

ORIGINAL ARTICLE

Comparison of the Diagnostic Power of Serum IL-6, IL-8 and TNF- α for the Idiopathic Anterior Uveitis in Children

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SUMMARY

Background: Uveitis is the inflammation of the uvea that often occurs in children. There are many causes of disease, but some of them do not have any reasons and are then called idiopathic uveitis. Cytokines play an important role in the regulation of the immune response. Determination of cytokine profiles could contribute to the explanation of the etiology of uveitis and could serve to evaluate the inflammation intensity as well as be helpful in the early diagnosis of this disease. The purpose of this study was to determine the serum level of selected inflammatory cytokines, such as interleukin-6 (IL-6), interleukin-8 (IL-8), and tumor necrosis factor α (TNF- α) and to compare their diagnostic power as a markers of idiopathic anterior uveitis in children.

Methods: The study was carried out on 28 children diagnosed with idiopathic anterior uveitis. The reference group comprised 30 healthy children. Serum IL-6, IL-8, and TNF- α concentrations were measured with specific enzyme-linked immunoassay (ELISA) methods.

Results: The mean values of IL-6, IL-8, and TNF- α in the children with idiopathic anterior uveitis were significantly higher than those found in the reference group. The highest sensitivity, specificity, accuracy, positive and negative predictive value, and likelihood ratio of a positive test result were achieved for IL-8. There was a significant difference between the area under the curve for IL-6 and IL-8.

Conclusions: Increased serum concentrations of interleukin IL-6, IL-8, and TNF- α may suggest that these cytokines induce inflammatory changes in the ocular surface. Analysis of cytokine levels showed that IL-8 has the highest diagnostic power and is the best marker for diagnosis of idiopathic anterior uveitis in children.

(Clin. Lab. 2017;63:xx-xx. DOI: 10.7754/Clin.Lab.2017.170629)

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KEY WORDS

inflammatory cytokines, idiopathic anterior uveitis

INTRODUCTION

Uveitis is the most common type of intraocular inflammation and main cause of visual morbidity. It usually develops as an isolated sickness, but can be linked with many other medical conditions. There are various causes leading to its development including systemic autoimmune disorders and infection, but some of the cases do not have any reasons (idiopathic uveitis). Immune-mediated systemic diseases include Behcet's disease, Vogt-Koyanagi-Harada disease, sarcoidosis and many others. Infectious causes include toxoplasmosis,

syphilis, brucellosis, and toxocariasis. So, the term *uveitis* is a general name and refers to inflammation of different etiologies. An ocular immune system exists in the human eye, its disturbance and resulting uncontrolled immune response may lead to inflammation and eyeball destruction. Inflammation is one of the earliest responses of the immune system to local injury or infection. Non-infectious or immune-mediated uveitis is caused by inflammatory factors in which cytokines play an important role. In the normal eye, cytokines take part in the regulation of the immune response and protect the eye from potential harmful microorganisms and non-infectious factors [1]. The following cytokines: interleukin-1 β (IL-1 β), IL-2, IL-4, IL-5, IL-6, IL-10, interferon- γ (IFN- γ), and tumor necrosis factor- α (TNF- α) were found in the ocular fluid or tissues in the inflamed eye [2,3]. Despite extensive studies, the etiology of uveitis is still unclear. According to most authors, uveitis is a T-cell mediated disease, and both T helper 1 (Th1) and Th17 cells play an important role in the pathogenesis of this sickness. It is believed that the onset of the sickness is associated with an impaired ocular immune system, and the illness is a result of inflammation and damage of the eyeball. A significant role has been attributed to pro- and anti-inflammatory cytokines namely IL-6, IL-8, IL-10, IL-15, IL-17, INF- γ and TNF- α [1,4,5]. An increased level of pro-inflammatory cytokines may cause recruitment of inflammatory cells, which can enhance the passage from the retina vessels and aggravate inflammation [3]. These cytokines elevate adhesion of the circulating lymphocytes to endothelial cells and thus play a major role in the breakdown of the blood-retina barrier [6]. They cause local tissue damage either through a direct cytotoxic effect or indirectly via chemotaxis of the cell with phagocytic properties [7]. The purpose of this study was to examine the serum level of selected inflammatory cytokines: IL-6, IL-8, and TNF- α and to assess their diagnostic power as markers of idiopathic anterior uveitis in children.

