ANALYSIS OF THYROID-STIMULATING HORMONE RECEPTOR ANTIBODIES IN VARIOUS THYROID DISEASES

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CERTIFICATE

This is to certify that this dissertation on " **Analysis of thyroid-stimulating hormone receptor antibodies in various thyroid diseases**" is a bonafide dissertation done by Dr.S.Zahir Hussain in Madras Medical College under the supervision and guidance of Prof. M. Chandrasekaran, and is submitted to "The Tamilnadu Dr. M.G.R. Medical University", Chennai in partial fulfillment of the requirement for the M.Ch (Endocrine Surgery) degree.

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ABSTRACT

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Objective:

To study the Thyroid Stimulating Receptor Antibodies (TSHRAb) level in various thyroid Disease by analyzing the TSH receptor antibodies level and comparing it with other thyroid antibodies and to analyze the importance of its estimation in various Thyroid Diseases with Hyperthyroidism or Hypothyroidism.

Design:

70 patients of various thyroid disease admitted in the period between 2008-2010 were included in the study. After classifying the patients on the basis of anatomical, physiological and pathological diagnosis, serum levels of thyroid-stimulating hormone receptor antibodies (TSHRAbs) were assayed along with antithyroperoxidase antibodies and antithyroglobulin antibodies. Clinical features like eye signs, tremors and thyromegaly were assessed. Results were tabulated and analyzed.

Results:

Out of the 70 patients included in this study, 29 patients were found to have elevated TSHRAb levels. Elevated TSHRAb levels correlated with serum alkaline phosphatase levels, hyperthyroidism, Graves' disease and ophthalmopathy. Out of 60 patients with multinodular goiter, 25 had elevated levels of TSHRAbs, which indicated the association of autoimmune thyroid disease (Graves' disease) with multinodular goiter.

Conclusion:

The levels of TSHRAb grossly parallel the degree of hyperthyroidism, the grade of thyromegaly and presence of ophthalmopathy. Elevated levels of Serum Alkaline phosphatase along with TSHRAb in hyperthyroidism may be taken as a marker for severity of Graves' disease.

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Introduction and objectives

1. INTRODUCTION AND OBJECTIVES

1.1 INTRODUCTION

Detection of thyroid antibodies can be helpful in establishing the diagnosis of autoimmune thyroid disease.

All autoimmune thyroid diseases share common immunological markers.[1] Antibodies against TSHR represent only one family of thyroid antibodies. Others include TPO antibodies and antithyroglobulin antibodies.

Historically, thyrotoxicosis in Graves' disease has been known to be caused by the action of THSR stimulation antibodies. Also, it was believed that these antibodies are never found in other diseases affecting the thyroid gland.[2] However, TSHR antibodies (TSHRAbs) have been found in about 20 % of other thyroid diseases.[3]

The nomenclature for the thryotropin receptor antibodies (TRABs) is complex and largely dependent on the assay used to detect these antibodies in serum. The assays that measure the displacement of radio-labelled TSH from its receptor by serum immunoglobulins detect TRAbs regardless of their functional activity. These antibodies have also been termed as TSH-binding Inhibitory Immunoglobulins (TBIIs).[4] TSH-stimulating antibodies (TSAbs) are essentially the cause of hyperthyroidism in Graves' disease. TSH-blocking antibodies (TBAbs) are the causative factor for atrophic auto-immune thyroiditis.[5] However, in vitro, the TSHRAbs might act as a stimulator or blocker depending upon other factors.[6]

THSR antibodies are pathogenically capable of activating or blocking TSH receptor function as demonstrated by the occurrence of trans-placentally transmitted hyperthyroidism or hypothyroidism in the fetus of mothers with high enough levels of stimulating or blocking TSHR antibodies in circulation.

Pathogenic antibodies could be reviewed as the ideal marker for the diagnosis and management of the corresponding autoimmune disease.

Indication for TRAb determination include the following :

- The detection or exclusion of autoimmune hyperthyroidism and its differentiation from disseminated autonomy of the thyroid gland. The presence of TRAb indicates that the patients thyrotoxicosis is of autoimmune etiology rather than due to toxic nodular goiter. Because the aim of treatment for Graves disease may differ from the treatment of other forms of thyrotoxicosis, an initial TRAb determination is clearly of value.

- Monitoring the therapy of Graves disease patients and prediction of relapse, thereby constituting an important decision-making aid in the management of the treatment. TRAb levels tend to fall during antithyroid drug therapy for Graves disease. Low levels or the absence of TRAb after a course of drug treatment may indicate disease remission, and therefore the withdrawal of therapy can be considered.

- TRAb measurement during the last trimester of pregnancy. Because TRAb are IgG-class antibodies, they cross the placenta and can cause neonatal thyroid disease. The measurement of TRAb during pregnancy in patients with history

of thyroid disease is therefore important in assessing the risk of thyroid disease in the neonate.

With this background, this study was conducted with an aim to study the TSH receptor antibody level in various thyroid diseases.

1.2 Objectives :

To study the TSH receptor antibodies level in various Thyroid Disease by analysing the TSH receptor antibodies level and comparing it with other thyroid antibodies and to analyze the importance of its estimation in Autoimmune Thyroid Diseases with Hyperthyroidism or Hypothyroidism

Chapter 2

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1 ANTI-TSH RECEPTOR ANTIBODIES IN CLINICAL PRACTICE

Autoimmune thyroid diseases are the more prevalent organ-specific autoimmune disorders in humans. They encompass a wide spectrum of clinical presentations, with hyperthyroid Graves' disease at one end and atrophic myxedema at the other. Whether Graves' disease and lymphocytic thyroiditis represent different aspects of the same disease or different diseases remains unclear. Autoimmune thyroid diseases share common immunologic markers-mono nuclear cell infiltration of the thyroid-and circulating antithyroid antibodies, the specificities of which might, in part, account for the diversity of these diseases. Autoantibodies against the thyroid-stimulating hormone receptor (TSH receptor antibodies, (TSHR-Ab) represent only one family of thyroid autoantibodies. Others include antithyroperoxidase (TPO-Ab), the former antithyroid microsomal antibodies, antithyroglobulin, and the recently identified anti-sodium/iodide symporter antibodies. Historically, TSHR-Ab have been associated with hyperthyroid Graves' disease.[7] Indeed, contrary to the other antithyroid antibodies, TSHR-Ab are pathogenic, capable of activating or blocking TSH receptor functions, as demonstrated by the occurrence of transplacentally transmitted hyperthyroidism or hypothyroidism in the fetus of mothers with high enough levels of circulating stimulating or blocking TSHR-Ab.

Pathogenic antibodies could be viewed as the ideal marker for the diagnosis and management of the corresponding autoimmune disease. The various conditions

discussed in this article that could benefit from the assay of TSHR-Ab include the following.

Graves' disease

TSHR-Ab at diagnosis of hyperthyroid Graves' disease

Diagnostic value

Severity marker

An aid to the choice of treatment

TSHR-Ab and the management of antithyroid drug (ATD) treatment

TSHR-Ab and ablative treatments

Special conditions

Graves' disease and pregnancy

Graves' disease in children and adolescents

Extrathyroidal manifestations of Graves' disease

Graves' disease and thyroid carcinoma

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Clinical usefulness of TSHR-Ab assays

Transient transplacental neonatal hypothyroidism

Spontaneous remission from autoimmune hypothyroidism

Depending on the clinical setting, the determination of either stimulating or blocking TSHR-Ab is discussed. The presence of TSHR-Ab is the hallmark of Graves' disease. Nevertheless, TSHR-Ab may be detected in other conditions, indicating the possible association of Graves' disease with other thyroid diseases.

2.2 AN OVERVIEW OF THE TSHR-Ab ASSAYS

Currently, two approaches are used to detect TSHR-Ab. One approach is based on the competition between the antibody and TSH for binding to the TSH receptor (TSH-binding inhibitory immunoglobulin (TBII). The other approach is based on the changes in the functional status of the receptor induced by the anitbody-receptor interaction. Both approaches are biologic methods. The competitive assay is commercially available using[8] bovine TSH and solubilized porcine TSH receptor. The functional assay measures the production/accumulation of cAMP by stably transfected chinese hamster ovary cells with the human TSH receptor. The competitive assay is not indicative of any functinal activity of the antibody. On the contrary, only functional assays can identify whether the antibody in agonistic (thyroid-stimulating antibody (TSAb) or antagonsitic [TSH stimulation blocking antibody (TSHAb)]. These TSBAb may be present in the same patients with stimulating antibodies, the effect of which is also inhibited, the overall activity being the algebraic sum of the two levels of activity.

