Research Article

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An exploratory study of neopterin and kynurenine pathway in pterygium

Abstract: Pterygium is an inflammatory, vascular and degenerative disorder with unknown aetiology. The aim of this study was to evaluate the changes in neopterin levels, reflecting T-cell immunity, and the kynurenine pathway, the main degradation process of tryptophan, in pterygium. For this purpose, neopterin concentrations were measured in serum and tear samples by enzyme-linked immunosorbent assay (ELISA) in pterygium patients (n=31) and control group (n=32). Kynurenine (KYN) and tryptophan (TRP) serum levels were simultaneously determined by high-performance liquid chromatography (HPLC) for evaluation of the kynurenine pathway. Serum neopterin concentrations and kynurenine to tryptophan ratio (KYN/TRP) as an index of tryptophan breakdown were found increased in pterygium compared to controls (p<0.05). Although there was a 3-fold difference observed between serum and tear neopterin levels, no significant relationship was found. It can be concluded that neopterin may be used as a nonspecific biomarker that reflects immunological activity in pterygium and has clinical potential for evaluation of pterygium pathogenesis. These immune- or inflammatory-mediated changes were also supported by an increased KYN/TRP ratio in pterygium patients.

Keywords: Ocular pathology; Tryptophan; Tear; Indoleamine 2,3-dioxygenase; Eye disease.

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Introduction

Pterygium is an inflammatory, fibrovascular and degenerative ocular disease. The disease has taken its name from the Greek word 'pterygion', meaning 'small wing' due to the triangular wing-shaped growth of conjunctival tissue on the cornea [1, 2]. In pterygium inflammation, uncontrolled cell division and angiogenesis are observed together which refer to neoplasia; however, pterygium is considered as neither a cancer, nor an inflammatory disease. According to some researchers, pterygium can be defined as a "neoplastic-like growth disorder" [3-5]. It is known that factors such as ultraviolet (UV) radiation, p53 tumour suppressor gene, immunologic and inflammatory mechanisms play a role in the pathogenesis, but its aetiology remains unclear [6-8]. The general approach in pterygium treatment is the removal of the tissue growth from the eye, but frequent recurrence rates of pterygium indicate that surgical treatment is not a permanent solution. Despite being a widely known disease for thousands of years, the uncertainty of its aetiology and high recurrence rates reveals the need for innovative biomarkers such as neopterin in clinical practice. It is known that neopterin is an unconjugated pteridine which is synthesized by guanosine triphosphate (GTP) via GTP cyclohydrolase I (GTP-CH-I) enzyme in stimulated monocytes and macrophages. Since the activity of the enzyme GTP-CH-I is increased by interferon-gamma (IFN-ɣ) stimulation, neopterin concentration is considered to be an important biomarker of immune activation and also inflammation. It expresses cellular immunity and is considered to be an important biomarker in the early diagnosis and prognosis of various pathologies [9, 10]. Another indicator that reflects cellular immunity is the enzyme indoleamine 2,3-dioxygenase (IDO), which plays a role in the kynurenine pathway leading to the formation of kynurenine (KYN) by consuming tryptophan (TRP) in the tissues [11, 12]. IDO-mediated tryptophan degradation functions as a defence mechanism that prevents the growth of malignant cells and pathogens by consuming tryptophan, an essential amino acid they require. Both increased KYN/TRP ratio and neopterin levels are the

parameters triggered by IFN-ɣ stimulation and used for assessment of immune system activation. It has been reported that elevated neopterin levels in pathologies such as infections, autoimmune diseases, cancers and graft versus host disorders are directly related to increased KYN/TRP ratio [13-16].

The aim of this study was to evaluate the possible role of neopterin and kynurenine pathways in pterygium disease. For this purpose, neopterin, tryptophan and kynurenine concentrations were measured in pterygium patients and healthy controls. The immunological activation status in pterygium was evaluated; serum and tear samples were used for systemic and local evaluation of immune activation, respectively.

Materials and methods

Subjects and samples

Only patients over the age of 18 with pterygium diagnosis confirmed by an ophthalmologist were included in this study as the pterygium group. The control group consisted of healthy subjects. Volunteers with any acute or systemic infections, malignant diseases or autoimmune diseases were not included in the study. The groups consisted of 31 pterygium patients (18 females, 13 males) and 32 healthy subjects (17 females, 15 males). Mean age (± SD) of the pterygium patients was 50.5 ± 14.2 years (range: 21–65 years), and control group was 34.2 ± 8.8 years (range: 20–54 years).

