Asian Biomedicine Vol. 6 No. 1 February 2012; 81-85
DOI: 10.5372/1905-7415.0601.130

Brief communication (Original)

The relationship between dry eye and lactoferrin levels in tears

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Background: Dry eye is a common ophthalmic problem and lactoferrin (LF) is one of the most important components of the immune system. Preliminary findings have suggested that LF concentration in tears may be linked to the risk of dry eye.
Objective: We investigated the relationship between dry eye and lactoferrin levels in tears.
Material and methods: LF levels in the tears of 40 patients with dry eye and 35 healthy controls were measured by radial immunodiffusion assay. Statistical analysis was used to study the correlation between LF levels and results of both Schirmer's and tear film break-up time tests and the age of the subject.
Results: The concentration of LF was significantly decreased in the tears of dry eye subjects compared with control subjects ($P<0.001$). There is a positive relationship between LF in tears and results from Schirmer's and tear film break-up time tests in non-Sj□gren's syndrome ($r=0.48$ and 0.78 respectively $P<0.001$), while there is a negative relationship between LF and age ($r= -0.74$, $P<0.005$).
Conclusions: Decreased LF in tears is a factor in the pathology of dry eye. When treating non-Sj□gren's syndrome, treatment with LF could be added to artificial tear treatment.

Keywords: Dry eye, lactoferrin, non-Sj□gren's syndrome, radial immunodiffusion assay.

Dry eye is an epiocular disease caused by instability of the tear film and superficial damage of the surface of the eye, produced by abnormalities in the quality, quantity or hydrodynamics of tears. Dry eye differs from a related condition known as ophthalmoxerosis. The former gives rise to symptoms of dry eye, but does not involve damage to the superficial parts of the eye. In the latter, such damage does exist alongside dry eye symptoms. Dry eye is the more common of the two and patients complain of dry, gritty eyes as well as the sensation of the presence of a foreign body in the eye and eye strain or fatigue. The etiopathogenesis of dry eye is not clear but the condition can have a profound effect on work, study and quality of life. The National Eye Institute of America categorizes dry eye into aqueous tear deficiency and hyperevaporation types [2]. The aqueous tear deficiency type can be further divided into Sj□gren's syndrome and non-Sj□gren's syndrome. This research focused upon patients with the non-Sj□gren's syndrome type of dry eye.

Lactoferrin (LF) is a glycoprotein with a molecular weight of 70-80 kD. It is found in the milk of some mammals and in secreted fluids such as tears, semen, and synovial fluid, and in neutrophil granules. LF can prevent bacterial growth by binding to ferric ions in the bacterium and its bactericidal action is enhanced in the presence of lysozyme [3]. LF's N-terminal peptide chain can break down protein molecules which plays a role in its antibacterial action [4]. LF also stimulates immunity by combining with lipopolysaccharide components of the microbial cell wall [5]. Thus, LF is one of the most important components of the immune defense system because of its anti-infective, anti-inflammatory, and immune stimulatory functions.

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To date, there are no unified diagnostic criteria for dry eye. Diagnosis is based upon symptoms and on tests such as Schirmer's test, the tear film break-up time (BUT) test, and fluorescein chromoscopy. All these examinations have low reproducibility and are readily influenced by external factors, although they do have the benefit of being simple and economical. Da Dalt et al reported that measurement of LF in tears had a sensitivity of 72% and a specificity of 95% in diagnosis of Sjögren's syndrome. In comparison, Schirmer's test gave a sensitivity and specificity of only 64% and 85% respectively. Though the sensitivity of ferning test was higher (92%) than other detection methods, its specificity was low. Accordingly, LF detection has become part of the routine diagnosis of dry eye because of its cost effectiveness and simplicity [6].

There has only been one previous report on the variation in LF concentrations in dry eye patients' tears [1]. Most researchers use immune methods, such as immunoelectrophoresis (IE), enzyme-linked immunosorbent assay (ELISA), and radial immunodiffusion to detect LF because of their sensitivity and accuracy. Radial immunodiffusion is an in vitro ultramicro method with high sensitivity, accuracy, and specificity. It can be performed in the general laboratory and is acceptable to patients because of its convenience. In order to explore the dependence of dry eye on LF levels, we determined LF concentrations in tears of both dry eye patients and normal controls using radial immunodiffusion assay. We then considered the relationship between LF in tears and dry eye morbidity, and the significance of LF in diagnosis and treatment of dry eye.

Materials and methods

Patients

Forty subjects undergoing outpatient treatment for dry eye were included. Right eye per patient was sampled (40 eyes). The average age (±SD) of the patients was 39±13 years (range 25-70 years). Fifteen of the subjects were male, while twenty-five were female. Their main symptoms were: eye strain, the sensation of a foreign body being present in the eye, dryness, burning sensations, swelling, aching, photophobia and eye reddening. Results on Schirmer's test were <5 mm and on BUT <10 s, and the fluorescence staining of the cornea and conjunctiva were positive.