Because competitive and functional assays do not necessarily measure the same type of interaction, it is not surprising that there is no continuous correlation between the results of the two assays. Nevertheless, sera with high levels of TBII are usually also positive in stimulation assays, except when TSBAb are predominant.

Methodologic improvements have recently been proposed, taking advantage of use of the recombinant human TSH receptor. Autoantibody binding to membrane-expressed TSH receptor can be detected by fluorescence[9] with good sensitivity or immunocytochemistry.[10] Immunoprecipitation of in vitro translated TSH receptor might represent a fruitful approach ; however, persisting difficulties associated with the large-scale production of recombinant human TSH receptor of suitable conformation and glycosylation still limit the development of direct and specific immunodetection of TSHR-Ab. Other approaches in progress are attempting to delineate functional epitopes on the TSH receptor and may lead to assays able to distinguish stimulating antibodies from blocking antibodies. In the meantime, a TBII assay with improved senstivity has been recently established that uses immobilized solubilized recombinant human TSH receptor and either I-labeled or chemiluminescent bovine TSH.[11] This new assay must be evaluated on a larger scale. Improvements in fucntional assays have also been proposed that would allow less cumbersome techniques with higher throughput.

Clearly, there is a need for standardization of the techniques and reagents used, and cooperative multilaboratory quality control studies need to be performed. Multicenter studies are also needed to evaluate the prevalence and levels of TSHR-Ab among patients from various populations because geographic or ethnic differences might account for divergences in the usefulness of TSHR-Ab assays.

2.3 GRAVES' DISEASE AND TSHR-Ab ASSAYS

Diagnosis of Hyperthyroid Graves' Disease

Diagnostic Value of the TSHR-Ab

The prevalence of TSHR-Ab at diagnosis of hyperthyroid Graves' disease ranges from 70% to 100%. . The initial TSAb activity averages 200% to 300% as expressed in percent increase of basal cAMP production. These relatively low levels of TSHR-Ab activity in routine assays are consistent with the result of quantitative studies indicating that serum from the average patient with Graves' disease contains TSHR-Ab concentration much lower than 1 to 5 µg/ml, whereas TPO-Ab may reach concentrations of 1 mg/mL. Moreover, as mentioned previously, optimization of the sensitivity of the TSHR-Ab assays is still in progress. With current methods, the proportion of patients with undetectable TSHR-Ab before treatment averages 10%.[12] In the study by Ilicki and co-workers,[12] in all of the initially TSHR-Ab-negative patients, TBII and TSAb became detectable 3 months after radioiodine therapy. On the basis that TSHR-Ab-negative cases usually present with smaller goiters and lower levels of thyroid hormones and radioiodine thyroid uptake, it has been proposed that they might correspond to an early stage of the disease. Such cases could be characterized by more marked lymphocytic infiltration of the thyroid than in TSHR-Ab positive case, which could implicate local cytokine production in thyroid regulation.

Because the presence of TSHR-Ab is specific for Graves' disease, TSHR-Ab positivity in patients with nodular or multinodular toxic goiter is indicative of the combination of the two diseases.[13] This occurrence is common in areas with mild

iodine deficiency but can be recognized chemically only in the case of associated features of Graves' ophthalmopathy.

TSHR-Ab as a Marker of Severity

It is common observation that the level of TSHR-Ab grossly parallels the degree of hyperthyroidism as assessed by the serum levels of thyroid homones.[16, 34, 43, 56] TSHR-Ab levels, by no means, can be considered to merely reflect the agonist-effect relationship in vivo; however, such quantitative correlation is generally observed in the transplacental model of fetal hyperthyroidism, which suggests the existence, in the diseased thyroid of the patients, of alterations capable of modulating the nature or the expression of the interaction of TSHR-Ab with thyroid cells. TSHR-Ab levels more likely reflect the intensity or duration of the intrathyroidal inflammatory autoimmune reactions.

In general, good agreement has been observed between TSHR-Ab and thyroid volume in untreated patients with Graves' disease.[14] Whether the controversial thyroid growth immunoglobulins have any relevance to the process of autoimmune goitrogenesis remains unsettled. Rieu and co-workers[15] found that the volume of both hyperplastic and nodular tissue was correlated with TSHR-Ab in untreated patients with typical Graves' disease, confirming the growth stimulation potential of these antibodies on any thyroid tissue.

TSHR-Ab as an Aid to the Choice of Treatment

Several studies have assessed the usefulness of TSHR-Ab level determination at diagnosis as an aid to to the management of patients. Because the basic therapeutic

alternative for hyperthyroid patients with Graves' disease is medical, with a high risk of relapse, or radical/ablative, it has been questioned whether the assay of TSHR-Ab contributes to the allocation of the patient to the optimum treatment arm. There is no simple answer to that question, and it seems that TSHR-Ab determination is only one of the indicators to identify patients who are likely to achieve remission without ablative treatment.

The more recently reported studies[16] performed in various parts of the world with very different iodine environments suggest that, when considered alone, TSHR-Ab positivity or titre before treatment is significantly correlated or uncorrelated to the post-ATD treatment outcome. The initial TSHR-Ab status has not had high enough positive and negative predictive values for remission or relapse after completion of ATD treatment to support, by itself, the treatment decision in most patients. Nevertheless, taking into account the titre of TSHR-Ab and other indicators such as age, gender, thyroid volume, the severity of hyperthyroidism, and possibly, the presence of ophthalmopathy, subgroups of patients can be identified with a high or low risk of relapse In a study by Michelangeli and colleagues[17] in Australia of 104 patients followed up for 15 months to 5 years, the relapse rate in patients with an intial TBII greateer than 60 U/L was 68% as compared with the average figure of 54% for the whole cohort. In a study by Benker and co-workers,[18] 45% of the patients with high levels of TBII were euthyroid after 3 weeks of ATD therapy as compared with 67% of patients who were negative or borderline for TSHR-Ab. In that study of the initial response to ATD treatment, other predictors such as goiter size, urinary iodine excretion, and the severity of hyperthyroidism were as significant, or even more, than the titre of TSHR-Ab.

Although it is not necessay for the diagnosis of Graves' disease, except in some cases of multinodular goiter, initial TSHR-Ab measurement is useful as a marker of disease severity and may, in combination with other clinical indicators contribute to the treatment decision.

2.4 TSHR-Ab And The Management of ATD Treatment

Because recent surveys of treatment policies have shown that nondestructive therapy for hyperthyroid Graves' disease remains the preferred modality in most centers outside North America, the optimization of ATD therapy remains a valid topic of clinical research to find ways to minimize the relapse rate. Many studies of the dose and duration of ATD treatment have been reported. One question merits consideration: could the determination of TSHR-Ab during ATD treatment contribute to the adaptation of the treatment plan to each case? Is it of benefit for the patient to monitor the level of TSHR-Ab during treatment? Two types of studies have been performed. In one type, the evolution of the level of TSHR-Ab, that is, the rate of fall of TSHR-Ab, has been used as an indicator for treatment withdrawal or correlated with the relapse rate.[18] In the other type of study using a fixed predetermined dose and duration of ATD treatment, the TSHR-Ab status at the end of the drug course has been correlated with posttreatment outcome.

Studies of the first type have found that the rate of fall of TSHR-Ab (TBII) during ATD treatment is predictive of subsequent outcome. In the series reported on by Michelangeli,[17] 73% of TBII-negative patients remitted compared with 28% of TBII-positive patients, after 12 months of treatment; corresponding percentages were

70% and 17% after 18 months. TBII values measured at 6 months of treatment were not discriminant.

