Persistently Elevated Levels of Serum Autoimmune Inflammatory Markers after Total Thyroidectomy for Hashimoto's Thyroiditis An Indicator of Prevailing Autoimmunity

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Abstract

Hashimoto's thyroiditis characterized by glandular lymphocytic infiltration with progressive parenchyma destruction and fibrosis. The autoimmunity was suggested by the reduced immune tolerance and the production of antibodies. The description of Th17 subpopulation has undesirable role in the pathogenesis of Hashimoto's disease. Among the cytokine secretion of Th17 is cell- type specific which carry its major effectors functions, IL17 and IL 22 are the most important**.**

Fifty two drug native patients with Hashimoto's thyroiditis were enrolled in the study. Preoperative, 6 weeks and 6 months postoperative serum assay of ATPO Ab, IL6, tumor necrosis factor TNF-**α**, IL17 A, IL22,IL23 was adopted. Surgery in form of total thyriodectomy was carried out for patients with suspicious nodule based on cytological examination or large symptomatic multinodular goiter.

The preoperative elevated Anti TPO Ab significantly higher levels than the 6 weeks and 6 months post operative measurement. Serum levels of IL 6 and TNF-α were significantly higher in the preoperative measurement than the 6 weeks post operative measurement, and higher than 6 months serum level for IL6 but not TNF –α. The IL 17A levels were significantly higher in the preoperative patients sera than the 6 wk post operative measurement, but not the 6 months measurement. No statistical difference was observed in the preoperative and post operative levels of IL 22. The preoperative serum Level of IL-23 was apparently high, and significantly different from 6 weeks and 6 months post operative measurement.

The measured selected immune parameters exhibit partial decrease in serum level which would not reach the normal circulatory levels 6 months after thyroidectomy.

Keywords: *Autoimmunity, Thyroidectomy, Inflammatory Markers.*

Introduction

Hashimoto's thyr0iditis (HT) is common autoimmune thyroid disorders with glandular lymphocytic infiltration with progressive parenchyma destruction and fibrosis[1]. HT affects 3–4% HT of the general population[2]. The disease is 10 times more frequent in females than males[3].The exact etiology of the disease is still elusive however genetic and environmental factors influence the development of autoimmunity against thyroid tissue[4]. The autoimmunity was suggested by the reduced immune tolerance and the production of antibodies[5,6]. The majority of patients have raised anti-thyroid peroxidase (anti-TPO)[7]. As destruction of thyrocytes progress followed by loss of thyroid hormone synthesis and eventual hypothyroidism[8]. Cell mediated autoimmune response is the main although not the sole pathogenic finding in HT, this response arise from a disruption of self-tolerance to thyroid antigenic structure[9,10]. In HT Th1 (T helper) lymphocytes generate an intense inflammatory infiltrate (predominantly lymphocytic) of thyroid gland, which initiate further thyroiditis and loss of thyrocytes. The activated cytotoxic lymphocytes

and macrophages, directly attack and destroy the thyroid follicular cells[11]. The suggested accelerated apoptosis seen in HT is thought to be induced, when thyrocytes express molecules involved in cell cycle and apoptosis (Fas receptor and also Fas ligand), a process mediated by cytokine released from Th1 and macrophage[12]. Th2 also induce B lymphocytes and plasma cells to produce thyroid targeted antibodies[13]. In fact, although the cell-driven destruction of thyrocytes is the main pathological finding, antibodies to TPO and Tg are also important components. Th1 cytokines stimulate the release of immunoglobulin (Ig) G1, whereas Th2 cytokines participate in production of IgG4[14,15]. TPO and Tg auto antibodies are of both IgG4 and IgG1 subclasses, indicating participation of Th2 and Th1 cytokines, including IL6 and TNF alpha[16]. The Th1 mediated disease is the most widely accepted, however the concept is further modulated by the description of Th17 subpopulation[17]. The Th17 cells mediate both normal and pathological immune response by its major role in immune response to the confounding extracellular pathogens and the disadvantageous role in the pathogenesis of several autoimmune diseases[18]. The cytokine secretion of Th17 is cell- type specific which carry its major effectors functions ,among which IL17 and IL 22 are the most important. Moreover, the Th17 cell expansion and survival is mainly mediated by IL-23[19]. The clinicopathological course of the disease is very variable ranging from subclinical state to overt thyroid failure[20]. Clinically HT present early with transient subclinical or overt hyperthyroidism for a variable periods followed by a stationary phase of euthyroid state eventually culminating on hypothyroidism, all stages of the disease may or not associated with goiter[21].