MATERIALS AND METHODS

Study subjects

The study group consisted of 28 children (aged 10 - 18) diagnosed with idiopathic anterior uveitis who attended to the Department of Pediatric Ophthalmology with Squint Treatment Unit at Medical University of Białystok Children's Clinical Hospital. The patients did not have concomitant systemic diseases and did not receive any treatment that alters cytokine expression, such as local, periocular or systemic immunomodulatory therapy. A diagnosis of uveitis was made according to the combination of clinical, instrumental, and laboratory tests [8]. The reference group (RG) comprised 30 healthy children of both genders from 9 to 18 years. Children were clinically free from infection at the time of surgery. The study was approved by the local Bioethics Committee at the Medical University of Białystok

(Approval No. R-I 002/249/2008).

Blood sampling

Blood samples were taken from a peripheral vein, on admission, before treatment. The serum was separated by centrifugation at 1,500 x g for 10 minutes at room temperature, collected, and stored at -80°C until analysis.

Laboratory tests

The level of IL-6, IL-8, TNF- α were determined using commercial enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN, USA). In this technique, monoclonal antibodies specific for IL-6, IL-8, and TNF- α have been pre-coated onto the wells of 96-well microplates provided in these sets. Next, standards and patient samples were pipetted into the wells and any cytokines present were bound by the immobilized antibodies. After a first incubation and washing away any unbound substances, enzyme-linked polyclonal antibodies (conjugate) specific for IL-6, IL-8, and TNF- α were added to the wells. After a second incubation and washing to remove any unbound antibody-enzyme reagent, a substrate solution (hydrogen peroxide and chromogen) was added to the wells. The enzyme acted on a substrate solution to produce color in proportion to the amount of cytokine present. The intensity of this colored product was directly proportional to the concentration of cytokines in the patient specimens. Readings were performed using an ELISA microplate reader (ANTHOS, Australia) at an absorbance value of 450 nm. The results were calculated based on the standard curve and expressed in pg/mL. The minimum detectable dose of IL-6 was less than 0.70 pg/mL, < 3.5 pg/mL for IL-8, and < 1.6 pg/mL for TNF- α .

Statistical analysis

The results were given as mean and standard deviation. To compare differences between two independent groups, the Mann-Whitney *U* test was used. The results were considered to be statistically significant when *p*-values were less than 0.05. To calculate the diagnostic accuracy of cytokines the ROC curve was used. Diagnostic sensitivity, specificity, positive (PPV) and negative predictive values (NPV) were counted using the following cutoff points (97.5th percentile in the reference group): 3.62 pg/mL, 9.92 pg/mL, and 2.50 pg/mL for IL-6, IL-8, and TNF- α , respectively. The likelihood ratio for positive test result (LR+) was calculated as the sensitivity divided by 1 minus specificity, and the likelihood ratio for negative test result as 1 minus sensitivity divided by specificity.

RESULTS

Interleukin results

The mean values of IL-6, IL-8, and TNF- α in the serum of children with uveitis were found to be significantly higher when compared to the reference group as follows: 5.27 ± 8.37 pg/mL vs. 1.19 ± 0.88 pg/mL, $p = 0.024$, 15.04 ± 12.55 pg/mL vs. 6.45 ± 1.50 pg/mL, $p = 0.003$, and 2.81 ± 2.95 pg/mL vs. 1.19 ± 0.58 pg/mL, $p = 0.013$, respectively (Table 1, Figure 1, Figure 2, Figure 3).