The duration of the course of ATD can be adapted to the TSHR-Ab status. In a study by Edan and co-workers,[8] 44 of 64 patients tested every 3 months in whom therapy was stopped when TSHR-Ab (TSAb) became negative were maintained on ATD for an average of 9 months (range, 3 to 18 months). Among these patients, the relapse rate was 41% in comparision with a rate of 92% for the patients who remained TSAb-positive after 18 months of treatment. The data reported by Cho and co-workers, who also compared fixed and adapted durations of ATD administration, are similar. Detailed analysis of these studies suggests that, even in the case of early disappearance of TSHR-Ab, the duration of ATD treatment should be 9 to 12 months to minimize the risk of relapse.

The studies investigating TSHR-Ab assays at the end of an ATD course of predetermined duration have been analyzed by Feldt-Rasmussen and colleagues.[28] This analysis clearly shows the value and limitations of the TSHR-Ab predictions. TSHR-Ab-negative patients had 65% less risk of relapsing than TSHR-Ab-positive patients. By increasing the cut-off level for TSHR-Ab positivity, it is possible to obtain a predictive value of a positive test of almost 1.00 but at the expense of lowering the predictive value of a negative test.[19] In the study by Vitti,[20] relapse was observed in 97.5% of the patients with high TSHR-Ab and in 41.4% of patients with low levels.

In contrast to the data reported by Hashizume and co-workers,[21] several studies have indicated that the administration of levothyroxine during and after

withdrawal of ATD treatment does not improve the outcome nor affect the evolution of TSHR-Ab levels.[22] Such trials have been based on the theoretic concepts of (1) the potential thyroidal immunosuppressive effect of ATD, leading to the use of a high dosage of the medication, and (2) the beneficial effect of "letting the thyroid test" by preventing any rise in TSH.

2.5 TSHR-Ab and Ablative Treatments

Radioiodine Treatment

Radioiodine efficiency does not seem to be dependent on TSHR-Ab status; however, a recent report identified a correlation between pretreatment TSHR-Ab level (both TBII and TSAb) and postirradiation outcome. There was an inverse relationship between the initial TBII level and the reduction in thyroid volume per megabecquerel retained. Also, residual hyperthyroidism was significantly correlated with higher pretreatment TSHR-Ab levels, and initial TSAb levels were higher in patients who became euthyroid when compared with patients who became hypothyroid. Initial TSHR-Ab levels seem to contribute to the thyroid resistance to radioiodine therapy. Supportive data have been reported by Chiovato and co-workers.[23] This resistance could result from increased I turnover or protection of thyroid tissue viability through reduced apoptosis or better regeneration.

Radioiodine treatment is associated with a transient increase in the level of TSHR-Ab and, in some cases, the appearance of blocking TSHR-Ab. The extent to which the postirradiation thyroid function evolution is dependent on changes in TSHR-Ab levels or activity is currently under study. Early transient hypothyroidism

might be associated with blocking TSHR-Ab, whereas the recovery of euthyroidism might be associated with an increase in stimulating antibodies. It is premature to recommend the routine assay of TSHR-Ab in the follow-up of radioiodine treatment.

Surgical Treatment

Subtotal thyroidectomy is effective when residual hormone output is insufficient to maintain hyperthyroid levels. TSHR-Ab determination has no practical routine usefulness in this setting except when it is of interest to study the evolution of circulating TSHR-Ab after removal of the thyroid (see further on), and when postsurgery outcome of thyroid function is partly related to TSHR-Ab status. After surgery, TSHR-Ab decline and become undetectable in most patients within 6 to 9 months.[24] The disappearance of TSHR-Ab from the circulation within a few weeks in many patients is in line with the concept of the target organ being the main site of autoantibody production; however this situation is not always true. Whether postoperative TSHR-Ab levels correlate with residual thyroid volume has not been studied thoroughly; however, it has been suggested that thyroidectomy could modulate immunologic activity of the disease.

The outcome after thyroidectomy is mainly dependant on the residual volume of the gland. Many retrospective studies have shown a correlation between postopertive recurrence of hyperthyroidism and the lack of decline of TSHR-Ab during ATD therapy before operation or the persistence of TSHR-Ab after operation. With the current trend toward more extensive removal of thyroid tissue in an attempt to prevent recurrences, TSHR-Ab determination is of no help in the management of patients, except in the perspective of subsequent pregnancy.

Special Conditions

Graves' Disease and Pregnancy

Graves' disease is common in women of reproductive age, with the prevalence rate of current or previous disease ranging from 0.5% to 1%. Hyperthyroid Graves' disease is estimated to occur at a rate of 0.5 to 2 per 1000 pregnancies. Although uncommon during pregnancy, this association has gained much attention as a complex situation with potential maternal and fetal complications. The prevention of this complication illustrates the benefits accrued from medical knowledge and expert multidisciplinary management. The main characteristics of Graves' disease during pregnancy are as follows:

1. The diagnosis of hyperthyroidism may be overlooked because mild clinical signs and symptoms may resemble the manifestations associated with pregnancy.

2. For reasons not completely elucidated, autoimmune hyperthyroid Graves' disease spontaneously improves during pregnancy so that, in a majority of patients, ATD treatment can be markedly reduced or even stopped. Nevertheless, in approximately 10% of patients, no improvement is observed, which requires a higher dosage of agents.

3. The fetal thyroid is affected by ATD and TSHR-Ab, which both cross the placental barrier readily, contrary to maternal thyroid hormones.

4. Because TSHR-Ab production may persist for several years after radical radioiodine or surgical treatment of hyperthyroid Graves' disease, euthyroid or T4-substituted women previously treated radically for Graves' disease may still have the risk of exposing the fetus to TSHR-Ab.

Although ATD treatment during pregnancy carries the risk of fetal hypothyroidism and goiter if not appropriately managed, feto-neonatal hyperthyroidism is observed in 2% to 10% of pregnancies in mothers with current or previous Graves' disease owing to maternal TSHR-Ab. It represents a serious condition with a 16% neonatal mortality rate and the risk of intrauterine death, stillbirth, and skeletal developmental abnormalities-including craniosynostosis. Feto-neonatal hyperthyroidism occurs in association with the highest levels of maternal TSHR-Ab[25], therefore, it is a predictable disease. Recently, the following guidelines for measurment of TSHR-Ab during pregnancy have been proposed in the American and European literature.

In the woman with antecedent Graves' disease in remission after ATD treatment, the risk for feto-neonatal hyperthyroidism is negligible, and systematic measurement of TSHR-Ab is not necessary. Thyroid function should be evaluated during pregnancy to detect an unlikely but possible recurrence. In that case, TSHR-Ab assay is mandatory.

In the women with antecedent Graves' disease previously treated with radioiodine or thyroidectomy and regardless of the current thyroid status (euthyroidism with or without thyroxine substitution), TSHR-Ab should be measured

early in pregnancy to evaluate the risk for fetal hyperthyroidism. If the level is high, careful monitoring of the fetus is mandatory for the early detection of signs of thyroid overstimulation (pulse rate > 170 bpm, impaired growth rate, oligoamnios, goiter). Cardiac echography and measurement of circulatory velocity may be confirmatory. Ultrasound measurements of the fetal thyroid have been defined from 20 weeks' gestational age but require a well-trained operator, and thyroid visibility may be hindered owing to fetal head position. Color Doppler ultrasonography is helpful in evaluating thyroid hypervascularization. Because of the potential risks of feto-neonatal hyperthyroid cardiac insuffiency and the lack of easy measurement of the degree of hyperthyroidism in the mother because of previous thyroid ablation, it may be appropriate consider direct diagnosis in the fetus. Fetal blood sampling through cordocentesis is feasible as early as 25 to 27 weeks' gestation with less than 1% adverse effects (fetal bleeding, bradycardia, infection, spontaneous abortion, in utero death) when performed in experienced centers. ATD administration to the mother may be considered to treat the fetal hyperthyroidism even though such treatment does not yet benefit from wide experience.