Aim : To evaluate some immune parameters in the sera of selected patients with Hashimoto's thyroiditis before and after total thyroidectomy.

Materials and Method

Fifty two patients with presumptive diagnosis of Hashimoto's thyroiditis were included in the study. The preoperative diagnosis was based upon clinical features, raised serum thyroid peroxidase Ab, cytology, TSH, thyroid hormone estimation and thyroid ultrasound. All patients were drug native euthyroid or mildly hypothyroid. Blood samples were collected in three different occasions, preoperatively as part of the assessment, 6wk and 6 months post operatively as part of the follow up, serum obtained for assay of ATPO Ab, IL6, tumor necrosis factor TNF-**α**, IL17 A, IL22, IL23. The indications for surgery was Hashimoto's thyroiditis with suspicious nodule based on cytological examination or large symptomatic multinodular goiter. Surgery in form of total thyroidectomy was successful in all patients and was followed by full Levo-thyroxin replacement. All resected specimens were subjected to histopathological examination which proved the diagnosis of Hashimoto's disease.

Inclusion criteria

Adults patients (18-60 years) with recent diagnosis of Hashimotos thyroiditis with euthyroid or subclinical hypothyroid state who were not subjected to any form of thyroid hormone replacement therapy.

Exclusion criteria

1. Patients with malignant disease including patients in whom thyroid malignancy was discovered after thyroidectomy.
2. Patients on thyroxin replacement.
3. Patients with advanced hypothyroidism.
4. Pregnancy.
5. Patients with chronic or autoimmune disease other than HT.
6. Patients on chronic steroid or immunosupreesive drugs.

All candidates informed about the details of the research including the hazards of surgery and the planned follow up, for which they signed a written consent. Levels of antithyroid peroxidase (anti-TPO) antibodies in the sera of all candidates was determined, using the diagnostic enzyme-linked immunosorbent assay (ELISA) kit (Monobind Inc., Lake Forest, USA). Values more than 40 IU/ml were considered positive.

IL-6 ,TNF- **α** ,IL-17A, IL-22, and IL-23 serum measurements were detected using human ELISA kit in accordance to the manufacturer's instructions (KOMABIOTECH- INC. Korea).

Statistical analysis

t-test for Independent sample was used for comparing the means of the two groups.

Results

Table 1. Age and Gender characteristics

Gender	Age in years			
	Mean	S.D	minimum	maximum
Female (n=44)	43.3	8.8	27	61
Male (n=8)	36.25	5,28	26	42
Total (n=52)	42.2	8,7	26	61

The preoperative elevated Anti TPO Ab showed higher levels than the 6 weeks and 6 months post operative measurement p value 0.001 and 0.0001 respectively. The 6 weeks serum level of anti TPO Ab was significantly higher than the 6 months measurement p value 0.0001. Serum levels of IL 6 were significantly higher in the preoperative measurement than the 6 wk and 6 months post-operative measurement p value 0. 01 and 0.003 respectively. Furthermore, no statistical difference was found between the 6 wks and the 6 months post operative measurement. The serum level of TNF-α in the preoperative measurement was significantly higher than the 6 wk post operative measurement p value 0.02 but no significant difference was found between the preoperative and 6 months measurement. The IL 17A levels were significantly higher in the preoperative patients sera than the 6 wk post operative measurement P value 0.04, but not the 6 months measurement. Furthermore no significant difference found between the 6 weeks and 6 months measurements. No statistical difference was observed in the preoperative and post operative levels of IL 22 The preoperative serum Level of IL-23 was apparently high, and significantly different from 6 weeks and 6 months post operative measurement p value =0.0001. No difference was found in serum level between the 6 weeks and 6 months measurements (Table 2).