ROC curves

Among cytokines, the highest sensitivity (53.6%), specificity (95.5%), accuracy (72.0%), positive predictive value (93.8%), negative predictive value (61.8%), and likelihood ratio of a positive test result (11.79) were obtained for IL-8 (Table 2). ROC analysis revealed that the area under curves (AUCs) for cytokines were as follows: for IL-6 - 0.644, IL-8 - 0.858, and TNF- α - 0.676 (Figure 4). There was a significant difference between the AUC for IL-6 and IL-8 (0.644 vs. 0.858; $p = 0.011$).

DISCUSSION

Uveitis is the inflammation of the uvea. It is an important cause of blindness in children in the developed world [9]. The risk of blurry vision or blindness in children may be greater than in adults because of delay in diagnosis (uveitis in children is often asymptomatic), established ocular pathology, and inadequate treatment. Due to the prolonged duration and increased risks of complications, this can lead to significant ocular morbidity and severe vision loss in 25 - 33% of cases [9, 10]. Pediatric uveitis accounts for 5 - 10% of all uveitis [11]. Anterior uveitis is the most common type of uveitis in children and chronic uveitis is more common in children compared to acute [12]. With regard to the etiology of uveitis, we have more than 85 different causes, but infections and autoimmune causes are the most common of them [13]. Among infectious causes are viral, parasitic, bacterial, spirochaetal, and others. In the group of autoimmune causes, juvenile idiopathic arthritis (JIA) and pars planitis were the most common reason of anterior, intermediate, and posterior uveitis and other rare causes were sarcoidosis, inflammatory bowel disease, Vogt-Koyanagi-Harada syndrome, Behcet's syndrome, Blau syndrome, and TINU (tubulointerstitial nephritis uveitis) syndrome [14,15]. The most common form of uveitis is idiopathic anterior uveitis [16].

The etiology of uveitis is still unclear. It has long been believed that the cell-mediated response with autoreactive effector CD4+ T lymphocytes and various cytokines released by inflammatory mediators play a key role in the pathogenesis of this disease [3,6,7,17]. Cytokines play an important role in maintaining lymphocyte homeostasis. In normal conditions, they take part in the regulation of the immune response and protect the eye

from infections and non-infections factors [1]. The spectrum of cytokines has been studied in uveitis, with reports of increased levels of inflammatory cytokines and decreased concentrations of regulatory cytokines over recent years [7,18,19]. Recent studies have identified various endogenous immune mechanisms and genetic factors that are involved in the pathogenesis of uveitis [20-22]. Determination of cytokine profiles could contribute to the explanation of the etiology of uveitis in children and could be helpful in the diagnosis of this disease.

The aim of this study was to determine the serum concentration of three cytokines: IL-6, IL-8, and TNF- α and to compare their diagnostic power as markers of idiopathic anterior uveitis in children. It is known that IL-6 is a multi-functional cytokine, which can be expressed by a wide variety of cell types, including T-cells [23]. It is a critical factor for Th 17 differentiation and a critical mediator for induction of inflammation [24]. This interleukin is a major regulator of the acute phase response. In uveitis patients, increased concentrations of IL-6 were shown in the aqueous or vitreous humor [19,24, 25]. The largest increase in IL-6 and TNF- α has been found in idiopathic uveitis and Behcet's disease [26-29]. In turn, IL-8 is a chemokine with a profound effect on neutrophils after secretion by lipopolysaccharide-stimulated monocytes. It acts chemotactic for T lymphocytes and can induce the surface to express adhesion molecules [30,31]. IL-8 participates in the pathogenesis of clinical uveitis and neutrophil attraction and activation may be involved in the process of tissue destruction in ocular inflammation [3]. In the inflamed peripheral retina and vitreous humor, the cells would produce a large amount of IL-8 that may be detectable in the blood. Increased IL-8 has been documented in experimental autoimmune anterior uveitis but without mRNA expression on T cells for its receptors CXCR1 and CXCR2 [3]. Raised expression of IL-8 was also found in patients with Behcet's disease [27]. Tumor necrosis factor (TNF- α) is a mediator of inflammation that plays a key role in the early stage of infections induced by bacteria, viruses, and parasites [3,32,33]. Increased synthesis of TNF- α is also observed in many autoimmune disorders, including uveitis, which is associated with considerable tissue damage.