In the woman with concurrent hyperthyroid Graves' disease, regardless of whether it has preceded the onset of pregnancy, ATD treatment should be adjusted to keep free T4 in the high-normal range to prevent fetal hypothyroidism. TSHR-Ab should be measured at the beginning of the last trimester, especially if the required ATD dosage is high. If the TSHR-Ab assay is negative or the level low, feto-neonatal hyperthyroidism is unlikely. If antibody levels are high (TBII is greater than or equal to 40 U/L or TSAb is greater than or equal to 300%), evaluation of the fetus for hyperthyroidism is mandatory. In this condition, there is usually a fair parallelism

between maternal and fetal thyroid function such that monitoring the ATD dosage according to the mother's thyroid status is appropriate for the fetus. In some cases in which a high dose of ATD (>300 mg/d of propylthiouracil (PTU) or > 20 mg/d of methimazole) is necessary, there is a risk of goitrous hypothyroidism in the fetus, which might be undistinguishable from goitrous Graves' disease. The correct diagnosis relies on the assay of fetal thyroid hormones and TSH, which allows for optimal treatment.

In the woman who has previously given birth to a newborn with hyperthyroidism, TSHR-Ab assay should be performed early in the course of pregnancy.

When the TSHR-Ab level is significant during late pregnancy, TSHR-Ab must be determined in cord blood in the newborn and then sequentially at 7- to 10-day intervals for 2 to 4 months to monitor the duration and dose of ATD treatment.

Although the bioassay for stimulating antibody is theoretically preferred to the radiocompetitive assay, several factors should be considered when selecting an assay.

1. When simpler stimulatory bioassays or direct epitope-specific assays become available, the practical advantages of the current commercial radiocompetitive assays may be overridden.

2. In the experience of most clinicians, assays for TBII serve the purpose of detecting transplacental feto-neonatal hyperthyroidism satisfactory, with the exception of the occurrence of natural stimulatory antibodies devoid of activity in the radiocompetitive assay.

3. A recent report suggests that there may be a shift from stimulating to blocking activity of TSHR-Ab during pregnancy with a decrease in TSAb despite unchanged TBII levels. This fascinating observation should be explored further.

The author suggests use of the radiocompetitive method for routine detection of TSHR-Ab. The minority of patients with positive sera should be tested subsequently in stimulation and blocking bioassay.

Graves' Disease in Children and Adolescents

Because hyperthyroid Graves' disease is usually more severe in younger patients, thyroid ablation is the most common therapeutic option. Long-term remission rates in children and adolescents are usually less than 30% to 40% and much lower in prepubertal (17%) than pubertal children(30%).[26] Predictors of early remission include age, body mass index, heart rate, goiter size, serum T4 and T3 concentrations, platelet count, the TSHR-Ab titer at diagnosis, changes in goiter size, and the time required for serum T4 and T3 concentration to normalize during ATD treatment.[32] In the previously mentioned collaborative study of 191 patients, because of insufficient data, it was not possible to include TSHR-Ab values in the multivariate analysis, precluding their evaluation as an independent predictor of outcome. Nevertheless, the low remission rate and the uncertainity regarding ATD treatment in this age group support the use of any method capable of identifying patients who will benefit from radical treatment. The predictive significance of the new-generation assays of TSHR-Ab is expected to be even more pertinent in children and adolescents than in adults.

Extrathyroidal Manifestations of Graves' Disease

Graves' ophthalmopathy, pretibial myxedema, and acropachy are observed in 60%, 2% to 5%, and less than 1% of patients with Graves' disease, respectively. Pretibial myxedema, often observed after radioiodine treatment and usually presenting with ophthalmopathy, is always associated with high titers of TSHR-Ab, although the link between the two is not understood. In patients who have pretibial myxedema of an unusual type, an assay of TSHR-Ab is useful for confirmation of the diagnosis.

Graves' ophthalmopathy is clearly not caused by anti- TSHR-Ab as shown in the materno-feto-neonatal model of transplacental hyperthyroidism; however, there is an association between TSHR-Ab and Graves' ophthalmopathy in epidemiologic and longitudinal studies.[27] The expression of the TSH receptor by activated retroocular fibroblasts suggests an implication of anti-TSHR-Ab or TSH receptor-specific T cells in this disease. The epitopes involved in retroocular autoreactivity could be non specific or neutral because sera from patients negative for classic TSHR-Ab have been shown to react with the TSHR receptor in Western blotting. In clinical practice, the detection of TSHR-Ab is useful in the patient with suspected euthyroid Graves' ophthalmopathy as one of the abnormalities characterizing subclinical thyroid disease. TSHR-Ab are detected in 32% to 40% of patients who have euthyroid Graves' disease. In some patients, the presence of TSHR-Ab may be the only detectable abnormality.

Graves' Disease and Thyroid Carcinoma

Thyroid carcinoma occus with higher than expected frequency (4% to 7%) and greater severity in patients with Graves' disease. TSHR-Ab can stimulate the function and growth of differentiated thyroid cancer metastases and anigiogenesis in the thyroid. In a recent publication, among 21 patients with thyroid carcinoma and Graves' disease. TSAb were present in all but one of the patients in whom recurrence developed.[28] Although no comparative study is available, it is likely that the presence of TSHR-Ab in such patients is a significant risk factor.

2.6 TSHR-Ab AND AUTOIMMUNE HYPOTHYROIDISM

Although stimulating TSHR-Ab are the hallmark of Graves' disease, blocking TSHR-Ab are present in some patients with autoimmune primary hypothyroidism. Indeed, the pathogenetic role of TSBAb in autoimmune thyroiditis is suggested not only by their prevalence but by their capacity to induce neonatal transient hypothyrodism.

Prevalence of TSHR-Ab in Autoimmune Thyroidits

Based on the extensive review published in 1995 by Ducornet and co-workers,[29] the prevalence of TBII ranges from 0% to 44% in goitrous thyroiditis (mean, 9%) and from 0% to 54% (mean, 21%) in atrophic thyroiditis (primary myxedema). For TSBAb, the corresponding figures are 0% to 44% (mean 12%) and 0% to 62% (mean 33%), respectively. Clearly, the prevalence of TSBAb is higher in nongoitrous than goitrous autoimmune thyroiditis and in overt than subclinical hypothyroidism or euthyroid thyroiditis.[30] Also, the prevalence of TSBAb seems

much greater in Asian than Caucasian populations. In contrast, TSBAb are uncommon in children.

Clinical Use of TSHR-Ab Assay in Patients with Autoimmune Hypothyroidism

Indentification of Transient Transplacental Neonatal Hypothyroidism

The hypothesis that maternally transmitted antithyroid anitbodies are the cause of familial congenitial hypothyroidism was first proposed by Beierwaltes and co-workers in 1959. It became progressively evident through subsequent studies that maternal antithyroglobulin and anithyroperoxidase antibodies are not pathogenic to the fetus. In 1980 Matsuura and co-workers[31] reported the first cases of transient neonatal hypothyroidism, owing to maternal TSBAb. Since then, the prevalence of TBII in newborns with congenital hypothyroidism has been estimated to range form 0.8% to 38%. In the mothers of hypothyroid newborns, TBII are detected in 5% and TSBAb in 4%. On the whole, transient anitbody-related neonatal hypothyroidism amounts to 1% of all causes of congenital hypothyroidism. Current assay methods do not permit the routine systematic detection of TSBAb in neonatal blood spots. Nevertheless, transient transplacental neonatal hypothyroidism is as predictable as feto-neonatal hyperthyroidism by the assay of maternal TBII and, if positive, by an assay of TSBAb during the last trimester. Epidemiologic data suggest that this screening is indicated in women with atrophic primary myxedema. There is a correlation between the severity of the hypothyroidism as assessed by thyroid function at birth and the degree of development of the inferior femoral epiphysis and the inhibitory activity of TSBAb, and thyroid uptake of radioiodine or pertechnetate is suppressed in this condition. Vigorous substitutive treatment of the neonate is

mandatory as early as possible when hypothyroidism has been expected or diagnosed at neonatal screening. The newborn should be monitored to detect disappearance of TSBAb at 7- to 15- day intervals. Substitutive treatment is generally maintained for several weeks or months after the 1.5- to 2- month period when the TSBAb assay has become negative.

