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AUTOIMMUNE THYROID DISORDERS - AN UPDATE

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ABSTRACT

Background : Autoimmune thyroid disease (AITD), a common organ specific autoimmune disorder is seen mostly in women between 30-50 yrs of age.Thyroid autoimmunity can cause several forms of thyroiditis ranging from hypothyroidism (Hashimoto's thyroiditis) to hyperthyroidism (Graves'Disease). Prevalence rate of autoimmune mediated hypothyroidism is about 0.8 per 100 and 95% among them are women. Graves' disease is about one tenth as common as hypothyroidism and tends to occur more in younger individuals. Both these disorders share many immunologic features and the disease may progress from one state to other as the autoimmune process changes. Genetic, environmental and endogenous factors are responsible for initiation of thyroid autoimmunity. At present the only confirmed genetic factor lies in HLA complex (HLA DR-3) and the T cell regulatory gene (CTLA 4). A number of environmental factors like viral infection, smoking, stress & iodine intake are associated with the disease progression. The development of antibodies to thyroid peroxidase (TPO) thyroglobulin (TG) and Thyroid stimulating hormone receptor (TSH R) is the main hallmark of AITD. Circulating T Lymphocytes are increased in AITD and thyroid gland is infiltrated with CD4+ and CD8+ T Cells. Wide varieties of cytokines are produced by infiltrated immune cells, which mediate cytotoxicity leading to thyroid cell destruction. Circulating antibodies to TPO and TG are measured by immunofluorescense, hemagglutination, ELISA & RIA. TSHR antibodies of Graves' disease can be measured in bioassays or indirectly in assays that detect antibody binding to the receptor.

KEY WORDS

Autoimmune thyroid disease, thyroid peroxidase antibodies, Thyroglobulin antibodies, and TSHR antibodies

INTRODUCTION

Autoimmune thyroid disease (AITD) is a common organ specific autoimmune disorder affecting mostly the middle aged women. About 2 to 4 percent of women and up to 1% of men are affected worldwide, and the prevalence rate increases with advancing age (1). AITD comprises a series of interrelated conditions including hyperthyroid Graves' disease (GD), Hashimoto's (goitrous) thyroiditis, atrophic autoimmune hypothyroidism, postpartum thyroiditis (PPT) and thyroid associated orbitopathy (TAO) .Out of all these diseases, Hashimoto's thyroiditis (HT) and Graves' disease (GD) are the commonest type and share many features immunologically. One form of the disease may change to other as the course of the immune process progresses. Autoimmune hypothyroidism (AH) affects about 5 to 10% of middle aged and elderly women. Graves' disease (GD) is about one tenth as common as hypothyroidism affecting mostly the younger individuals (2). Many of these patients progress to hypothyroidism either spontaneously after treatment with antithyroid drugs or iatrogenically after radioiodine therapy or surgery. The development of antibodies to thyroid peroxidase (TPO), thyroglobulin (TG) and thyroid stimulating hormone receptor (TSH-R) is the main hallmark of AITD (3).

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ETIOLOGY

The etiology of AITD is multifactorial. Susceptibility to the disease is determined by a combination of genetic, environmental and constitutional factors.

Genetic Factors:

Numerous studies show a higher frequency of AITD in family members of patients with autoimmune hypothyroidism and Graves' disease (4). Both types of the disease cluster together in families, provides additional support that these conditions share common etiologic and pathogenic features. The autoimmune polyglandular syndrome type 2, involves the occurrence of autoimmune thyroid dysfunction with other autoimmune diseases. (Type 1 diabetes

mellitus, Addison's disease, pernicious anemia & vitiligo). Shared genetic factors are likely in this group of autoimmune disorders.

