Original Article
Changes of autoantibodies and intercellular adhesion molecule-1 in patients with Graves disease after clinical treatment

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Abstract: Objective: To study the changes of autoantibodies and intercellular adhesion molecule-1 (ICAM-1) in patients with Graves disease (GD) after clinical treatment. Methods: A total of 68 patients with GD admitted to our hospital from August 2018 to August 2019 were selected as the research objects. The thyroid peroxidase antibody (TPOAb), thyroid stimulating antibody (TSAb), and antithyroglobulin antibody (TgAb), ICAM-1, insulin-like growth factor 1 (IGF-1), Interleukin-6 (IL-6), Interleukin 17 (IL-17) before and after treatment were examined. Results: The levels of TSAb, TgAb and TPOAb after treatment were remarkably lower than those before treatment (P<0.001); the levels of ICAM-1, IGF-1, IL-17 and IL-6 after treatment were noticeably lower than those before treatment (P<0.001); the FT3 and FT4 levels of patients after treatment were significantly lower than those before treatment (P<0.001), and the FSH level was significantly higher than that before treatment (P<0.001). Conclusion: Clinical treatment can remarkably reduce the levels of autoantibodies, ICAM-1 and IGF-1 in GD patients, improve thyroid function, and relieve inflammation. The detection of the above indicators can provide guidance for the progression and treatment of GD.

Keywords: Graves disease, autoantibodies, intercellular adhesion molecule-1

Introduction

Graves disease (GD), also known as toxic diffuse goiter, is a common immune disease. Its clinical manifestations are complicated with multi-system syndrome which not only present at the thyroid, but also include hypermetabolic syndrome, eye signs, skin signs, etc. [1, 2]. GD may affect multiple tissues and organs, and even with a longer treatment period and a higher recurrence rate [3]. In recent years, the prevalence of GD has been increasing year by year and has become a major public health concern. Therefore, it is extremely important to seek an effective treatment method to reduce the recurrence rate of GD and to improve the prognosis of the disease, which has become a hot issue in the current medical research. Radioactive 131 iodine therapy is currently a common method for the treatment of GD, and the strong radiation ability may cause thyroid filtering cells degenerated and necrotic, and reduced secretion of thyroid hormone [4-6].

Extensive studies have found that the recurrence of GD is related to the patient's autoimmune status. Intercellular cell adhesion molecule-1 (ICAM-1) is a human immunoglobulin factor. When it is highly expressed, it can reduce the adhesion between diseased cells in the body, causing the diseased cells to fall off and flow into the blood vessels. It will further work with lymphocytes to make the diseased cells escape from immune surveillance, and complete the metastasis, enabling the diseased cells to spread [7-9]. Autoantibodies refer to antibodies produced against one's own tissues, organs, cells and cell components. The growth, development and survival of organisms rely on the maintenance of a complete autoimmune tolerance mechanism. Once the integrity of self-tolerance is destroyed, the body will have an autoimmune response to produce autoantibodies [10, 11]. GD is a metabolic disease that affects the immune and metabolic system. Generally, patients would be accompanied by the decrease in the concentration of

insulin-like growth factor 1 (IGF-1). Interleukin 17 (IL-17) is a T lymphocyte cytokines expressed downstream of the subgroups of cytokines, and it can activate the inflammatory response through a variety of ways, has a chemotactic effect on inflammatory cells, and can directly participate in the inflammatory response and the tissue damage process caused by the immune response. Serum Interleukin-6 (IL-6), a cytokine, is synthesized and secreted by monocyte-phagocytes, vascular endothelial cells, etc., and participates in the regulation of immune system functions. We aimed to explore the treatment on immune status by observing patients' autoantibodies, ICAM-1 and thyroid function changes before and after clinical treatment on 68 patients with GD treated in our hospital from August 2018 to August 2019. This is the first attempt to provide simple detection markers for the progress of GD from the protein level, and provide a basis for early intervention and treatment of patients.

Materials and methods

General information

A total of 68 GD patients admitted to our hospital from August 2018 to August 2019 were selected as the research objects.

Inclusion criteria

① Met GD clinical diagnostic criteria [11]; ② With complete clinical data; ③ All received clinical treatment; ④ This study was approved by the hospital ethics committee, and the patient and their family members knew the purpose and process of the study and signed an informed consent.

