues may also cause dry eye. Our test method combined the cytological examination of conjunctival IC with flow cytometry measurements of apoptosis and directly assessed the condition of epithelial apoptosis in living patients. Because endonuclease stops the DNA chain of degradation of apoptotic cells between

nucleosomes, apoptotic cells can be detected by measuring the quantity of hypodiploid DNA inside the cells. As shown by the results, the percentage of conjunctival epithelial cells undergoing apoptosis was clearly higher in patients with dry eye than in patients without dry eye, while the percentage of conjunctival epithelial cells undergoing apoptosis was higher in patients with dry eye and systemic autoimmune disease than in the control group with dry eye. The amount of apoptosis can influence the occurrence of dry eye; an increase in apoptosis can cause dry eye, and the combination of abnormal immunity and dry eye can further aggravate apoptosis. Various markers of immune activation in conjunctival epithelial cells (e.g., human leukocyte antigen — antigen D relate [HLA-DR]) have significant correlations with the expression of apoptotic factors (Fas, Fasligand and apoptotic marker AP02.7, etc.). Many studies have indicated that apoptosis on the ocular surface may activate certain signal channels, which results in an imbalance between the inducing and inhibiting factors of apoptosis. An animal model has verified that an inflammatory factor of dry eye can aggravate the apoptosis of conjunctival goblet cells by activating apoptosis channels.³²

Our study verified that dry eye morbidity is higher in patients with systemic autoimmune disease than in normal patients and confirmed, through cytological analysis of the ocular surface, that the occurrence rate of ocular surface apoptosis in patients with systemic autoimmune disease and dry eye is clearly increased. Considering that both immunity and apoptosis influence the morbidity of dry eye, conjunctival impression sampling provides a visual analysis of the shape and function of ocular surface cells and suggest that further research on the role of cytokines in immunity is needed.

Conclusion

The morbidity of dry eye is higher in subjects with systemic autoimmune disease than in healthy controls. The cell injury on the ocular surface was more serious in subjects with dry eye in systemic autoimmune disease than in subjects with dry eye in healthy controls.

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Conflict of Interest: None.

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