Spontaneous Remission from Autoimmune Hypothyroidism

In the few series reported, reversibility of autoimmune hypothyroidism has been observed in 0% to 24% of patients. Takasu and co-workers found TSBAb in 10% of patients with goitrous autoimmune thyroiditis and in 25% of patients with the atrophic form. During a maximum follow-up period of 11 years, 15 of the 21 patients with blocking antibodies became negative. Among them, six patients remained euthyroid after thyroxine treatment withdrawal. In contrast, in the six TSBAb-positive patients with atrophic thyroiditis, blocking anitbodies and hypothyroidism persisted for the whole follow-up.[32] Measurement of TSBAb is not a specific marker of spontaneous recovery from hypothyroidism because disappearance of the anitbody is not necessarily parallel to the disappearance of hypothyroidism. Moreover, systematic screening for spontaneous recovery from hypothyroidism does not seem justified based on epidemiologic grounds nor is it cost-effective.

TSHR-Ab and Atypical Thyroid Autoimmune Patterns

Fluctuating Thyroid Function. Spontaneous evolution of hyperthyroid Graves' disease to hypothyroidism has been characterized. In many cohorts with long-term follow-up of 10 to 15 years, this evolution occurs in 2.1% to 2.8% of

patients treated with ATD.[33] Different mechanisms may be involved. Although the development of destructive autoimmune thyroiditis has been observed, the presence of TSBAb is demonstrated in 20% to 40% of cases.[34] TSBAb may result from a conversion of the bioactivity of TSAb or may coexist with TSAb at the time of hypothyroidism. TBII are usually detectable. In most cases, hypothyroidism, is definitive; thus, the presence of TBII is not an indicator of subsequent normalization.

More than 60 cases of fluctuating thyroid function have been reported, most of which have been associated with the spontaneous evolution of hypothyroidism to hyperthyroidism.[29] In a few cases, cycles of transition from hypothyroidism to hyperthyroidism or the converse have been observed.[35,36] Determination of the TSHR-Ab, TBII, and stimulating/blocking antibodies is useful in an attempt to characterize these unusual anecdotal cases, although there is not always a concordance between thyroid status and the net bioactivity of TSHR-Ab.

2.7 **Hypothyroid Graves' Disease**.

Hypothyroid Graves' disease is defined as the development of Graves' ophthalmopathy in a hypothyroid subject. The association of infiltrative ophthalmopathy with primary hypothyroidism is rare.[37] In a recently reported series of five patients, all were positive for TSAb but not for TSBAb. Thyroid function fluctuated in four patients. All of the patients had high levels of antithyroglobulin and antithyroperoxidase antibodies. In these cases, TSHR-Ab assay confirmed the association of Graves' disease and autoimmune thyroiditis.

2.8 Painless Thyroiditis.

TSHR-Ab are uncommon in postpartum of thyroiditis; thus, they are not predictable for recovery. In the sporadic forms of painless thyroiditis. TSHR-Ab are detected in 6% to 20% of cases. In some case reports, determination of TSHR-Ab and sometimes of TSBAb has been useful for diagnosis. In the few instances associated with hypothyroidism, the disappearance of TSBAb justifies a substitutive treatment withdrawal trial.

Chapter3

RESEARCH METHODOLOGY

3. RESEARCH METHODOLOGY

3.1 Place :

Department of Endocrine Surgery

Rajiv Gandhi Government General Hospital Chennai

All study patients were inpatients in ward 135.

3.2 Patients and Methods :

70 patients of various thyroid disease admitted between 2008-2010 were included in the study.

3.2.1 Criteria for inclusion :

All patients who had clinical hypothyroidism or hyperthyroidism or thyroid nodules with biochemical evidence of antibody positivity were included in the study.

3.2.2 Investigations :

The following investigations were done for all the patients

- Complete Haemogram
- Thyroid function tests (T3,T4,TSH,FT3,FT4)
- Anti-TPO antibodies
- Anti thyroglobulin antibodies
- TSHR antibodies
- Serum Calcium
- Serum Alkaline Phosphatase

3.2.3 Consent :

Informed consent was obtained from patients prior to inclusion in the study.

3.2.4 Ethical committee approval:

The ethical committee of Madras Medical College has approved the protocol and study (No. 01092010) dated 14.09.2010

3.2.4 Statistical Analysis

Data was analyzed using Pearson correlation and cross tabulations.

Chapter 4

RESULTS AND DISCUSSION

4. RESULTS AND DISCUSSION

4.1 Test Summary

A Total number of 70 patients with various thyroid disorders were included in this study. Out of this 62 were women and 8 were men. The age of the patients ranged from 13 years to 67 years with a mean age of 38 years.

Out of these patients, 8 had diffuse goiter, 60 had multinodular goiter and 2 had a solitary thyroid nodule at presentation

On the basis of size of the swelling the patients were graded into Grade I (5 Patients) Grade II (48 Patients) and Grade III (17 Patients)

13 patients were hypothyroid, 27 were hyperthyroid and 30 were euthyroid.

The patients were categorized on the basis of histopathology reports 35 had nodular colloid goitre, 24 had thyroiditis, 3 patients had Graves disease, 6 had papillary carcinoma and two had follicular adenoma

Among the patients with thyrotoxicosis, 9 had eye signs at presentation while 22 had tremors.

TABLE – 1

CORRELATION OF ANTIBODIES TO VARIOUS THYROID ANTIGENS WITH ANATOMICAL DIAGNOSIS

ANATOMICAL DIAGNOSIS	AMA		ATG		TSHRAb	
	Negative	Positive	Negative	Positive	Negative	Positive
Diffuse Goiter (n=8)	1	7	2	6	4	4
MNG (n=60)	10	50	19	41	35	25
SNT (n=2)	1	1	2	0	2	0

Among the patients with diffuse goitre, 7 were positive for AMA, 6 for ATG and 4 for TSHRAb. Out of the 60 patients with MNG, 50 were positive from AMA, 41 for ATG, 25 for TSHRAb and 1 patient with solitary thyroid nodule was positive for AMA. No patients with SNT were positive for ATG or TSHRAb (Table – 1)

FIGURE – 1

CORRELATION OF ANTIBODIES TO VARIOUS THYROID ANTIGENS

WITH ANATOMICAL DIAGNOSIS

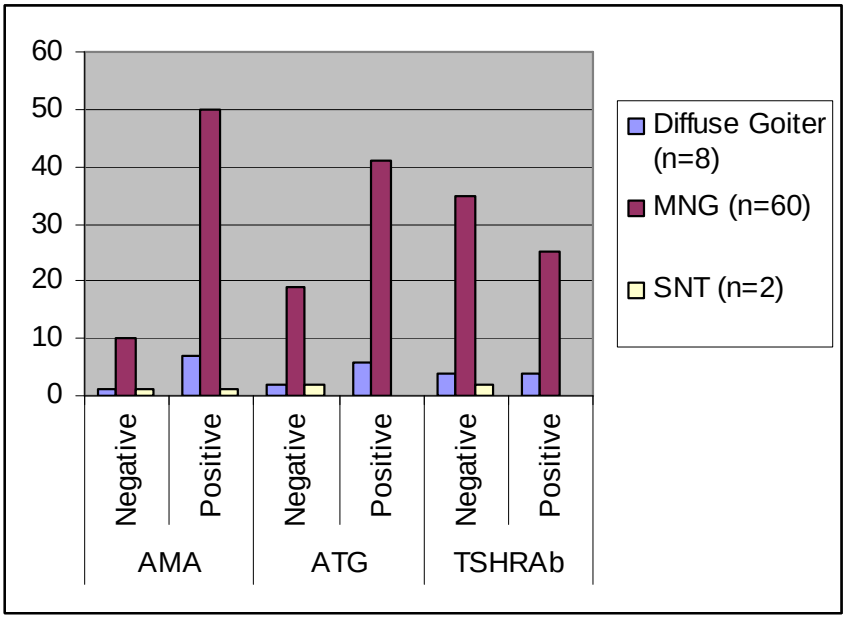

TABLE – 2

CORRELATION OF SERUM LEVELS OF VARIOUS THYROID ANTIBODIES WITH FUNCTIONAL STATES OF THYROID

Physiological Diagnosis	AMA		ATG		TSHRAb	
	Negative	Positive	Negative	Positive	Negative	Positive
Hypothyroid (n=13)	3	10	2	11	10	3
Hyperthyroid (n=27)	1	26	5	22	5	22
Euthyroid (n=30)	8	22	16	14	26	4