Twin studies show increased concordance of GD and AH in monozygotic (MZ) twins, compared with dizygotic (DZ) twins. A recent investigation based on a population of 8,966 Danish twins has shown that concordance for GD in MZ twins was 35% compared with 3% in DZ twins (5). Various techniques have been employed to identify the genes contributing to the etiology of AITD, including candidate gene analysis and whole genome screening. These studies have enabled the identification of several loci (genetic regions) that are linked with AITD, and in some of these loci putative AITD susceptibility genes have been identified. Some of these genes/loci are unique to Graves' disease (GD) and Hashimoto's thyroiditis (HT) and some are common to both diseases, indicating that there is a shared genetic susceptibility to GD and HT. The putative GD and HT susceptibility genes include both immune modifying genes (e.g. HLA, CTLA-4) and thyroid specific genes (e.g. TSHR, TG). Most likely these loci interact and their interactions may influence disease phenotype and severity (6).

The most important susceptibility factor so far recognized is association of AITD with HLA-DR alleles. These MHC class-II genes play a critical role in the initiation of adaptive immune response. HLA-DR3 is the best-documented genetic factor for GD and AH in Caucasians (7). In non-white populations, GD is associated with different HLA alleles. For example, it is associated more with HLA B35, B46, A2, and DPB1*0501 in Japanese; (8) A10, B8, and DQw2 in Indians; (9) and DR1 and DR3 in South African blacks (10).

The cytotoxic T-lymphocyte antigen-4 (CTLA-4) gene, encoding a negative regulator of the T-lymphocyte immune response, had been reported to be associated and/or linked to AITD. Recently, AITD susceptibility in the Caucasians was mapped to the 6.1-KB3'UTR of the CTLA-4 gene, in which the three single nucleotide polymorphisms (SNPs) CT60, JO31, and JO30 were strongly associated with AITD. The SNP, JO31 was most significantly associated with AITD in the Japanese, whereas the association of the JO30 with AITD was not observed (11). Ueda H & coworkers have identified polymorphism of CTLA-4 gene that is a candidate gene for common autoimmune disorders like GD, AH and Type-1 Diabetes (TID). The disease susceptibility was mapped to a non-coding 6.1 KB 3' region of CTLA-4, the common allelic variation of which was correlated with lower mRNA levels of the soluble alternative splice form of CTLA-4 (12). It was recently shown, that the A/G single nucleotide polymorphism (SNP) at position 49 in exon 1 of the cytotoxic T lymphocyte-associated molecule-4 gene in 148 Chinese Graves' disease (GD) patients, was associated with the relapse of the hyperthyroidism after antithyroid withdrawal. A/G polymorphism of the cytotoxic T lymphocyte-associated molecule-4 gene affects the progress of GD. The G/G genotype is associated with poor outcome (13).

Environmental Factors:

Iodine: It has been well documented that the incidence of AITD is proportional to dietary iodine content. In Europe the prevalence of GD increases with national iodine intake programs. Iodine increases the antigenicity of TG with exacerbation of experimental thyroiditis in animals. Recent in vitro studies in NOD.H2^{h4} mouse have shown that high iodine doses alone may damage thyrocytes and enhances the disease progression in a dose-dependent manner (14).

Infection: No convincing evidence has indicated a role infection in AH except congenital rubella syndrome. An association has been proved between Yersinia infection and GD (15). Yersinia contains proteins that mimic TSH-R immunologically. Recently, retrovirus has received attention but the results are conflicting (16).

Stress: Some studies suggest an association between antecedent major life events and Graves' disease, but a causal role of stress in autoimmune process remains to be clearly established. Smoking is a minor risk factor for the development of thyroid ophthalmopathy (17). The female preponderance of thyroid autoimmunity is most likely due to the influence of sex steroids. Estrogen use is associated with a lower risk, and pregnancy with a higher risk for developing hyperthyroidism (18).

PATHOLOGY

In Hashimoto's thyroiditis there is an extensive infiltration of thyroid by lymphocytes, plasma cells and macrophages. There is formation of germinal center and giant (Langerhans) cell can occur. The thyroid follicular cells are destroyed to a variable extent, depending on the chronicity of the disease. During this process the remaining cell become hyperplastic and undergo oxyphilic metaplasia, which gives rise to the so-called Askanazy or Hurthle cells.