The biological samples were collected early morning to eliminate circadian rhythm variability. Blood samples were drawn into laboratory tubes (ISOLAB Laborgeräte GmbH, Eschau, Germany) and centrifuged at 1500 g for 10 minutes to separate serum fractions. Tear samples were collected using a capillary tube from the 1/3 lateral lid margin tear meniscus of the eye with the pterygium. No anaesthesia or invasive procedures were performed to collect tear samples. Tear samples of the control group were always collected from the right eye to provide standardisation. No pre-treatment was applied to the tear samples. All serum and tear samples were kept away from direct UV exposure and stored at –20°C until required.

Ethical approval: The research related to human use has been complied with all the relevant national regulations, institutional policies and in accordance the tenets of the Helsinki Declaration, and has been approved by the local ethics committee of the University (#GO 17/435-17/May 16th, 2017).

Informed consent: Informed consent has been obtained from all individuals included in this study

Measurements

Serum and tear neopterin concentrations were determined by enzyme-linked immunosorbent assay (ELISA) kits obtained from IBL (Hamburg, Germany) and performed in strict accordance to the manufacturer's instructions. The optical density was measured by using an ELISA microplate reader (Spectra Max M2, Molecular Devices Corporation, Sunnyvale, CA). The neopterin levels were expressed as nanomole per litre (nmol/L).

Tryptophan and kynurenine concentrations in serum samples were simultaneously measured by high-performance liquid chromatography (HPLC, HP Agilent 1100, Vienna, Austria) as described in the previous studies [17, 18]. Briefly, samples were passed through a C18, ODS column (250 × 4.6 mm, 5 μm) with a dihydrogen phosphate buffer. Tryptophan and kynurenine were measured by fluorescence (excitation 286 nm, emission 366 nm) and UV detectors (at 360 nm), respectively. The tryptophan and kynurenine concentrations were both expressed as micromole per litre (μmol/L). Calculation of the kynurenine to tryptophan ratio (KYN/TRP), as an index of tryptophan breakdown and reflection of the activity of IDO, was expressed as μmol/mmol.

Statistical analysis

All results were expressed as mean ± standard error of the mean (SEM). Mann Whitney U test was used for statistical comparisons between study groups. Correlations between the parameters were evaluated by Spearman's rank correlation (Rs) analysis. P-values <0.05 were considered statistically significant.

Results

The results were summarised in Table 1. Serum neopterin levels, serum kynurenine concentrations and KYN/TRP ratios were significantly increased in pterygium patients compared to the control group (all, p<0.05).

Tear neopterin levels in control subjects were higher than the pterygium patients. However, the difference was not significant (p>0.05). There was also no significant difference in tryptophan levels between the study groups (p>0.05).

Table 1: Neopterin, tryptophan and kynurenine levels and KYN/TRP ratios from control and pterygium patient groups.

Parameters	Controls (n=32) Mean ± SEM Range Median	Pterygium patients (n=31) Mean ± SEM Range Median
Neopterin [nmol/L]		
Serum	6.52 ± 0.50 2.75 – 13.99 6.22	8.57 ± 0.41* 4.02 – 14.76 7.85
Tear	3.60 ± 0.46 0.49 – 10.82 3.17	3.24 ± 0.43 0.79 – 9.50 2.35
Tryptophan [µmol/L]	65.60 ± 2.08 35.86 – 81.94 65.66	65.44 ± 1.56 42.33 – 86.54 65.43
Kynurenine [µmol/L]	2.23 ± 0.12 1.14 – 3.97 2.03	2.75 ± 0.09* 1.89 – 3.80 2.61
KYN/TRP [µmol/mmol]	34.54 ± 1.89 20.92 – 63.25 32.65	42.73 ± 1.58* 25.35 – 57.98 41.66

*Significantly higher than control group, $p < 0.05$.

Table 2: Correlation between the parameters in study groups.

		Kynurenine	KYN/TRP	Neopterin Tear
Controls (n=32)	**Neopterin**			
	Serum	Rs=0.448 p<0.05	Rs=0.447 p<0.05	Rs=0.227 p>0.05
	Tear	Rs=0.087 p>0.05	Rs=− 0.071 p>0.05	
Pterygium patients (n=31)	**Neopterin**			
	Serum	Rs=0.576 p<0.05	Rs=0.477 p<0.05	Rs=− 0.017 p>0.05
	Tear	Rs=0.219 p>0.05	Rs=0.244 p>0.05	

The correlations of the measured parameters were shown in Table 2. Serum neopterin levels were found to be approximately three times higher than tear neopterin levels in pterygium patients. Despite the correlation between serum and tear neopterin levels, this was not statistically significant (p>0.05).