Among the patients with hypothyroidism (n=13), 10 patients were positive for AMA, 11 patients for ATG and 3 patients for TSHRAb. Among the hyperthyroid (n=27), 26 patients were positive for AMA, 22 patients for ATG and 22 patients for TSHRAb. In Euthyroid patients (n=30), 22 patients had increased AMA, 14 patients had increased ATG, and 4 were positive for THSRAb.(Table – 2)

FIGURE – 2

CORRELATION OF SERUM LEVELS OF VARIOUS THYROID ANTIBODIES WITH FUNCTIONAL STATES OF THYROID

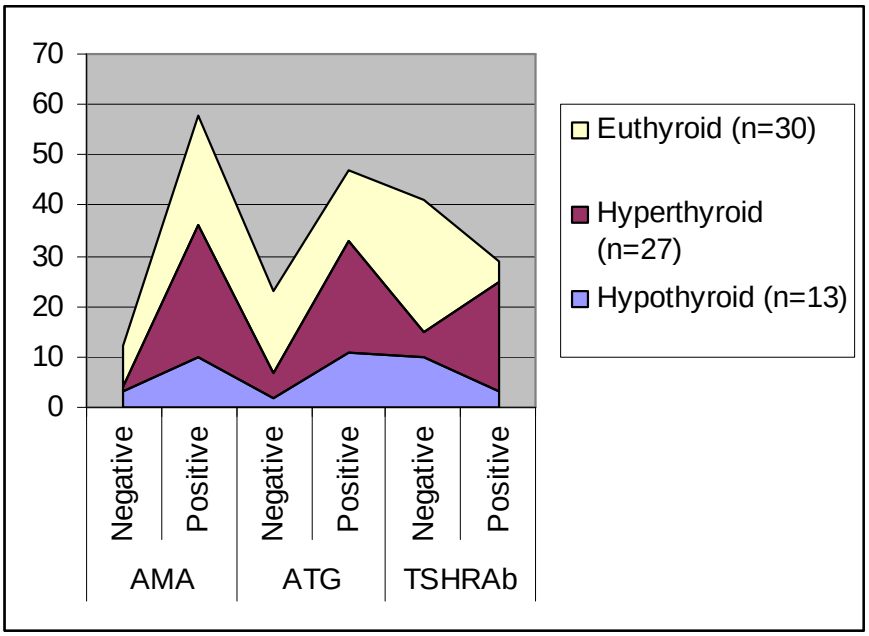

TABLE – 3

CORRELATION OF LEVELS OF ANTIBODIES WITH PATHOLOGICAL DIAGNOSIS

Pathological Diagnosis	AMA		ATG		TSHRAb	
	Negative	Positive	Negative	Positive	Negative	Positive
Nodular goiter (n=35)	5	30	12	23	19	16
Thyroiditis (n=24)	3	21	6	18	15	9
Graves disease (n=3)	0	3	0	3	0	3
Papillary carcinoma (n=6)	3	3	3	3	5	1
Follicular adenoma (n=2)	1	1	2	0	2	0

Among the nodular goitre (n=35) 30 were AMA positive and 23 were ATG positive and 16 were TSHRAb positive. Among the thyroiditis (n=24) 21 were AMA positive 18 were ATG positive and 9 were TSHRAb positive. In graves disease (n=3) all were ATG, AMA and TSHRAb positive. In papillary carcinoma patients (n=6) 3were both ATG and AMA positive and 1 was TSHRAb positive. In follicular neoplasm (n=2) 1 was AMA positive and the other were ATG and TSHRAb negative.(Table – 3)

FIGURE – 3

CORRELATION OF LEVELS OF THSRAb WITH PATHOLOGICAL DIAGNOSIS

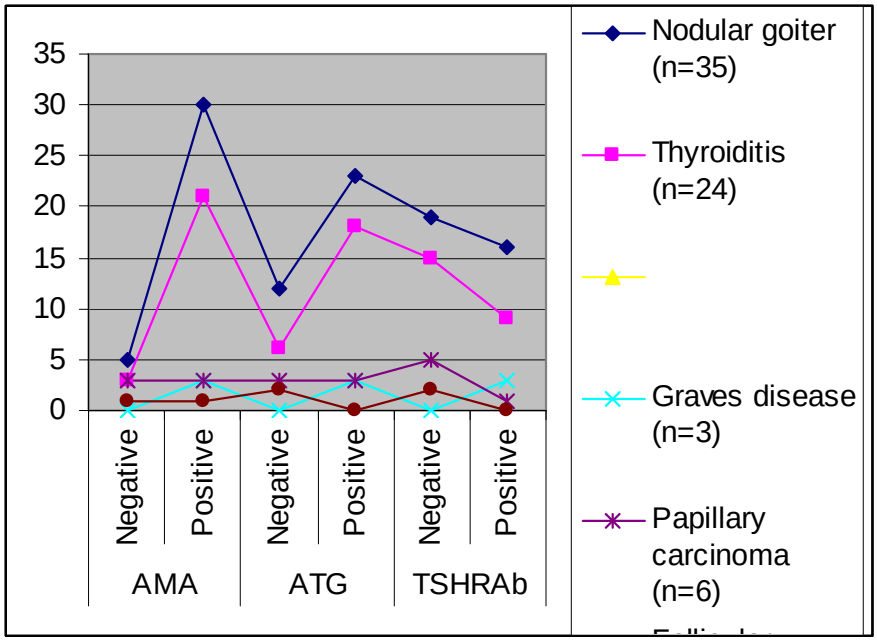

TABLE – 4

MEAN AGE AND LEVELS OF THYROID HORMONES AND THYROTROPIN IN TSHRAb POSITIVE PATIENTS

	MEAN	STD DEVIATION	STD. ERROR
AGE	35.86	10.91	2.02
T3	213.35	145.01	26.92
T4	13.15	8.01	1.48
TSH	5.26	16.51	3.06
FT3	9.36	7.95	1.47
FT4	3.03	2.51	0.46

A total of 29 patients were positive for TSHR Ab in this study. The mean age of these patients was 36 years. The mean value of T3 (Tri-iodothyrosine) was 213.35 ng/ml. The mean value of T4 (Total thyroxine) was 13.15 µg/dl. The mean value of FT3 was 9.3 pg/ml. Mean value of FT4 was 30 ng/dl. The mean value of TSH (thyrotropin) was 5.26 mIu/ml. (table – 4)

TABLE - 5

CORRELATION OF THYROMEGALY WITH TSHRAb LEVELS

THYROMEGALY	TSHRAb NEGATIVE	TSHRAb POSITIVE	TOTAL
GRADE I	5	0	5
GRADE II	27	21	48
GRADE III	9	8	17
TOTAL	41	29	70

None of the patients with Grade I thyromegaly had elevated levels of THSRAb. Out the 48 patients with Grade II thyromegaly, 21 were positive for TSHRAb. Among 17 patients with Grade III thyromegaly, 8 patients had elevated TSH receptor antibody. (Table 5)

