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Short-term effect of radioactive iodine therapy on CXCL-10 production in Graves' disease

Abstract

Purpose: To observe the short-term dynamic change in serum CXC chemokine ligand-10 (CXCL10) levels in patients with Graves' disease (GD) before and after iodine therapy and to analyze the relationship between CXCL10 levels and clinical disease indices.

Methods: ELISA was used to determine serum levels of CXCL10 in 43 patients with GD shortly before radioiodine therapy and on days six, 14, and 60, post-therapy.

Results: Patients with newly diagnosed GD showed significantly higher levels of serum CXCL10 compared with the control group ($P < 0.01$). The serum CXCL10 level increased slightly on day six after treatment of radioactive iodine ($P < 0.01$). There was no significant statistical difference in serum CXCL10 levels pre-treatment and on day 14 post-treatment. A significant reduction in serum CXCL10 level was observed on day 60 ($P < 0.01$). GD patients with exophthalmia showed higher serum CXCL10 level than GD patients without exophthalmia. No correlation was found between levels of CXCL10 and FT3, FT4 or TSH at any time point, but significant positive correlation was shown between thyroid peroxidase antibodies (TPOAb) and CXCL10 ($r = 0.50$, $P < 0.01$).

Conclusion: CXCL10 participates in the early inflammatory response after radioactive iodine therapy in patients with Graves' disease and shows a strong association with the autoimmune process.

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Graves' disease (GD), which causes hyperthyroidism, is a common autoimmune disease of the thyroid. GD shows striking immunological disorders including the production of activating antibodies, abnormalities in the circulating T cell population and goitres with lymphocytic infiltration. The thyroid antibodies may stimulate thyroid function, resulting in unrestrained thyrocyte growth, excessive thyroid hormone production, thyrotoxicosis, diffuse hyperplasia and enlargement of the thyroid gland. Recently, it has been reported that T cells, involved in GD, may change throughout the course of the disease, and that T cells are critical in the induction, development and maintenance of GD.

Chemokines are a group of low molecular weight peptides that are able to recruit specific leukocyte subtypes to inflammation sites [1]. Several studies suggest that chemokines may be crucial for the recruitment and/or homing of T cells in the inflamed tissues. Recent experimental evidence has shown that CXC- α chemokines, especially CXC chemokine ligand-10/inducing protein-10 (CXCL-10/IP-10), play an important role in the initial phases of autoimmune thyroid disorders [2-6]. In GD, CXCR3, the receptor of CXCL-10, is expressed in endothelial and inflammatory cells, thyrocytes [2,5] and serum CXCL10 levels are significantly increased in GD patients with recent onset of disease [2].

Iodine-131 (^{131}I) therapy is increasingly being used as a first-line treatment for hyperthyroidism in Graves' disease (GD). Previous studies have evaluated the effect of radioactive iodine therapy on cytokine production in GD patients, but with contrasting results [7, 8]. The aim of our study was to identify the short-term effect of radioiodine on serum CXCL10 levels and to evaluate the relationship between CXCL10 levels and clinical disease indices.

Materials and Methods

Patients

GD was diagnosed on the basis of clinical, biochemical and immunological features, as well as from scintiscans. The selected 43 GD patients in this study were newly diagnosed and were not being treated with antithyroid drugs. Patients were referred to the inpatient clinic at the People's Hospital of Linyi City for first-time radioiodine treatment of hyperthyroidism: 34 women and 9 men, aged 20–57 years (mean, 35.88 ± 9.79 years). The duration of the disease was from two weeks to two years (mean, 6.06 months). The control group matched approximately for age and sex comprised 23 female and 7 male subjects, aged 22–54 years (mean, 36.28 ± 7.26 years) who were all in good health. All subjects gave informed consent. The

study was approved by ethical committee review. All GD patients were treated with a single oral dose of radioiodine (^{131}I).

Blood samples

Blood were obtained from control group and patients shortly before radioiodine therapy and on days six, 14 and 60 post-therapy. The blood samples were centrifuged at 2500 g for 15 minutes and the supernatants were stored at -80°C for later testing.