Similarly, in the control group, there was no correlation in tear and serum neopterin levels; while serum neopterin
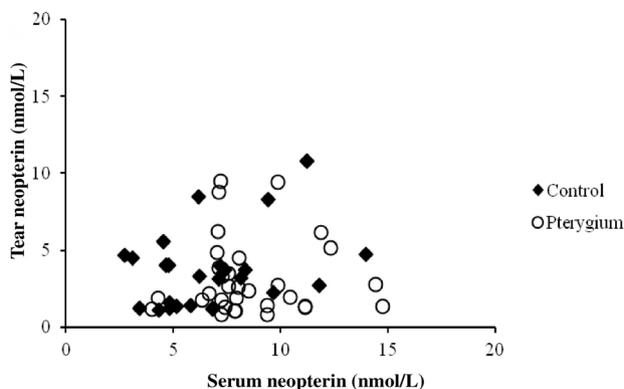

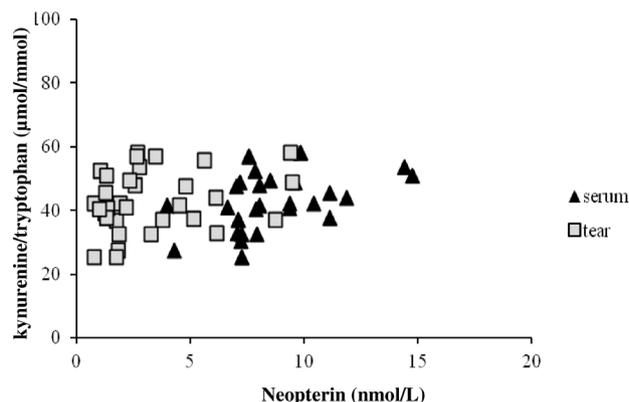**Figure 1:** Correlation between serum and tear neopterin levels.

Figure 2: Correlation between kynurenine/tryptophan ratio and neopterin levels in pterygium patients.

levels were approximately twice as high in serum than in tears. The correlation between serum and tear neopterin levels were shown in Figure 1.

A significant positive correlation existed between serum levels of neopterin and KYN/TRP (p<0.05); however, no correlation was found between serum KYN/TRP ratio and tear neopterin levels (p>0.05). These correlations were shown in Figure 2.

Discussion

Inflammatory molecules such as IFN-ɣ, induces IDO expression and neopterin biosynthesis. Together, evaluation of neopterin and IDO provides a better understanding of the immunological status of the organism [13, 19, 20]. The aim of this study was to investigate the role of neopterin and kynurenine pathway in the pathogenesis of pterygium with an immunologic and/or inflammatory point of view.

There are few studies in which neopterin levels and kynurenine pathway are evaluated in eye diseases; such as the study performed by Palmer et al. [21]. They reported increased urine neopterin levels in retinal vasculitis, which is an inflammatory eye disease. In cataract, it was reported that there was a positive correlation between neopterin and kynurenine levels in serum samples [22]. This is the first study to determine serum and tear neopterin levels and serum tryptophan degradation in pterygium disease. In the present study, it was shown that increased serum neopterin concentrations in pterygium disease and neopterin concentrations were correlated with KYN/TRP ratios, which reflect IDO activity in pterygium. These results are parallel with previous studies in other eye disorders and similar to the reports in patients with uveitis [18, 21-23].

Analysis of serum and tears were used for comparison of systemic and local immunologic state in pterygium, respectively. Neopterin concentrations were elevated in tears, a valuable biological fluid for analyses due to the small volumes necessary to collect in comparison to blood or urine. According to the results, tear neopterin levels of patients and healthy subjects were not significantly different. In contrast to serum neopterin, tear neopterin did not increase with KYN/TRP ratio. The results show that neopterin and IDO activation, which reflects the degree of cellular immune activation, was elevated in pterygium, but this activation can be expressed systemically, not locally. Although pterygium is a local disease, the results reflect systemic immunity rather than local immunity in our study.

In our previous study [24], it was reported that serum neopterin and tear neopterin levels were significantly positively correlated in healthy subjects, and neopterin levels in serum were approximately 3-fold higher than in tears. On the other hand, in this present study, there was no significant correlation between serum and tear neopterin levels in either patients or healthy subjects. An approximate 3-fold difference was found between serum and tear neopterin. Tears are an unfamiliar biological sample for most assays, the components of tears and its secretion are changed in pterygium. Therefore, tear and serum levels may not correlate in this study and do not reflect each other exactly. Besides tears, other local biological fluids like aqueous humour and vitreous humour should be evaluated as further evaluations in pterygium; but it should be noted that these methods are invasive.

The most important problem with pterygium is recurrence. Therefore, the success of the pterygium treatment is expressed by the low recurrence rates.

As a T-cell immunity biomarker, neopterin levels may contribute to the prediction of pterygium progression. Also, considering the uncertain mechanisms of pterygium, neopterin can be expressed as a biomarker in the aetiology evaluation, and this study may contribute to the literature in terms of the immune and inflammatory mechanisms of pterygium.

A limitation of this study was the lack of individual evaluation of environmental factors such as sunlight exposure of volunteers, which is very effective in pterygium. A further study involving the assessment of risk factors would be more extensive.

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