We have found increased levels of IL-6, IL-8, and TNF- α in children with idiopathic anterior uveitis in comparison with the reference group. The concentrations of cytokines in our study are comparable to those reported by other authors [5,18,34,35]. Kramer et al. showed significantly elevated serum levels of IL-6 and IL-8 in patients with active noninfectious uveitis and decreased concentrations of these interleukins during uveitis in remission compared to the controls [35]. They concluded that interleukin IL-6 takes part in modulation of inflammation in the chronic uveitis, and the parallel increase in IL-8 may suggest innate immune mechanisms in the acute disease. No consistent pattern was seen for TNF- α [35]. De Boer et al. studied IL-8 which appears to be a

Table 1. The results of cytokine concentrations in the study and reference groups.

| Group | Interleukin-6 (pg/mL) | Interleukin-8 (pg/mL) | Tumor necrosis factor- α (pg/mL) |
|--------------------|--------------------------------|----------------------------------|---|
| Reference (n = 30) | 1.19 \pm 0.88 | 6.45 \pm 1.50 | 1.19 \pm 0.58 |
| Study (n = 28) | 5.27 \pm 8.37 p = 0.024 * | 15.04 \pm 12.55 p = 0.003 * | 2.81 \pm 2.95 p = 0.013 * |

Data are reported as mean and standard deviation. * - p < 0.05 the differences between study and reference group (Mann-Whitney U test).

Table 2. Diagnostic usefulness of cytokine concentrations in children with uveitis.

| Cytokine | Cutoff (pg/mL) | Sensitivity (%) | Specificity (%) | ACC (%) | PPV (%) | NPV (%) | LR+ | LR- |
|---------------|----------------|-----------------|-----------------|---------|---------|---------|-------|------|
| IL-6 | 3.62 | 35.7 | 95.7 | 62.7 | 90.9 | 55.0 | 8.21 | 0.67 |
| IL-8 | 9.92 | 53.6 | 95.5 | 72.0 | 93.8 | 61.8 | 11.79 | 0.49 |
| TNF- α | 2.50 | 32.1 | 91.3 | 58.6 | 81.8 | 52.5 | 3.70 | 0.74 |

Abbreviations: IL-6 - interleukin 6, IL-8 - interleukin 8, TNF- α - tumor necrosis factor α , ACC - accuracy, PPV - positive predictive value, NPV - negative predictive value, LR - likelihood ratio.

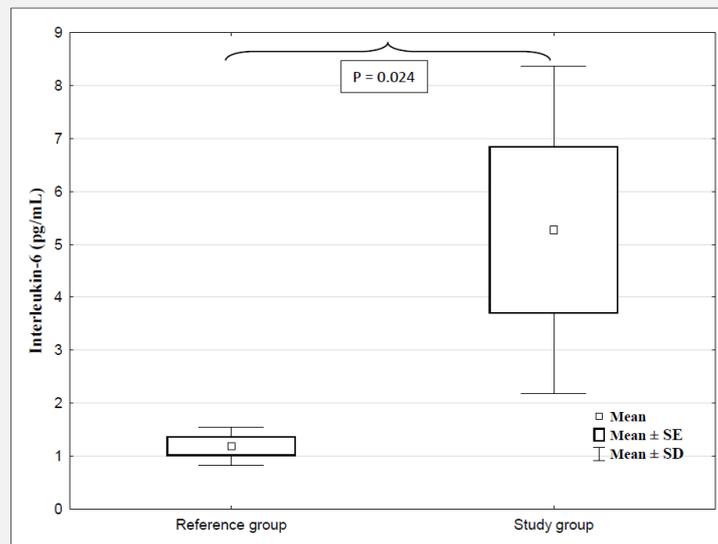


Figure 1. The serum concentration of IL-6 in children with uveitis and reference group.