The pathologic features of Graves' disease are often obscured by prior treatment with antithyroid drugs. There is hypertrophy and hyperplasia of the thyroid follicles, the epithelium is columnar and the colloid shrinks. In addition a variable degree of lymphocytic infiltration is present, sometimes with germinal center formation.

Autoimmune features:

All forms of thyroid autoimmunity are associated with

a lymhocytic infiltrate in the thyroid, and these lymphocytes are largely responsible for generating both T and B Cell-mediated autoreactivity. Other sites such as thyroid draining lymph nodes and bone marrow may also contain thyroid autoreactive lymphocytes in AITD. The initial autoimmune response by CD4+ T cells appears to up regulate the secretion of IFN-g resulting in the enhanced expression of MHC class II molecules on thyrocytes. This most likely triggers expansion of autoreactive T cells and gives rise to the characteristic inflammatory response and as the disease progresses; thyrocytes are targeted for apoptosis resulting in hypothyroidism. Another contributing factor to the observed hypothyroidism in Hashimoto's thyroiditis patients could be the circulating TSH inhibitory antibodies. Graves' disease on the other hand represents the other end of spectrum wherein the patients suffer from hyperthyroidism. The activation of thyroid specific CD4+ T cells leads to the recruitment of autoreactive B cells and the mounting of thyroid stimulatory immune response via anti-thyroid antibodies (19).

Autoantibodies:

i. Thyroid Peroxidase (TPO) antibodies:

TPO is the key thyroid enzyme catalyzing both the iodination and coupling reaction for the synthesis of thyroid hormone. It is membrane bound and found in the cytoplasm and in high concentration on the apical microvillar surface of thyrocytes. It is of mol wt between 100 to 105-kDa and previously was known as thyroid microsomal antigen (20). Multiple T & B Cell epitopes exists within the molecule and the antibody response to TPO is restricted at the level of the germ line heavy and light chain variable (V) region (21).

Anti-TPO autoantibodies are found in over 90% of patients with autoimmune hypothyroidism and Graves' disease. Together with TG antibodies these are the predominant antibodies in AH. Anti-TPO antibodies are mainly of the IgG class with IgG_1 and IgG_4 subclasses in excess (22).

ii. Thyroglobulin (TG) Antibodies

TG is a 660-kDa glycoprotein composed of two identical subunits of 330 kDa each. It is secreted by the thyroid follicular cells into the follicular lumen and stored as colloid. Each TG molecule has around 100 tyrosine residues, a quarter of which are iodinated. These residues couple to form the thyroid hormones triiodothyronine (T_3) and thyroxin (T_4). The sequence of human TG has been determined (23). When TSH stimulates the thyroid cell, TG is endocytosed and hydrolyzed in lysosome releasing T_3 & T_4.The exact location of T and B cell epitopes within TG is uncertain (24). A key T cell epitope in the spontaneous thyroiditis of OS chickens contains iodine, and it has been found out that poorly iodinated TG is only weakly immunogenic (25).

Thyroglobulin autoantibodies are found in less than 60% of patients with lymphocytic thyroiditis and 30% of Graves' disease patients. They are polyclonal and mainly of IgG class with all four subclasses represented. TSH regulates the cell surface expression of TPO and TG altering the mRNA transcription of these two proteins, possibly at the gene promoter level. These effects are mimicked by auto antibodies (both blocking and stimulating) in the sera of the patients with GD (26).

iii. Thyroid Stimulating Hormone receptor (TSH-R) Antibodies

TSH-R is the prime autoantigen in Graves' disease and atrophic thyroiditis. It is located on the basal surface of thyroid follicular cells. In Graves' disease thyroid stimulating antibodies (TSAbs) bind to the receptor and stimulate the thyroid cell to produce excessive amount of thyroid hormones resulting in hyperthyroidism. In patients with atrophic thyroiditis the major antibody is the TSH-R blocking antibody. After binding to the receptor this antibody blocks the binding of TSH to its receptor, thus preventing stimulation of thyroid cell. This results in diminished thyroid hormone output, atrophy of thyroid gland and the clinical state of hypothyroidism.