Exclusion criteria

① Combined with malignant tumor; ② With severe organic diseases such as brain, heart, liver, and kidney; ③ Poor treatment compliance; ④ Combined with mental and other cognitive impairment.

Methods

The patient took orally methimazole tablets (SFDA approval number: H11020885; Manufacturer: Beijing Taiyang Pharmaceutical Co., Ltd.; Specification: 5 mg*50 tablets), the dose was 30 mg/d, the maintenance dose was 5 mg/d; oral propylthiouracil tablets (SFDA approval number: H20150035; Manufacturer: Helbrand Pharmaceutical Factory, Germany; Specification: 50 mg*100 s), the dose was 300 mg/d, the maintenance dose was 50 mg/d; oral prednisone acetate tablets (SFDA approval number: H33021207; Manufacturer: Zhejiang Xianju Pharmaceutical Co., Ltd.; Specification: 5 mg*100 s), the dosage was 15 mg/d, gradually decreasing, and the use cycle was 3 months; oral levothyroxine sodium tablets (SFDA approval number: J20160065; Manufacturer: Germany Merck; Specifications: 50 μg*100 s), 25-50 μg/d, combined with Leucogen (SFDA approval number: H320254-44; Manufacturer: Jiangsu Jibeier Pharmaceutical Co., Ltd.; Specification: 20 mg*48 tablets) for supplementary treatment. The blood routine was monitored during the treatment and the medication was continued for 6 months [12-14].

Observation indicators

3 ml fasting venous blood was collected from patients before and after treatment, and centrifuged to obtain upper serum. The ST-1545 electrochemiluminescence analyzer (purchased from Beijing Xuxin Instrument Equipment Co., Ltd.) was used to determine the changes in thyroid peroxidase antibody (TPOAb), thyroid stimulating antibody (TSAb), antithyroglobulin antibody (TgAb) and ICAM-1 levels; An automatic chemiluminescence immunoassay analyzer (purchased from Wuhan Mingde Biotechnology Co., Ltd.) was used to determine free triiodothyronine (FT3), hypersensitivity thyroid-stimulating hormone (hTSH) and free thyroid hormone (FT4) value; SpectraMax M5/M5e microplate reader (manufacturer: Meigu Molecular Instruments Co., Ltd.) was used to determine the level of interleukin-17 (IL-17) and insulin-like growth factor 1 (IGF-1), and the level of IL-6 was determined by radioimmunoassay. The kit was purchased from Shanghai Jining Industrial Co., Ltd., which was operated strictly in accordance with the kit instructions.

Statistical methods

The experimental data were all statistically analyzed and processed by SPSS21.0 software, and GraphPad Prism 6 (GraphPad Software, San Diego, USA) is used to plot pictures. The enumeration data were compared
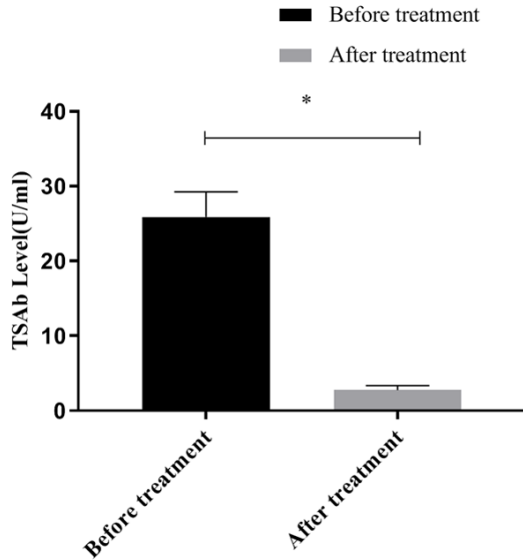
Figure 1. Comparison of TSAb levels before and after treatment ($\overline{x} \pm s$). Note: The abscissa represents before and after treatment, and the ordinate represents TSAb level (U/ml); The TSAb levels of patients before and after treatment were (23.49±4.76) U/ml and (2.32±0.86) U/ml, respectively. There is a significant difference in the TSAb level of patients before and after treatment (t=36.091, *P<0.05).
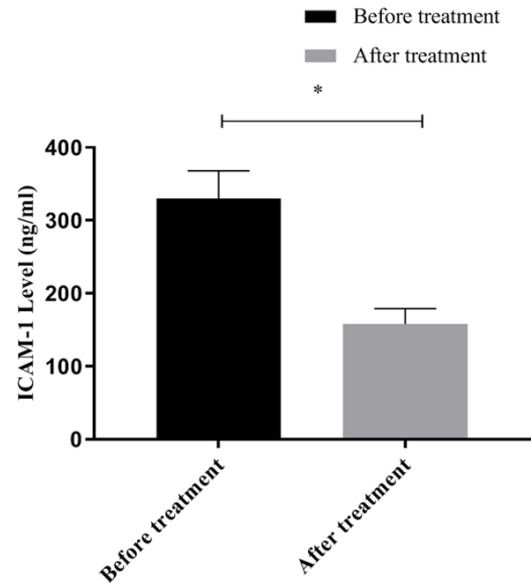
Table 1. Comparison of TgAb and TPOAb levels before and after treatment ($\overline{x} \pm s$, IU/ml)