strong chemoattractant and activator for neutrophils [18]. They observed increased level of this cytokine in vitreous fluid (median: 33 pg/mL) in 45% of patients with uveitis and in 26% with non-inflammatory eye dis-

ease (median: 25 pg/mL) and suggested that IL-8 participates in the pathogenesis of clinical uveitis and that neutrophil attraction and activation may be involved in the process of tissue destruction in ocular inflammation.

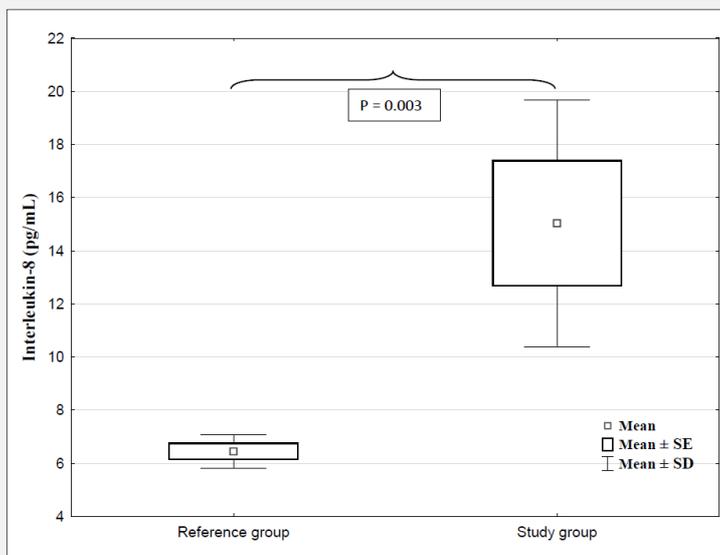


Figure 2. The serum concentration of IL-8 in children with uveitis and reference group.

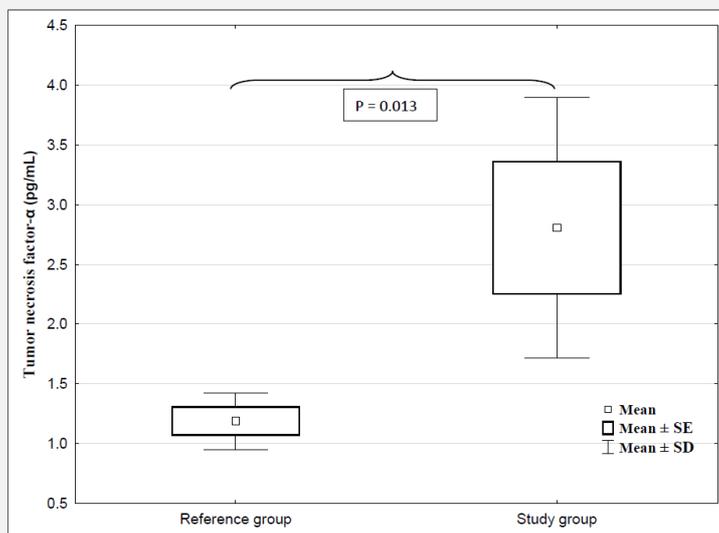


Figure 3. The serum concentration of TNF-α in children with uveitis and reference group.