TSH-R has 398 amino acid extra cellular domains, a 266 amino acid transmembrane domain (organized in seven loops) and an 83 amino acid intracellular domain. Antibodies binding to the amino terminal area are stimulatory where as those binding to amino acids 261 to 370 or 388 to 403, near the cell surface, have blocking activity (27). As with TPO multiple T and B cell epitopes has been defined within the molecule.

iv. Other antibodies

Na^+ / I^- symporter (NIS) is the fourth major thyroid autoantigen; first demonstrated using cultured dog thyroid cells. Around a third of Graves' disease sera and 15% of Hashimoto's sera contain antibodies that inhibit NIS mediated iodide uptake in vitro (28). Antibodies to thyroid hormone can be found in 10% to 25% of patients with AITD and non-specific autoantibodies against DNA, tubulin and other cytoskeletal proteins can also be detected in a small proportion of patients.

MECHANISM OF THYROID CELL INJURY

Several antibody and cell-mediated mechanisms contribute to thyroid injury in autoimmune hypothyroidism. Different groups have analyzed partners in the diseased thyroid glands. In general, in case of Hashimoto's thyroiditis, the expressions of death receptors such as CD95 and death receptor

ligands such as CD95L and TRAIL in the thyroid tissue appear to be much higher compared to their normal counterparts. Also, the expression of positive effectors of apoptosis such as caspase 3 and 8, as well as Bax and Bak appear to be relatively high in thyroiditis samples as compared to controls. This expression pattern clearly supports enhanced apoptosis as the mechanism underlying the loss of thyrocytes in Hashimoto's thyroiditis. In contrast, the opposite trend appears to be the norm in Graves' disease. One striking feature is the highly elevated expression of negative modulators of apoptosis such as cFLIP, Bcl-2 and Bcl-XL and close to normal expression of the caspases in thyrocytes analyzed from Graves' patients, clearly supporting a role for apoptosis inhibitory mechanisms in these patients. Although in both cases there is significant expression of Fas/CD95 and its ligand, only in Hashimoto's thyroiditis, the thyrocytes undergo apoptosis. Recent work by Stassi *et al.* solved the puzzle by exposing the role of cytokines in the development of autoimmune disorders (29). In case of Hashimoto's thyroiditis, a TH1 disease, the cytokine IFN-g appears to play a crucial role in the pathology of the disease by enhancing the expression of caspases and there by sensitizing cells to FAS mediated apoptosis. In contrast in the TH2 mediated Graves' disease, the cytokines IL4, and IL-10 strongly up-regulate the expression of two anti-apoptotic proteins Bcl-XL and cFLIP, which offer resistance to Fas mediated apoptosis. This again proves the necessary modulatory roles played by the TH1 and TH2 cytokines in the development of autoimmune disorders (19).

B Cell responses

TG and TPO antibodies occur in very high concentration in patients with Hashimoto's thyroiditis and primary myxedema. These antibodies are less common, but still frequent in Graves' disease, where as TPO rather than TG antibodies are frequent in postpartum thyroiditis (30). Both of the antibodies show partial restriction to the IgG1 and IgG4 subclass (21). TG antibodies usually mediate Antibody mediated cytotoxicity (ADCC), where as TPO antibodies form terminal complement complexes within the thyroid gland. Cell mediated injury may be necessary for TPO antibodies to gain access to their antigen and become pathogenic (31).

Thyroid stimulating antibodies (TSAbs) of Graves' disease are detected in 95% of cases. These antibodies are often k chain restricted and of the IgG1 subclass. TS-Abs also occurs in 10% to 20% of patients with autoimmune hypothyroidism (AH) but their effects are obscured by TSH-R-blocking antibodies and destructive processes (27). TSHR is a member of G protein coupled receptor family. It's stimulation by TSH leads to intracellular signaling by cyclic adenosine mono phosphate (cAMP) pathway.