Thirty five healthy controls were selected (35 eyes). The average age (±SD) of the patients was 36±12 years (range 22 to 69 years). Sixteen of the 35 subjects were male, and 19 were female. Slit-lamp examination revealed controls to be free of ocular disease. None were wearing contact lenses, or using eye or general medications, particularly any that would affect lacrimal secretions, and none had eye-related symptoms (by patient report). All were free of general disease. Sampling was carried out in accord with the Helsinki declaration. Written informed consensus was obtained from all of the subjects. The research protocol was reviewed and approved by the ethical committees of the Affiliated First Hospital of Zhengzhou University.

Materials

^{125}I-LF was obtained from Atomic Energy Research Establishment (Peking, China), Rabbit-anti-human LF antibody, goat-anti-rabbit antibody and the radial immunodiffusion reagent kit were from Bios (Peking, China).

Methods

After stopping eye drops for two weeks, both dry eye patients and normal controls were given the following tests: Schirmer's test, BUT test, fluorescence staining of the cornea and conjunctiva.

A small amount of cooling oil was smeared on the skin of subjects' lower eyelids. A volume of 80-100 ul tears was collected from the conjunctival sac using microcapillary tubes, and separately placed in 5 ml aseptic intravenous transfusion tubes. The intravenous tubes were sealed by heating their opening, and stored at -30°C until analysis.

Ten ul calibration solution and 100 ul of rabbit-anti-human LF antibody were added to a set of tubes (S_0-S_6) containing normal saline buffer which were then placed in a 37°C water bath for 30 minutes after mixing well. A set of quality control tubes was prepared as above. Tear samples (10 ul) were then taken from the aseptic intravenous transfusion tubes and added to detector tubes. Then ^{125}I-LF (100 µl) was added to all the tubes and placed at 4°C for 24 hours after mixing. Goat-anti-rabbit antibody (0.3 ml) containing 0.05 mol/L EDTA and 100 µl normal rabbit serum, diluted to 1:400, was then added to each tube and stored at 4°C for 24 hours. Each tube was centrifuged for 20 minutes at 1800 rpm and the supernatant discarded. A γ counter was used to determine the

deposition in each tube. Thus, the LF content was calculated. With those data, a standard curve was drawn, from which the LF concentration was determined. Radial Immunodiffusion kit was the same batch number.

All data was handled by SPSS10.0 statistical soft ware. Measurement data was indicated by mean and standard deviation ($\bar{x}\pm s$) were calculated and data was also analyzed by the t-test, linear correlation and linea regression.

Results

The mean LF concentration in the experimental group was 1.10□0.79 mg/ml, compared with 1.95±0.25 mg/ml in the control group. There is a significant difference between the two groups (t=6.80, P<0.001). There were no significant differences in age and sex between the two groups (P>0.05).

There was a direct correlation between LF concentration and results of Schirmer's test (r=0.48, t=3.39, P<0.005) as can been seen in **Figure 1**. Regression equation: Y=0.91+0.07X.

There was a direct correlation between LF concentration and BUT time (r=0.78, t=7.74, P<0.005) (**Figure 2**). Regression equation: Y = 0.83+0.07X.

There was an inverse correlation between LF concentration and age (r= -0.74, t=6.88, P<0.005) (**Figure 3**). That was, LF concentration in tears decreases at a rate of 0.02 mg/ml per annum. Regression equation: Y=1.88 - 0.02X.

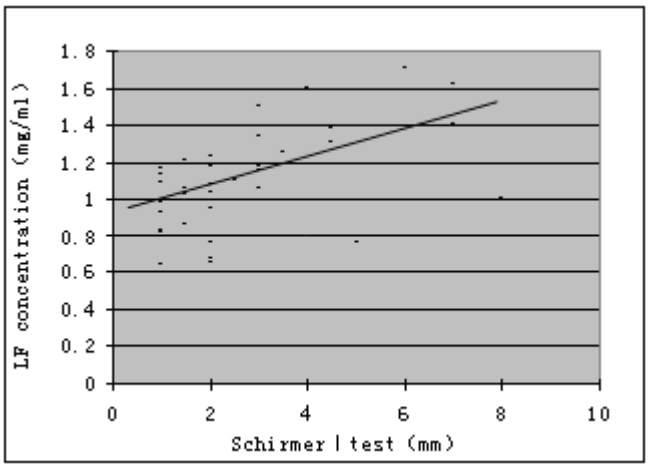

Figure 1. Relationship between LF concentration and Schirmer's test.

