Efficacy of Corticosteroids in the Treatment of Autoimmune Uveitis and its Effect on T Helper Cell and S100 Protein Levels in Peripheral Blood

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To explore the efficacy of glucocorticoids in treating uveitis and their effect on T helper cell and S100 protein levels in peripheral blood. Sixty patients with uveitis were included in the uveitis group and given glucocorticoid therapy. The curative effect was evaluated after treatment. In addition, 60 healthy subjects were included in the non-uveitis group. The expression levels of T helper cell and S100 protein (S100A12, S100A8/A9) in peripheral blood of non-uveitis group and uveitis group were measured and compared, and the correlation between expression levels and clinical data was analyzed. After treatment of 60 patients with uveitis, 40 experienced complete recovery, 18 showed positive response to the treatment, while 2 cases did not respond effectively. The effective rate was 96.67 %. Before treatment, the T helper 1, T helper 17, S100A12, and S100A8/A9 levels were significantly higher in the uveitis group than in the non-uveal inflammation group ($p<0.05$). After treatment, T helper 1, T helper 17, S100A12, S100A8/A9 levels decreased significantly in the uveitis group ($p<0.05$) and approached the non-uveal inflammation group ($p>0.05$). The t-test showed that T helper 1, T helper 17, S100A12, S100A8/A9 levels were significantly associated with uveitis patients ($p<0.05$), and T helper 1, T helper 17, S100A12, S100A8/A9 levels were significantly higher in active patients. Glucocorticoid has good effect on uveitis. T helper 1, T helper 17, S100A12, S100A8/A9 and uveitis have a certain correlation, the level of T helper 1, T helper 17, S100A12, S100A8/A9 also decreased after treatment, which is a potential biomarker of uveitis.

Key words: Uveitis, immunodeficiency, T helper 1, T helper 17, retina, inflammation

Uveitis is a common eye disease, referring to the inflammation occurring in the tissues of the iris, ciliary body, choroid, vitreous, retina, and retinal vessels[1]. Initial symptoms of uveitis are mild and may manifest as eye pain, light sensitivity, tearing, and blurred vision. As the condition progresses, intraocular structures will gradually be damaged by inflammation, leading to visual impairment and even blindness. Therefore, early detection and timely treatment are particularly crucial[2]. Glucocorticoids are the first-line medications for treating uveitis, preventing severe visual loss and significantly improving visual outcomes[3]. However, their use is limited by side effects, such as diabetes, gastrointestinal ulcers, Cushing's syndrome, hypertension, and osteoporosis[4]. Most uveitis cases are autoimmune diseases primarily mediated, developed, and progressed by Clusters of Differentiation (CD) 4 T helper cells (Th cells). Th cells can be classified into different subtypes based on the types of cytokines they secrete. In the past decade, research on pathogenic T cells has mainly focused on Th1 and Th17 cell subtypes[5,6]. In addition, due to the inflammatory nature of uveitis, S100 proteins serve as important biomarkers for measuring intraocular inflammation. In recent years, several studies abroad have reported elevated levels of S100 proteins in uveitis patients[7]. Biomarkers for uveitis contribute to early clinical diagnosis and treatment monitoring, holding significant value in clinical practice. However, there is limited reporting on this topic in China. Therefore, we investigate the efficacy of glucocorticoid therapy for uveitis and its impact on peripheral blood helper T cells and S100 protein levels, aiming to provide a theoretical basis for clinical applications. Included 60 patients