FIGURE - 4

CORRELATION OF THYROMEGALY WITH TSHRAb LEVELS

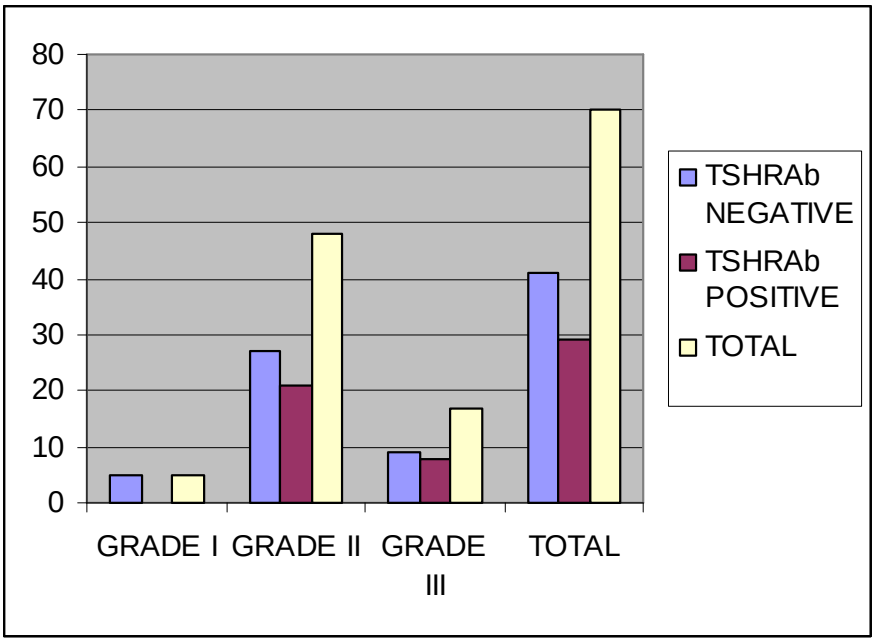

TABLE - 6

CORRELATION OF MEAN VALUES OF VARIOUS ANTIBODIES WITH THYROID FUNCTIONAL STATUS

		N	Mean	Std. Deviation	Std. Error
ATG	1	13	429.52	333.89	92.60
	2	27	596.14	769.45	148.08
	3	30	362.52	746.58	136.30
AMA	1	13	235.54	380.63	105.56
	2	27	212.94	224.70	43.24
	3	30	174.03	299.36	54.65
TSHrAb	1	13	1.94	4.907	1.36
	2	27	20.33	16.12	3.10
	3	30	.84	1.40	.25

NOTE 1-HYPOTHYROIDISM

2- HYPERTHYROIDISM

3- EUTHYROID

TSHR antibody levels were elevated in patients with hyperthyroidism (mean value 20.33 IU ± 3.10). Mean TSHRAb levels in hypothyroid patients was 1.9 ± 1.3 and 0.8 ± 0.25 in euthyroid patients (Table 6)

TABLE - 7

CORRELATION OF MEAN VALUES OF TSHRAb LEVELS WITH PATHOLOGICAL DIAGNOSIS

		N	Mean	Std. Deviation	Std. Error
TSHrAb	NODULAR GOITRE	35	8.04	13.16	2.22
	THYROIDITIS	24	9.24	15.47	3.15
	GRAVES DISEASE	3	17.75	6.15	3.55
	PAPILLARY CARCINOMA	6	7.00	16.16	6.59
	FOLLICULAR ADENOMA	2	.30	.00	.00

TSHRAb levels were high (17.7 ± 3.5) in patients with histopathological diagnosis of Graves disease (Table -7)

In patients with pap ca (n=6) the one patient who had elevated TSHRAb had clinical evidence of hyperthyroidism.

FIGURE – 5

CORRELATION OF MEAN VALUES OF TSHRAb LEVELS WITH PATHOLOGICAL DIAGNOSIS

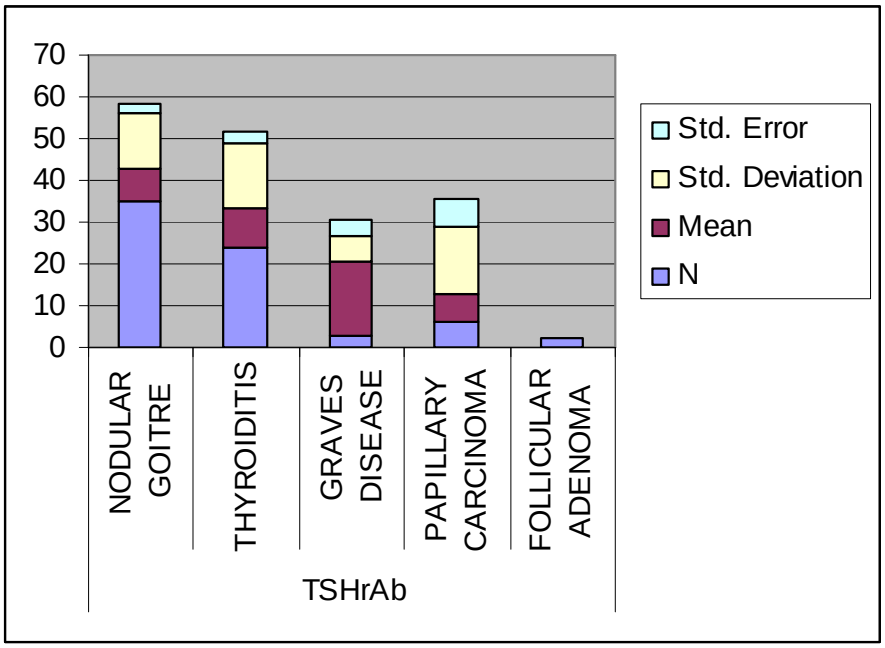

TABLE - 8

CORRELATION OF TSHRAb LEVELS WITH ATG AND AMA

	TSHRAb Negative	**TSHRAb Positive**	**Total**
TSHRAb only	5	2	7
TSHRAb + AMA	10	6	16
TSHRAb + ATG	5	0	5
TSHRAb + AMA + ATG	21	21	42
Total	41	29	70

Among the 70 patients 5 patients were negative for all the three abs-THSRAb, AMA and ATG. 2 patients had elevation of only TSHRAb with normal ATG and AMA levels. 10 patients had elevation of AMA only. 6 patients were positive for both AMA and TSHRAb. 5 patients were positive for ATG only. None of the patients had elevated TSH and ATG levels with normal AMA levels. 21 patients were Negative for AMA,ATG and TSHRAb and 21 patients had elevated TSHRAb, AMA and ATG levels.(Table – 8)

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FIGURE - 6

CORRELATION OF TSHRAb LEVELS WITH VARIOUS ANTIBODIES

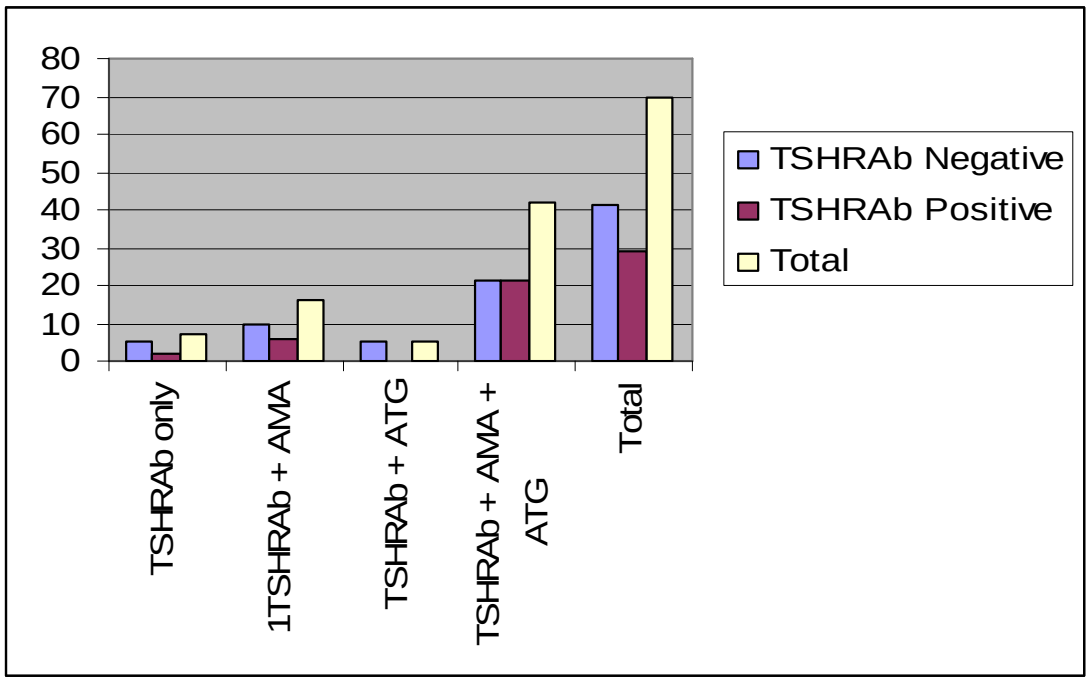

TABLE - 9

CORRELATION OF EYE SIGNS WITH TSHRAb LEVELS

	TSHRAb		AMA		ATG	
	Positive	Negative	Positive	Negative	Positive	Negative
Eye Sign (n=9)	8	1	9	0	7	2