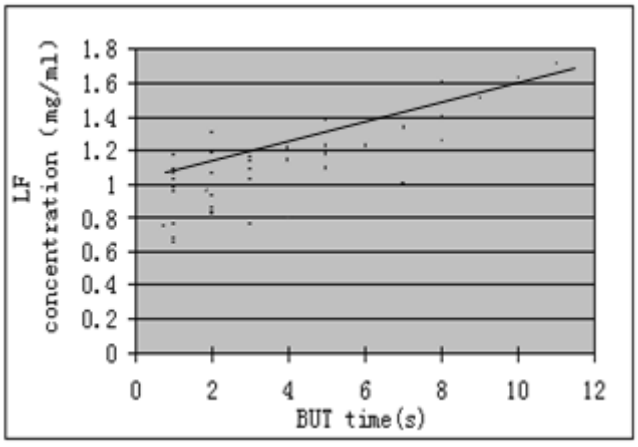

Figure 2. Relationship between LF concentration and BUT time.

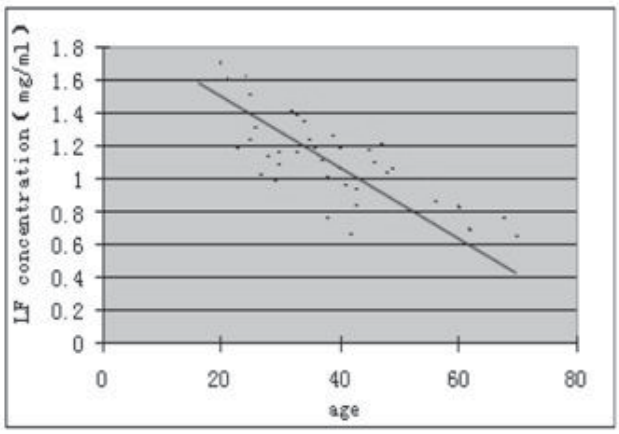

Figure 3. Relationship between LF concentration and age of subject.

Discussion

Lactoferrin concentrations in vivo vary with sites. There are at least 500 proteins in tears but LF is one of the most important, accounting for 21% of total [7]. Fullard indicated that proteins secreted by the lacrimal gland in basal tears were similar to those in reflex tears [8]. However, Stuchell et al discovered that the LF concentration of reflex tears was higher than that of basal tears [9]. This conclusion came from immunoelectrophoresis measurements. Our research was performed through radial immunoassay, which detected reflex tears of xeroma patients and those of normal controls. Values were significantly higher than those found by Janssen [10]. We attribute these differences to the use of different empirical methods and experimental conditions. We would expect LF levels to increase with an increase in reflex tears, as most LF in tears comes from lacrimal gland cells.

Schirmer's test is one of the most useful methods for studying lacrimal gland secretory function. Our research showed that both tear fluid and LF in dry eye were lower than in normal controls, and there was a direct correlation between LF concentration and Schirmer's test results in the dry eye group. When stimulated, both tears and tear proteins secreted by the lacrimal gland were correspondingly increased, which demonstrated that lacrimal gland function of non-Sj□gren's syndrome is normal. However, Tsubota had already pointed out that lacrimal gland function of non-Sj□gren's syndrome was not normal, while further investigations were needed to explain why tear and its proteins decreased [11].

The BUT test is an indicator of mucin secretion and it is also the only index to measure the stability of the lacrimal film directly. The mechanism of lacrimal film rupture is as follows. First, the lacrimal film reconstitutes when blinking. During the pause between blinks, part of the tear evaporates and part of it flows back into the fornix. With lacrimal film's thinning, lipid layer is contiguous to mucin. When lipid touches mucin's critical point, breakup appears [12].

In our research, there was a direct correlation between LF concentration and BUT time, which was consistent with Yoshiki's finding [1]. Thus, we concluded that LF is one of the most essential components of lacrimal film. We also found that the more severe the dry eye symptoms were, the lower the LF concentration of the tears. That is, LF examination is important, and can be used as an index to assess the severity of disease.

Findings on the relationship between age and LF concentrations vary. Yoshiki et al considered that age had no influence on LF concentrations in tears [1], while McGill et al found that LF values decrease linearly with age at a rate of 0.01 mg/ml per annum. The inverse correlation between LF concentration and age probably occurs because of decline in the secretory function of the lacrimal gland. After age 40, there is an obvious decrease in LF concentrations in tears, which is consistent with the findings of McGill et al [13].

Conclusion

In this research, radial immunodiffusion proved a satisfactory method for detection of LF concentrations.

Besides, detection of LF in dry eye had higher positive rate. Detection of LF concentration in tears is a feasible auxiliary method for the diagnosis of dry eye. Radial immunodiffusion can readily be applied routinely, as it is simple and easy.

Tear film is one of the most important components of the ocular defensive system. It not only lubricates the cornea and conjunctiva but provides them with nutrition to prevent drying and secondary infection. Accordingly, it is very important for visual acuity protection to preserve the quality of the tear film. Morbidity from dry eye is increasing, so it is important to have a unified approach to diagnosis and therapy. Our research suggests that bioactive tear protein could supplement artificial tears as a treatment of non-Sj□gren's syndrome dry eye.

The authors have no conflict of interest to report.

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