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diagnosed with uveitis who sought medical attention at the First Affiliated Hospital of Huzhou University between October 2021 and December 2022, forming the uveitis group. In inclusion criteria, the patients diagnosed with uveitis by ophthalmologists according to the American SUN criteria; non-immunodeficient individuals and non-pregnant or lactating women and informed consent and voluntary participation. In exclusion criteria, the suspected tuberculosis infection or tuberculosis patients; patients with hepatitis B or abnormal liver function; patients with malignant tumors were excluded. Collected data included patient's birthdates, gender, and affected eye locations. Additionally, 60 healthy individuals were included, forming the non-uveitis group; no history of eye or systemic diseases and no recent use of local or systemic medications. Oral administration of glucocorticoids at 5-10 mg/dose, once a day, with the dosage adjusted by the doctor based on the patient's condition. Simultaneously, gastroprotective agents, calcium supplements, and potassium supplements such as sustained-release potassium chloride tablets were administered to prevent adverse reactions. Efficacy was categorized into three levels; marked effective, effective, and ineffective based on changes in patient's visual acuity and visual field symptoms. Remarkable efficacy indicates an improvement of more than 3 lines on the Snellen chart for unaided vision and an expansion of the visual field by 10° or more. Effective denotes an improvement of more than 1 line on the Snellen chart for unaided vision and a visual field expansion of 5°-10°. Ineffective signifies that symptoms persist after treatment, with no change or even a decline in visual acuity and visual field. Vitreous opacity scored according to the Hackett-McDonald scoring system. Determination of Th1 and Th17 cell ratios by the venous blood obtained from fasting patients. 200 µl of whole blood were diluted 1:1 with 1640 culture medium, and then Phorbol Myristate Acetate (PMA), working solution and ionomycin working solution were added. The mixture was cultured at 37° with 5 % Carbon dioxide (CO2) for 5 h. After incubation with the appropriate concentrations of antibodies, flow cytometry was used for detection following lysis and washing. Measurement of S100A12 and S100A8/A9 levels by the fasting venous blood was collected, and serum was obtained after centrifugation. S100A8/A9 and S100A12 are two subtypes of S100 proteins associated with autoimmune diseases. The concentrations of S100A8/A9 and S100A12 in the serum were measured utilizing an Enzyme-Linked Immunosorbent Assay (ELISA), following the guidelines provided in the assay kit. This study applied Statistical Package for the Social Sciences (SPSS) 26.0 for data processing. Measurement data are expressed as "x±s", and comparisons between groups are made using t-test. The correlation analysis between Th1 cells, Th17 cells, S100 protein levels and clinical data also used t-test. $p<0.05$ was used as the criterion for determining the significance of differences. After treatment with glucocorticoids, 40 of 60 patients with uveitis were cured, 18 were significantly effective, and 2 were ineffective. The effective rate of treatment was 96.67 %. The vitreous opacity score before treatment was 3.04±0.25 points, and the vitreous opacity score after treatment was 2.18±0.16 points. The difference was statistically significant ($p<0.05$). Before treatment, the proportion of Th1 and Th17 cells and the levels of S100A12 and S100A8/A9 in the uveitis group were significantly higher than those in the non-uveitis group ($p<0.05$). After treatment, the proportion of Th1 and Th17 cells and the levels of S100A12 and S100A8/A9 in the uveitis group were significantly lower than those before treatment ($p<0.05$), and were close to those in the non-uveitis group ($p>0.05$) as shown in Table 1. Correlation analysis results showed that the levels of Th1, Th17, S100A12, and S100A8/A9 were significantly related to whether the patients with uveitis were in the active stage ($p<0.05$). The proportion of Th1 and Th17 cells in patients in the active stage and the levels of S100A12 and S100A8/A9 Significantly higher as shown in Table 2. Corticosteroids are commonly used in the treatment of uveitis, administered through various routes such as local instillation, intravitreal injection, oral ingestion, or intravenous injection. They work by inhibiting the synthesis of inflammatory mediators and inducing the expression of anti-inflammatory agents, while also suppressing delayed-type hypersensitivity reactions. However, their long-term high-dose use is limited due to potential side effects. In this study, oral corticosteroid treatment was administered to uveitis patients. After treatment, among the 60 uveitis patients, 40 were cured, 10 showed remarkable improvement, 8 were effective, and 2 were ineffective, resulting in a high treatment effectiveness rate of 96.67 %. The vitreous opacity score also significantly decreased after treatment, indicating a favorable therapeutic outcome. The pathogenesis of uveitis is not fully understood, but it

is widely accepted that neutrophils and T cells mediate autoimmune inflammatory responses, leading to non-granulomatous uveitis and retinal vasculitis. Increasing evidence suggests the involvement of Th cells in the occurrence and development of uveitis. Th cell subtypes, such as Th1 and Th17, play distinct roles based on the cytokines and specific transcription factors they secrete. Th1 cells predominantly produce Interferon (IFN) and Interleukin (IL)-2, promoting B cell antibody production, activating macrophages, and inducing T lymphocyte-mediated cytotoxic effects and cellular immunity. Th17 cells are implicated in the pathogenesis of various autoimmune diseases, and pathogenic Th17 cells are thought to induce immune cell infiltration and tissue damage by secreting pro-inflammatory cytokines, including IL-17A, IL-17F, IL-22, and granulocyte-macrophage colony-stimulating factor. Numerous studies using experimental autoimmune uveitis animal models have demonstrated that retinal antigen specific Th1 and Th17 cells can induce autoimmune uveitis in naive mice through adoptive transfer[5,8]. In this study, compared to the non-uveitis group, the proportions of Th1 and Th17 cells in uveitis patients were significantly elevated before treatment. After treatment, the proportions of Th1 and Th17 cells significantly decreased in uveitis patients ($p<0.05$), approaching those of the non-uveitis group ($p>0.05$). Correlation analysis revealed a significant association between the proportions of Th1 and Th17 cells and whether uveitis patients were in the active phase. Patients in the active phase had significantly higher proportions of Th1 and Th17 cells ($p<0.05$), confirming that in actual clinical samples, uveitis patients exhibit a reduction in Th1 and Th17 cell proportions, with higher levels observed in those in the active phase. S100 protein subtypes have been proven valuable as biomarkers for inflammation or autoimmune diseases[9]. In humans, the S100 protein family consists of 21 subtypes with structurally similar components, expressed specifically in different cell types, exhibiting diverse functions[10]. Among the S100 subtypes, S100A8/A9 forms a heterodimeric complex expressed primarily by monocytes and granulocytes, while S100A12 serves as a neutrophil-specific activation marker. When released by activated phagocytes, S100A8/A9 and S100A12 serve as pro-inflammatory ligands that engage with Toll-Like Receptor (TLR)-4 or receptors for advanced glycation end products. This interaction initiates inflammatory reactions and various other functions. The presence of these proteins in serum is indicative of the overall activity of immune disorders[11-14]. Recent studies have reported elevated levels of S100A12 in the serum of adults with active autoimmune uveitis[15]. Other studies have indicated an elevation in plasma levels of S100A8/A9 in uveitis patients. Importantly, after glucocorticoid treatment, the levels of S100A8/A9 were observed to synchronously decrease with the severity of intraocular inflammation[16]. In this study, compared to the non-uveitis group, the uveitis group exhibited significantly elevated levels of S100A12 and S100A8/A9 before treatment ($p<0.05$). After treatment, the levels of S100A12 and S100A8/A9 significantly decreased ($p<0.05$), approaching those of the non-uveitis group ($p>0.05$). Correlation analysis revealed a significant association between the levels of S100A12 and S100A8/A9 and whether uveitis patients were in the active phase ($p<0.05$), with significantly higher levels observed in patients in the active phase. These results suggest abnormal upregulation of S100A12 and S100A8/A9 in uveitis patients, with higher levels in those in the active phase. In summary, glucocorticoid treatment shows promising results in uveitis. The proportions of Th1 and Th17 cells, as well as the levels of S100A12 and S100A8/A9 proteins, demonstrate certain correlations with uveitis, and these indicators tend to decrease after treatment, suggesting their potential as biomarkers for uveitis. However, this study has limitations, such as a relatively small sample size. Additionally, serum markers may not precisely reflect local ocular inflammation, and this study did not assess the expression of S100A12 and S100A8/A9 in aqueous humor samples.