In the serum, IL-8 concentration was raised in 30% of patients with non-inflammatory eye disease, and was 42 pg/mL. This value was about 3-fold higher than that in our studies, but our method for the determination of

IL-8 was nearly 3 times more sensitive than that one (detection limit: 3.5 pg/mL vs. 10 pg/mL). It is interesting that increased IL-8 levels were always accompanied by increased IL-6 levels but not vice versa and IL-6 lev-

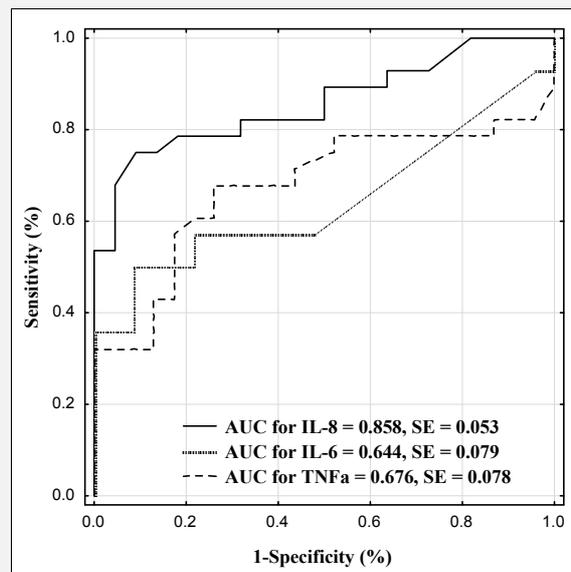


Figure 4. The ROC curve for cytokine concentrations in children with uveitis.

els were more frequently increased than IL-8 concentrations [18]. In turn, we observed that IL-8 and IL-6 were both elevated in about 54%, but IL-8 concentrations were more frequently elevated than IL-6 levels. Klock et al. showed an increase in serum IL-8 levels in patients with idiopathic intermediate uveitis, sarcoid uveitis, and with HLA-B27 associated acute anterior uveitis [34]. The levels of circulating IL-8 were different and medians were as follows 700, 1,600, and 2,914 pg/mL, respectively. They found that elevated serum IL-8 concentrations were related with disease activity in idiopathic intermediate uveitis and could be a marker of developing systemic disease [34]. Sijssens et al. were the first authors who analyzed a wide profile of 16 immune mediators: intraocular cytokines, chemokines, and soluble adhesion molecules in aqueous humor of 25 children with uveitis [5]. They found an increase in the levels of cytokines IL-6, IL-8, IL-10, and TNF- α in the study group compared with controls [5]. The observation that higher levels of cytokines were detected in active uveitis and patients treated with methotrexate compared with samples collected during ocular surgery (quiet uveitis) and not treated with methotrexate might implicate that these cytokines are mainly involved in active uveitis. The results may suggest uvea degradation and cytokine migration from the eye to the blood vessels [5]. In our study, the most significant changes in the serum cytokine level profiles were observed for IL-6 (more than 4-fold increase in concentration), followed by IL-8, and TNF- α (more than 2-fold increase). The increasing levels of these cytokines may lead to the adhesion of

neutrophils to endothelium and thus contribute markedly to the breakdown of the blood-retina barrier *via* their degranulation. The frequency of cytokine results exceeding the mean value of the reference group was highest for IL-8 and was approximately 90%, and further diminished to 75% for TNF- α and about 57% for IL-6. Taking into account the alterations of areas under the ROC curves (AUCs), cytokines were placed in the downgrade scale as follows: IL-8 (0.858), TNF- α (0.676), and IL-6 (0.644). There was a significant difference between the AUC for IL-6 and IL-8. These results indicate that the IL-8 has the highest diagnostic power for the diagnosis of idiopathic anterior uveitis in children.

CONCLUSION

In conclusion, increased serum concentrations of interleukin IL-6, IL-8, and TNF- α may suggest that these cytokines induce inflammatory alterations in the ocular surface. Analysis of cytokine levels showed that IL-8 has the highest diagnostic accuracy and is the best marker for diagnosis of idiopathic anterior uveitis in children.

Declaration of Interest:

The authors declare that they have no conflict of interest.

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