T-Cell response

Both CD4+ and CD8+ T-Cells occur in thyroid lymphocytic infiltrate with a preponderance of CD4+ cells. There is an increase in activated T Cell expressing markers like HLA-DR. A wide array of cytokines including IL-2, Interferon (IFN-g), Tumor necrosis factor (TNF-a), IL-4, IL-6, IL-10, IL-12, IL-13 and IL-15 are produced by the lymphocytes with some variation between patients (32). Thyroid cells express MHC class-II molecules as well as other immunologically important molecules and behaves as an antigen-presenting cell (APC). Expression of ICAM-1, LFA-3 and MHC class I molecules by thyrocytes is enhanced by IL-1, TNF-a & IFN-g (33). This response increases the ability of cytotoxic T cells to mediate lysis. Thyroid cell destruction is mediated both by perforin containing Cells which accumulate in the thyroid and by Fas dependent mechanisms (34,35). Cytokines and other toxic molecules such as nitric oxide and reactive oxygen metabolites probably also contribute directly to cell mediated tissue injury. (Fig 1 & Fig 2)

Humoral immunity exacerbates cell-mediated damage in a secondary fashion, both by direct complement fixations (TPO antibodies) and by ADCC (36). Complement attack initiated via the classic or alternative pathway, impairs the metabolic function of thyroid cells and induces them to secrete IL-I, IL-6, reactive oxygen metabolites and prostaglandin. All of these enhance the autoimmune response.

As well as T and B cell, dendritic cells and monocyte/ macrophages accumulate in the thyroid. Presumably they play a major role as APC & capable of providing co-stimulatory signals. Thyroid cell-derived monocyte chemoattractant-I, produced after TNF-a, IFN-g, or IL-I stimulation, is likely to be responsible for the accumulation of monocytes, which are important source of cytokines (37).

CLINICAL FEATURES

The main clinical features of hypothyroidism are summarized in Table-1. Patients with Hashimoto's thyroiditis may present a goiter which varies from small to large in size. It is usually firm and painless often with an irregular bosselated surface. The goiter in case of GD is diffusely enlarged and firm in consistency. Increased blood flow may be manifested by a thrill or bruit. The clinical features of hyperthyroidism are summarized in Table –2.

DIAGNOSIS OF AITD

Diagnosis of AITD is based upon clinical features & supported laboratory investigations. The patient may be euthyroid, hypothyroid or hyperthyroid, according to disease type and stage. Investigations used to determine the existence and cause of both hypo and