Thyroid function tests

Free T₄, Free T₃ and TSH were measured by chemiluminometric immunoassay. Serum thyroid peroxidase antibodies (TPOAb) were measured by radioimmunoassay. Serum CXCL10/IP-10 levels were measured by ELISA (human IP-10 immunoassay, R&D Systems, USA). Intra- and interassay coefficients of variation (CV) were all below 10%.

Statistical analysis

Differences between patients and controls were analyzed by Student's *t* test, and T paired test were used in the analysis among patients at different time points before and after treatment. Correlations among CXCL10 levels, thyroid function and autoantibody levels were determined by linear regression analysis. *P* value of less than 0.05 was accepted as statistically significant. Statistical analyses were carried out by SAS software (version 8.0).

Results

The levels of FT₃ and FT₄ in the newly diagnosed GD patients were significantly higher immediately before therapy (mean, 24.04 ± 5.22 pmol/L and 77.42 ± 28.99 pmol/L, respectively) compared with those of the control group (mean, 4.66 ± 0.49 pmol/L, 13.65 ± 1.23 pmol/L, respectively) and fell to 7.00 ± 5.81 pmol/L and 19.87 ± 7.13 pmol/L, respectively by day 60 after the therapy. TSH in patients before treatment was lower than that of control group (mean, 0.006 ± 0.001 uIU/L, 2.21 ± 1.11 uIU/L, respectively) and became nearly normal (5.76 ± 1.56 uIU/L) on day 60 (Fig.1A). The serum TPOAb levels increased gradually after therapy and became positive in 86.05% of subjects on day 60 after therapy (Fig.1B).

The serum CXCL10 levels were significantly higher in newly diagnosed GD patients in comparison with the control group ($t = -3.94$, $P < 0.01$) (Fig.2A). Serum CXCL10 levels increased slightly on the sixth day after radioactive iodine

FIGURE 1. (A) Levels of FT3, FT4 and TSH in the control group and in GD patients before radioiodine therapy and 60 days after therapy; (B) Serum levels of TPOAb in the control group and in patients before radioiodine therapy and 60 days after therapy.

treatment, relative to pre-treatment levels ($t=-4.172$, $P<0.01$), decreased by day 14 to levels not significantly different from pretreatment levels, then decreased further to below pre-treatment levels by day 60 after therapy ($t=4.212$, $P<0.01$) (Fig.2B). Prior to therapy, the serum levels of CXCL10 in GD patients with exophthalmia were higher than those without exophthalmia (Fig.2C).

Correlation analysis

There was no correlation between levels of CXCL10 with FT3, FT4 or TSH at any time point ($P>0.05$). TPOAb level was positively related with CXCL10 ($r=0.50$, $P<0.01$).

Discussion

Iodine-131 (^{131}I) therapy is increasingly being used as a first-line treatment for hyperthyroidism in Graves' disease (GD). There is general consensus that ^{131}I constitutes a safe and highly cost-effective therapeutic option, devoid of major side effects. Nevertheless, there are some aspects related to ^{131}I therapy that remain controversial (9). In our study, thyroid hormone levels become nearly normal by day 60 and the serum levels of TPOAb increase gradually after therapy. The destruction of thyroid follicular cells and the release of thyroid antigens after ^{131}I treatment, with the ensuing effects on circulating markers of autoimmunity [9], is the probable cause of these changes in hormone and antibody levels. Therefore, it is reasonable to ex-

pect that chemokine profiles and the autoimmune process might also be affected.