Time	n	TgAb	TPOAb
Before treatment	68	42.36±8.54	27.43±5.42
After treatment	68	31.26±4.35	24.07±5.31
t		9.551	3.652
P		0.001	<0.001

using x^2 test, which were represented by [n (%)], and measurement data were examined by paired t test within the group, which were reported as (x ± s). When P<0.05, the difference was statistically significant.

Results

Clinical data

The research objects, including 36 males and 32 females, with an average age of (41.36±6.53) years and an average course of (20.31±3.46) months.

Comparison of TSAb levels before and after treatment

The TSAb level of patients after treatment was significantly lower than before treatment (P< 0.05), as shown in **Figure 1**.

Figure 2. Comparison of ICAM-1 levels before and after treatment ($\overline{x} \pm s$). Note: The abscissa represents before and after treatment respectively, and the ordinate represents the level of ICAM-1 (ng/ml); The ICAM-1 levels before and after treatment were (303.34±53.73) ng/ml and (143.54±29.38) ng/ml respectively; There is a significant difference in ICAM-1 levels before and after treatment (t=21.518, *P<0.05).

Comparison of TgAb and TPOAb levels before and after treatment

The TgAb and TPOAb levels of patients after treatment were remarkably lower than those before treatment (P<0.05), see **Table 1**.

Comparison of ICAM-1 levels before and after treatment

The ICAM-1 level of the patient after treatment was notably lower than that before treatment (P<0.05), as shown in **Figure 2**.

Comparison of thyroid function before and after treatment

The FT3 and FT4 levels of the patients after treatment were significantly lower than those before treatment (P<0.05), and the FSH levels were significantly higher than that before treatment (P<0.05), see **Table 2**.

Comparison of IL-17 and IL-6 levels before and after treatment

The IL-17 and IL-6 levels of patients after treatment were significantly lower than those before treatment (P<0.05), see **Table 3**.

Table 2. Comparison of thyroid function before and after treatment between the two groups ($\bar{x} \pm s$)

Time	n	FT_3 (pmol/L)	FT_4 (pmol/L)	FSH (μ*IU/ml*)
Before treatment	68	21.48±4.63	64.38±3.27	0.04±0.01
After treatment	68	5.94±1.06	17.25±1.21	7.35±0.54
t		29.979	111.456	111.610
P		0.002	<0.001	0.001

Table 3. Comparison of IL-17 and IL-6 levels before and after treatment ($\bar{x} \pm s$, pg/ml)

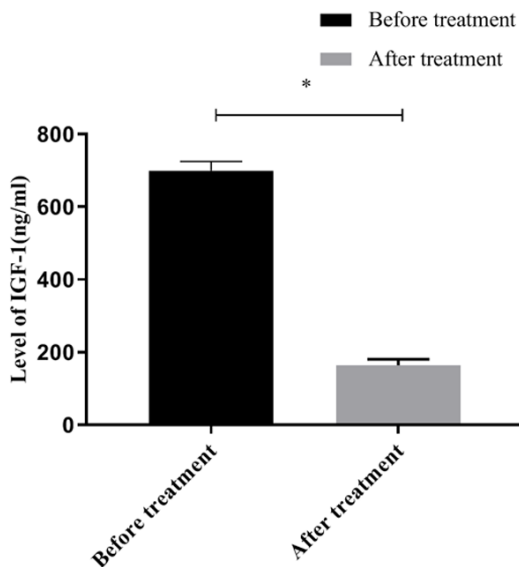| Time	n	IL-17	IL-6
Before treatment	68	629.54±89.77	162.74±31.26
After treatment	68	209.17±41.35	127.59±19.27
t		38.073	7.893
P		<0.001	<0.001