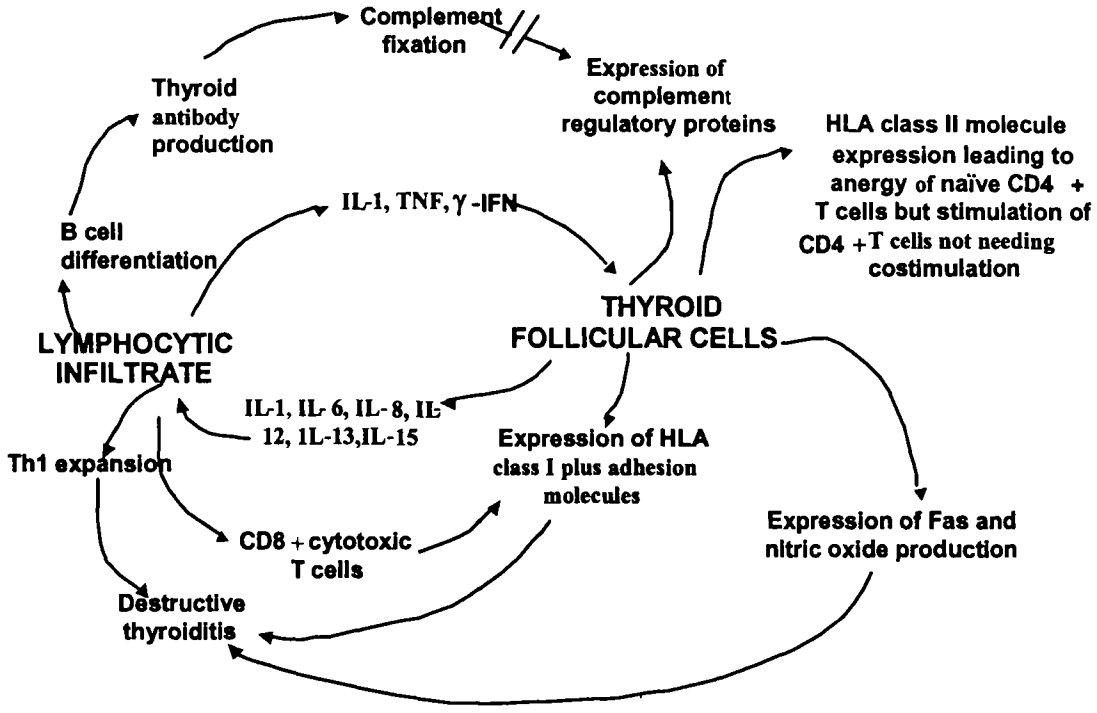


Fig 1. Interaction between thyroid cells and the immune system via cytokines.

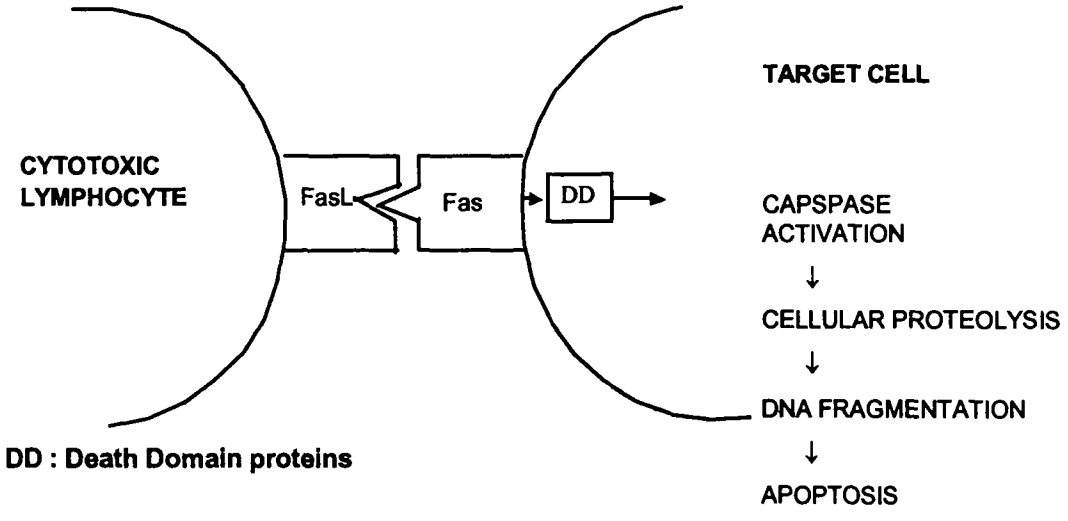


Fig 2. Interaction between Fas ligand (FasL) on cytotoxic lymphocyte and Fas on a target cell leading to apoptosis