TABLE 1: CHANGES IN Th CELL PROPORTION AND S100 PROTEIN LEVEL BEFORE AND AFTER TREATMENT

Indicator	Non-uveitis group	Uveitis Group	
		Before treatment	After treatment
Th (%)	6.96±2.81	8.70±2.23*	7.11±2.23
Th17 (%)	2.36±0.31	4.46±0.39*	2.42±0.37
S100A12 (ng/ml)	792.15±86.31	1211.97±105.95*	827.35±111.06
S100A8/A9 (ng/ml)	77.78±5.28	108.48±8.26*	84.58±8.53

Note: *$p<0.05$, compared with non-uveitis group

TABLE 2: CORRELATION BETWEEN Th CELLS, S100 PROTEIN AND CLINICAL DATA BEFORE TREATMENT

Clinical information	n	Th1 (%)	Th17 (%)	S100A12 (ng/ml)	S100A8/A9 (ng/ml)
Sex	792.15±86.31	792.15±86.31	792.15±86.31	792.15±86.31	792.15±86.31
Male	31	8.52±2.01	4.46±0.37	1206.06±100.37	109.32±7.27
Female	29	8.86±2.55	4.47±0.43	1218.29±113.04	107.57±9.24
t		0.5737	0.0287	0.4438	0.8194
p		0.5684	0.9772	0.6588	0.4159
Age diagnosed	792.15±86.31	792.15±86.31	792.15±86.31	792.15±86.31	792.15±86.31
≤50 y	30	8.97±2.57	4.42±0.38	1208.2±106.67	111.22±8.98
>50 y	30	8.43±1.97	4.49±0.41	1215.73±106.9	107.73±10.52
t		1.292	0.9699	0.273	1.309
p		0.1990	0.3341	0.7858	0.1958
Active stage	792.15±86.31	792.15±86.31	792.15±86.31	792.15±86.31	792.15±86.31
Yes	36	9.76±1.99	4.66±0.31	1188.68±115.28	106.41±9.26
No	24	7.06±1.62	4.16±0.32	1246.9±80.3	111.57±5.28
t		5.531	5.937	2.148	2.459
p		<0.0001	<0.0001	0.0359	0.0165
Recurrence	792.15±86.31	792.15±86.31	792.15±86.31	792.15±86.31	792.15±86.31
Yes	12	9.35±2.12	4.62±0.34	1217.76±121.86	109.06±11.66
No	48	8.52±2.29	4.42±0.4	1210.52±102.98	108.33±7.33
t		1.133	0.1066	0.2101	0.2722
p		0.2617	1.6390	0.8343	0.7864
Affected area					
Both eyes	8	8.81±2.51	4.33±0.47	1243.61±113.1	108.61±5.71
Left eye	27	8.72±2.26	4.48±0.37	1219.03±100.64	108.64±9.74
Right eye	25	8.61±2.29	4.47±0.4	1194.22±110.32	108.26±7.43
F		0.029	0,.4424	0.7614	0.01475
p		0.9714	0.6447	0.4717	0.9854

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Conflict of interests:

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