Table 2. The pre and postoperative measured parameters, expressed by mean ±SD.

parameter	Preoperative	6 wk	6 month
Anti-TPO(Iu⁄ml)	708.3±194.4	317.9±154.1	180.4±90
IL 6(pg/ml)	93 ± 12.18	30.3±9.05	21.2±9.9
TNF-α (pg/ml)	29.7 ± 14. 2	19.03±10.8	23.6±13.45
IL 17 A(pg/ml)	7.8±5.7	5.7 ±3.64	6.7 ±4.4
IL22(pg/ml)	30.9±16.2	28.8±20.1	27.5±17.3
IL23(pg/ml)	87.76±11.7	45,9±9.8	42,3±7.9

Discussion

In 2003 the discovery of Th17 cells, a unique CD4+ T-cells sub population have been found to have both beneficial and harmful immunological function, especially as a result of extravagant Th1 responses[22,23]. These lymphocytes are the major source for production of cytokines from IL-17 family namely the IL17A and IL17 F, together with IL21 and IL22, which particularly accentuate the immune state by further release of other pro inflammatory cytokines like IL-Beta, TNF-**α**, and chemokines which are involved in cellular induced tissue damage[24].The IL-17A is the well known form in this family and has established proinflammatory effect in HT[25]. Many cytokines have been suggested to have a potential role in the Th17 cells differentiation in humans among which the IL23, IL21, IL6 and TNF-α are the most important players[26]. It has been found that patients suffering from HT found to have abnormally raised levels of Th17 cells and Th-17- related pro inflammatory cytokines[27]. Our findings was supportive of the persistently high serum level of ATPO antibody following total surgical thyroid ablation.

The pro inflammatory IL6 and TNF alpha has been found to have crucial role in the pathogenesis of HT, most of the published data agreed with the finding of increases serum level in patients with HT irrespective to the clinical state[28,29]. To date no available data regarding the serum level of these proinflammatory cytokines in thyroidectomised patients with HT. Yet no available research has depict the changes in the IL17 serum level in

patients with HT after thyroidectomy. IL22 has a potential role in autoimmune disorders including HT, through its ability to induce other pro inflammatory cytokines[30]. Our results are associated with only modest increased serum level of IL-22 in HT patients preoperatively, results which has been demonstrated by[31]. Moreover our results declared no difference in serum level of IL-22 results between pre and post operative measurement in our sample. IL-23 is known to have potent stimulatory effect on Th-1 to differentiate, producing the Th-17 subpopulation. Ruggeri et al has recorded a significant increase in serum level as compared with healthy control[32], While results displayed by Fatemeh et al declare a non significant difference in serum level of IL-23 between patients with HT and healthy control[31]. Our data revealed an abnormally high serum levels of IL-23 in the pre operative measurement which was statistically different from the post operative one, in which the levels approximately halved and remained static in an abnormally higher level for the following 6 months period. These parameters are indicative that the immune process is still working in spite of removal of the thyroid gland. The mechanisms, by which the auto reactive T cells escape deletion earlier and subsequent anergy, and activated thereafter remain elusive. Furthermore it has been found that patients with HT have elevated levels of Th-17 and its associated pro inflammatory cytokines both in the thyroid and peripheral blood[27]. The natural T regulatory cells regarded by many as the natural suppressor of T reactive cells and responsible for maintaining the peripheral tolerance[33], In HT they found to be dysfunctional and loss their immunosuppressive function and in the affected patients, and possibly converted to a pro inflammatory cells (Th- 1 and Th -17)[34]. On the other hand the coexistence of HT and other autoimmune diseases like diabetes and Addison disease and the existence of autoimmune polyendocrine syndrome type II, supplement the involvement of generalized autoimmune process rather than isolated thyroid targeted autoimmunity[35]. Moreover the development of Hashimotos related encephalopathy which has immunologically based pathogenesis, may uncover the prevailing autoimmunity[36].

Conclusion

The level of selected circulating immune parameters which was essentially elevated before thyroidectomy in patients with Hashimoto's thyroiditis, exhibit only partial reduction after total surgical removal of the gland. Therefore, in autoimmune disease extirpation of the target organ will not ablate the autoimmunity in particular patient.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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