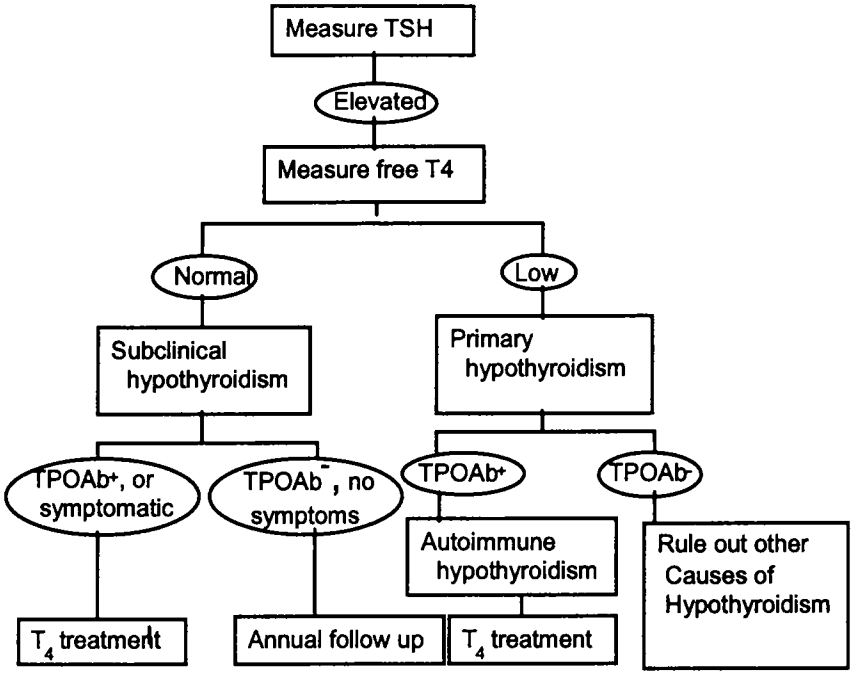
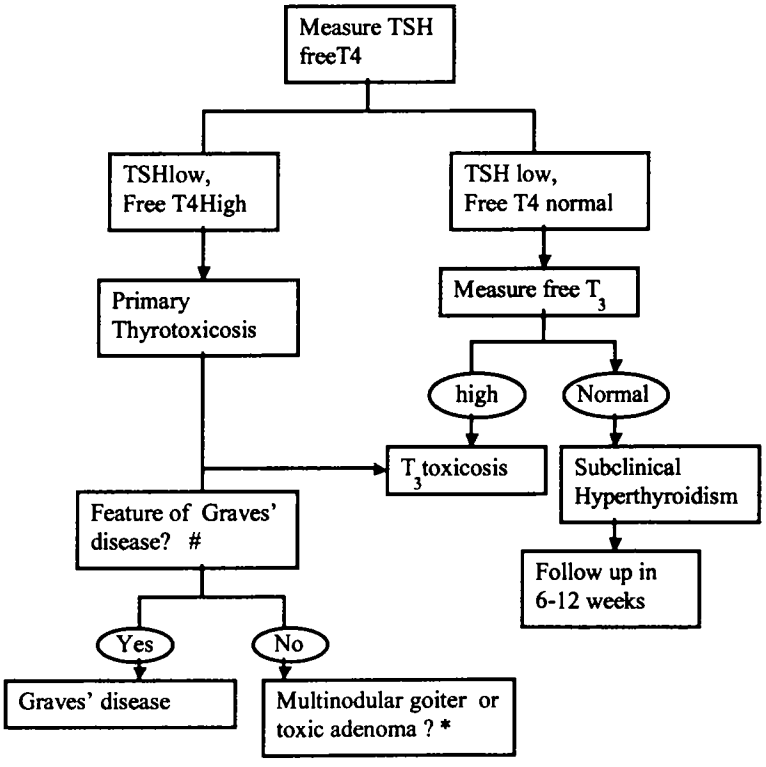


Fig 3. Evaluation of hypothyroidism



Diffuse goiter, positive TPO antibodies, ophthalmopathy * Confirmed by Radionuclide scan

Fig 4. Evaluation of hyperthyroidism

TABLE I. Signs & Symptoms of Hyperthyroidism

Symptoms	Signs
Weight Loss	Fine hair,thin skin
Fatigue	Muscle weakness
Menstrual irregularities	Low cholesterol
Heat intolerance	Glucose intolerance
Increased Sweating	Tachycardia
Hyperdefecation	Widened pulse pressure
Nervousness	Tremor,rapid deep tendon reflexes
Restlessness	Stare, Lid lag.