Among the 9 patients with eye signs, 8 had elevated TSHRAb, out of which one patient was hypothyroid. All 9 patients had elevated AMA levels. Among the 22 patients with tremors, 19 had elevated TSHRAb (Table 9)

TABLE - 10

PEARSON CORRELATION OF SAP AND SERUM CALCIUM WITH TSHRAb, AMA AND ATG LEVELS

		TSHrAb	CALCIUM	SAP
ATG	Pearson Correlation	.082	-.342**	.159
	Sig. (2-tailed)	.501	.004	.189
	N	70	70	70
AMA	Pearson Correlation	.035	-.222	.153
	Sig. (2-tailed)	.773	.064	.207
	N	70	70	70
TSHrAb	Pearson Correlation	1	-.052	.335**
	Sig. (2-tailed)		.667	.005
	N	70	70	70

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

When Ca and SAP levels were correlated with hyperthyroidism, eye signs, tremors and TSHRAbs and histo-pathological diagnosis of Graves' disease, SAP was elevated in patients with hyperthyroidism, tremors, eye sign and elevated TSHRAb and Graves' pathology. Elevated Ca levels correlated with elevated ATG levels. Ca levels did not correlate with any of these entities. (Table 10)

4.5 DISCUSSION

TSHRAb is a useful diagnostic tool for diagnosis of Graves' disease. The prevalence of TSHRAb in hyperthyroid and Graves disease is 70-100 %

In the present study all the patients with histopathological diagnosis of Graves' disease were positive for TSHRAb (Prevalence 100 %)

Though presence3 of TSHRAb is specific for Graves' disease, some studies show the TSHRAb positivity present in significant proportion of toxic MNG.[13]

In the present study, TSHRAb positivity was seen in 26.05 % of patients (16 patients out of 60).

It is a common observation that the level of TSHRAb grossly parallels the degree of hyperthyroidism as assessed by serum levels of thyroid hormones.[38,39]

In the present study also, the mean value of TSHRAb was significantly higher in patients with hyperthyroidism (20.33 IU ± 3.10). So, TSHRAb levels may be taken as a marker of severity of the disease.

In general, good agreement has been observed with TSHRAb levels and thyroid volume.[40] Rieu and co workers[15] found that the volume of both hyperplastic and nodular goiter correlated with TSHRAb levels, confirming the growth stimulation potential of these antibodies on thyroid tissue.

In the present study, TSHRAb levels were significantly higher in patients with Grade II and Grade III thyromegaly.

TSHRAb assay is very specific and sensitive for hyperthyroid Graves' disease. Up to 98% of untreated patients are positive for TSHRAbs with very few false-positive results.[41]

In the present study, all the 3 patients who had histo-pathological diagnosis of Graves' disease had elevated TSHRAb levels. 3 patients who were hypo-thyroid (out of 13) were positive for TSHRAbs, probably indicating false-positive results.

Hypothyroid Graves' disease is defined as development of Graves' ophthalmopathy in hypothyroid subjects. The association of infiltrative ophthalmopathy with hypothyroidism is rare.[37] In these patients, TSHRAb assay confirms the association of Graves' disease with autoimmune thyroiditis.

Out of the patients with eye signs in the present study, one patient had hypothyroidism. The elevated TSHRAb levels in this patient indicates co-existence of autoimmune thyroiditis with Graves' disease.

Clinical sign sand symptoms of ophthalmopathy are present in about 50% of the patients with Graves' disease.[42, 43] In the present study, all three patients with histopathological diagnosis of Graves' disease had eye disease and elevated TSHRAb levels.

In some studies, the level of TSHR gene expression was found to be higher in the orbital tissue of patients with Graves' ophthalmopathy when compared with normal orbital tissues or tissue from patients with inactive eye disease.[44,45,46] In the present study, out of 9 patients with ophthalmopathy, 8 patients had elevated TSHRAb levels as well. This may indicate active orbital autoimmune response.

When present, the Graves' ophthalmopathy coincides with the onset of thyrotoxicosis in about 40% of cases, follows in another 40% of case and precedes onset of thyrotoxicosis in 20%.[47,48] Even when the onset of the two disorders doesn't coincide, each occurs within 18 months from the onset of the first manifestation.

In the present study, one patient with ophthalmopathy who was negative for TSHRAbs might have developed the eye disease before the onset of thyrotoxicosis.

Antithyroid antibodies in serum should be determined routinely in the work-up of the patients with multinodular goiter. This recommendation is based mainly on the fact that Hashimoto's thyroiditis may be mistaken for simple multinodular goiter and that these antibodies may be recognized as increased risk for ^{131}I-induced hypo-thyroidism as well as Graves' disease.[49]

In the present study, out of 60 patients with multinodular goiter 25 had increased TSHRAb levels. Had TSHRAb estimation not been done, possibility of Hashimoto's thyroiditis and Graves' disease might have been missed.

Thyroid carcinoma occurs with higher-than-expected frequency (4% - 7%) and greater severity in patients with Greaves' disease. TSHRAb can stimulate the

function and growth of differentiated thyroid cancer metastases and angiogenesis in the thyroid. In a recent publication, among 21 patients with thyroid carcinoma in Graves' disease, TSHRAbs were positive in all but one of the patients in whom, recurrence developed.[28]

In the present study, out of 6 patients with papillary carcinoma, TSHRAbs were elevated in one patient, who had clinical and biochemical evidence of hyper-thyroidism and who later turned out to have inoperable metastatic disease.

Hyperthyroid Graves' disease is usually more severe in younger patients. Long-term remission rates in children and adolescents are usually less than 30%-40% and much lower in pre-pubertal age (17%).[26] The low remission rate and the uncertainty regarding anti-thyroid drug treatment in this age-group support the use of any method capable of identifying patients who will benefit from radical treatment. The predictive significance of the new-generation assays of TSHRAb is expected to be even more pertinent in children and adolescents than in adults.

In the present study, 3 children who were in pre-pubertal age-group were found to have increased TSHRAb levels with toxicity. These patients are candidates who require treatment in the form of total thyroidectomy.

IN the present study, levels of serum Alkaline phosphatase correlated with hyperthyroidism, tremors, eye sign and elevated TSHRAb and Graves' pathology.

Assays for TSHRAb should not be considered a magic tool for the management of autoimmune thyroid disease, but as one indicator among others

(e.g. thyroid volume, basal TSH, etc.) in a complex and multifactorial situation. The contribution of TSHRAb assays to the prediction of post-treatment outcome is significant but limited. Such assays are useful in clinical practice when the risks of the wrong therapeutic decision are significant. More informative is the longitudinal study of TSHRAb levels when compared with a single, end-of-treatment determination.

Chapter 5

CONCLUSION

5. CONCLUSION

- TSHRAb is a useful diagnostic tool for diagnosis of Graves' disease.

- Though it has been considered to be specific for Graves' disease, increased levels were found in patients with multinodular goiter. Estimation of anti-thyroid antibodies in patients with multinodular goiter is highly recommended to recognize the association of autoimmune thyroiditis and Graves' disease in multinodular goiter.

- The levels of TSHRAb grossly parallel the degree of hyperthyroidism and the grade of thyromegaly.

- Elevated TSHRAb levels in patients with ophthalmopathy indicate active eye disease. In euthyroid patients with ophthalmopathy, increased levels of TSHRAb may predict the future occurrence of Graves' disease. In patients with elevated levels of TSHRAb, AMA and ATG, the risk of development of eye signs is significantly higher.

- Elevated levels of TSHRAbs in patients with papillary carcinoma indicates poor prognosis.

- Level of Alkaline phosphatase in serum correlates with serum levels of TSHRAb and severity of disease. So elevated levels of Serum Alkaline phosphatase along with TSHRAb in hyperthyroidism may be taken as a marker for severity of Graves' disease.

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