Chemokines are a family of cytokines initially characterized by their capacity to induce chemotaxis, or directed leukocyte migration [10, 11]. CXCL10/ IP -10 are CXC chemokines that are induced by interferon- γ during inflammation and that display potent lymphocyte chemotactic activity [12, 13]. The CXCR3 chemokine receptor is specific for the CXC chemokines CXCL10/ IP -10 [13,14]. CXCL10/ IP -10 binding to the CXCR3 receptor is considered important in delivering specific signals for selective homing of activated/effector cells to some inflammatory sites [15]. Recent studies have implicated both mRNA and protein expression of the chemokines CXCL10/ IP -10, and its receptor, CXCR3, could be readily detected in the thyroid glands of patients suffering from GD. In addition, CXCL10/ IP -10 levels can be measured in the serum of GD patients. Expression of CXCL10/ IP -10 and CXCR3 has been shown to be poor or absent in normal thyroid tissue from patients undergoing thyroidectomy. CXCL10/ IP -10, usually localized to infiltrating lymphocytes and macrophages, as well as to resident epithelial follicular cells, maximal expression of CXCL10/ IP -10 was found in the thyroid gland of patients with recent-onset GD and was correlated with interferon- γ [2]. Taken together, these data suggest a pathogenic role for these chemokines in the recruitment of activated T cells in inflamed thyroid tissue and suggest that the detection of CXCL10/ IP -10 may represent a useful clinical marker in GD.

FIGURE 2. (A) Serum CXCL10 levels in the control group and in newly diagnosed GD patients; (B) Serum CXCL10 levels in patients shortly before radioiodine therapy and at six, 14, and 60 days afterward; (C) Pre-therapy serum levels of CXCL10 in GD patients with exophthalmia and without exophthalmia.

Antonelli *et al.* have shown that in patients with Graves' disease, serum CXCL10 is significantly reduced after thyroidectomy [16] or ^{131}I therapy [17]. These results suggest that the thyroid gland is the main source of circulating CXCL10 in patients with Graves' disease. Antonelli *et al.* also found that serum levels of CXCL10, evaluated in hyperthyroid GD patients at the time of diagnosis, was reduced significantly after restoration of euthyroidism by MMI treatment; however, patients with toxic nodular goiter showed no obvious change when rendered euthyroid by the same medication. Therefore, the marked reduction in circulating concentrations of CXCL10 in GD patients after MMI treatment could be interpreted as resulting from an immunomodulatory action of the drug rather than from the restoration of euthyroidism [18, 19]. Increased plasma CXCL10 would promote infiltration of activated lymphocytes into the thyroid gland, and represents a potential indicator of disease activity in Graves' disease; thus, increased circulating CXCL10 in patients with Graves' disease may be specifically sustained by the autoimmune, inflammatory process.

In present study, the serum CXCL10 levels were significantly higher in newly diagnosed GD patients in comparisons with the control group. Of note, a significant correlation was observed between serum levels of CXCL10 and TPOAb but not with the thyroid hormone levels. The levels of CXCL10, which first increased on day six after treatment with radioactive iodine, may relate to the autoimmune response induced by ^{131}I . The subsequent reduction of circulating CXCL10 levels after destruction of thyroid tissue by ^{131}I therapy can be explained by removal of the large part of either intrathyroidal lymphocytes and/or thyrocytes, which may be the main source of CXCL10. Thus, the dynamic change of CXCL10 levels can be considered a marker of a more aggressive autoimmune process leading to thyroid destruction [3,4].

Significantly higher pre-therapy circulating CXCL10 levels were also seen in GD patients with ophthalmopathy (GO) compared with the patients without ophthalmopathy. It is well known that cytokines play a crucial role in the pathogenesis of GO; Antonelli *et al.* showed similar results and a significant association between CXCL10 levels and activity of GO, suggesting that the presence of orbital inflammation may be responsible for an increase in circulating concentrations of CXCL10 (20). It is worth noting that there are currently no established, reliable serum markers to evaluate the activity in GO. Thus, it would be helpful to investigate the clinical significance of serum chemokine detection [21].

The present study confirmed that CXCL10 participates in the short term effects of radioactive iodine therapy and shows a

strong association with the autoimmune process; therefore, observation of the dynamics of these changes in serum CXCL10 levels may be helpful to understand the inflammatory changes after iodine treatment. A longitudinal study would be required to determine the changes in CXCL10 and thyroid autoantibody levels, and to observe the effects of these changes on clinical outcome after ^{131}I therapy.

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