TABLE-2 Signs & Symptoms of Hypothyroidism

Symptoms	Signs
Weight gain	Growth retardation
Easy fatigue	Deep, hoarse voice
Lethargy	Dry coarse skin
Cold intolerance	Myxedema
Hair Loss	High cholesterol
Constipation	Bradycardia
Depression	Slow reflex relaxation

Table-3

Free T3	1.4–4.4	PG/ML
Free T4	0.71–1.85	NG/DL
TSH (3rd generation)	0.400–4.000	μIU/ML
Test technique ➡ CHEMILUMINESCENCE		
TPO antibodies	<35 negative 35–50 equivocal >50 positive	U/ML
TG antibodies	<225 negative 225–325 equivocal >325 positive	IU/ML
Test Technique ➡ ELISA		

hyperthyroidism are summarized (Fig 3 & Fig 4).

AITD is detected most easily by measuring circulating antibodies against TPO & TG. A negative test for both the antibodies virtually excludes AITD, as 98% of patients are positive for either antibody. TPO Ab is more specific and sensitive than TG Ab in diagnosis of AH. Elevated TSH with TPO antibodies is the gold standard for diagnosis of chronic HT (Hashimoto's thyroiditis). TSH Abs that stimulates the TSH-R in GD is measured to predict neonatal thyrotoxicosis. They can be measured by radio receptor assays or bioassays. Thyroid panel tests to detect AITD are described in Table-3.

TREATMENT

Patients with Autoimmune hypothyroidism and TSH-R-blocking antibodies enter remission after T_4 treatment. Goiter size in HT reduces by around a third over a 2-year period and thyroid autoantibodies can remain elevated or decline with T_4 supplementation (38)

Treatment with antithyroid drugs (carbimazole, methimazole, propylthiouracil) for Graves' disease leads to a decline in TSAbs and other thyroid antibodies. There is also a decline in severity of thyroiditis as well as other immunologic changes (39). Propranolol or other long acting beta-blockers, such as atenolol may be useful to control adrenergic symptoms especially in early stages before antithyroid drugs take effect. Radioiodine causes progressive destruction of thyroid cells and can be used as initial treatment or for relapses after a trial of antithyroid drugs.

Further understanding of the immunologic basis of Graves' disease will ultimately lead to immunologically based treatment aimed at reinducing tolerance to TSH-R. Already, pilot studies have been performed to assess the potential for oral tolerance with the use of TG (40), and it is possible that any such treatment might have benefit for ophthalmopathy as well. On the other hand, T_4 is such simple treatment for autoimmune hypothyroidism that at present, novel treatments are most unlikely.

AITD AND NEOPLASMS:

Thyroiditis and thyroid antibodies are found in a quarter to a third of patients with thyroid cancer, and such patients have an improved prognosis (41). Preexisting Hashimoto's thyroiditis is the major risk factor for the development of non-Hodgkin's lymphoma of thyroid (42). Studies show an increased frequency of autoimmune thyroiditis in women with breast cancer (43).

CONCLUSION

Autoimmune thyroid disease is the result of a complex interaction between genetic and environmental factors. The disease results when the autoreactive lymphocytes escape tolerance or ignorance. Both cell mediated and humoral immune responses contribute to tissue injury in autoimmune hypothyroidism. In

Graves' disease, production of TSAbs leads to hyperthyroidism. The multistep development of disease suggests that it will be possible to restore normal tolerance and treat Graves' disease immunologically. Current approaches to medical therapeutic intervention in AITD include the use of monoclonal antibodies to selectively deplete specific T lymphocytes subsets and blocking of the T-Cell receptor MHC interaction, by vaccination with chemically altered auto antigens.

ABBREVIATIOINS

CTLA-4 (Cytotoxic T- lymphocyte antigen-4), **ADCC**(Antibody dependant cell mediated cytotoxicity) **ICAM** (Intercellular adhesion molecule), **LFA** (Lymhocyte function associated antigen)

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