Figure 3. Comparison of IGF-1 levels before and after treatment ($\bar{x} \pm s$). Note: The abscissa represents before and after treatment, and the ordinate represents the level of IGF-1 (ng/ml); The IGF-1 levels of the patients before and after treatment were (682.17±35.62) ng/ml and (152.86±23.15) ng/ml respectively; There is a significant difference in IGF-1 levels before and after treatment (t=102.745, *P<0.05).

Comparison of IGF-1 levels before and after treatment

Compared to that before treatment, the patient's IGF-1 level after treatment was markedly lower (P<0.05), as shown in **Figure 3**.

Discussion

Most patients with GD are accompanied by hyperthyroidism. To date, the pathogenic mechanism of the disease is poorly understood. It is speculated that it is related to the inflammation of the orbit and external muscle connective tissue. Inflammation will cause the volume of the patient's orbital contents to increase, which will induce eyelid retraction and eye movement disorder. The pathogenesis is usually under the stimulation of chemokines and adhesion factors [15, 16]. We measured the levels of autoantibodies, serum inflammatory factors and ICAM-1 before and after treatment in GD patients, and observed the changes of the above indicators in GD patients, further revealing the impact of clinical treatment on GD patients.

Subsequently, we found that the TSAb, TgAb, and TPOAb levels of patients after treatment were significantly lower than before treatment, indicating that clinical treatment can dramatically improve the immunological indicators of GD patients and promote clinical efficacy. IL-17 and IL-6 are known to be involved in the entire pathogenesis of GD. IL-17, as an inflammatory cytokine produced by T cells, is an important marker of the body's inflammatory response. It can stimulate the activation of T cells by amplifying and stimulating molecules such as ICAM-1 induction, and cause neutrophils to accumulate in the inflammation zone [17-19]. This experiment further confirmed that the levels of IL-17 and IL-6 before treatment in GD patients were significantly higher than those after treatment, and it is assumed that two serum inflammatory cytokines are involved in the pathogenesis of GD, suggesting clinical treatment can remarkably reduce their expression, relieve the inflammatory response, and improve the prognosis.

ICAM-1 is an important adhesion molecule that mediates the adhesion reaction. It is lowly expressed in resting vascular endothelial cells. As a transmembrane protein between endothelial cells and white blood cells, this molecule plays a role in promoting the migration of

white blood cells and endothelial cells [20, 21]. Clinical studies have found that ICAM-1 is expressed at a high level in patients with thyroid-related eye diseases, and is correlated with the severity of the disease. This study shows that ICAM-1 after treatment is significantly lower than that before treatment, whereas it was previously reported that ICAM-1 is highly expressed in liver cancer patients [22]. After the patients received antiviral drugs, the ICAM-1 level was (144.28±31.54) ng/ml, which was significantly lower than that before treatment (315.22±33.23) ng/ml, indicating that ICAM-1 is involved in the pathogenesis of many diseases in the human and is expressed at a high level. Ataam et al. [23] pointed out that the expression level of IGF-1 in orbital fibroblasts of GD patients was three times that of healthy people, and the level of IGF-1 was positively correlated with the migration and proliferation of fibroblasts. The content of IGF-1 in serum will increase with the aggravation of the disease, and it is speculated that it can be used as an important biological marker to mirror GD disease. The limitation of this study is that the number of patients included is small, there is no long-term follow-up, and the study is a self-controlled study, lacking a control group. Therefore, the sample size in the future studies needs to be conducted, and controlled and long-term trials are also needed.

To conclude, clinical treatment can remarkably reduce the levels of autoantibodies and ICAM-1 in GD patients, promote the recovery of thyroid function, and reduce the body's inflammatory response. It is expected to provide more references for the clinical prognosis evaluation of GD by measuring the above indicators.

Disclosure of conflict of interest

None.

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