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# Thyroid Peroxidase (TPO) and Thyroid Stimulating Hormone Receptor (TSHR) Based Detection on Grave for Pregnant Women

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## Abstract

Graves' Disease is a form of specific autoimmune disorder in the thyroid organ characterized by thyroid-stimulating antibodies (TSAb). Pregnant women are the most susceptible to GD due to hormonal changes and tolerance of immune responses during pregnancy. The incidence of prematurity, low birth weight (LBW), and neonatal thyrotoxicosis risk are the most complications that can be acquired if treatment is late and inadequate. It has implications for increased fetomaternal morbidity and mortality. Apart from being a biomarker for definitive diagnosis, TSAb testing is also beneficial for assessing treatment response and predicting relapse of GD (relapse) after oral anti-thyroid treatment. GD patients with high TPOAb titers also tend to have a high relapse rate. However, the evaluation of both TSAb and TPOAb examinations during and after treatment is rarely done routinely due to the examination's high cost. This works proposed developing TSHR and TPO antigen-based rapid diagnostic tests through the immunochromatography method to address the challenges of financing and limited laboratory facilities in the area. Besides, understanding the importance of examining thyroid antibodies (TSAb and TPOAb) and interpretation in clinical practice is still a matter of debate in clinical circles, so it requires in-depth information.

**Keywords:** Graves' Disease, thyroid-stimulating antibodies, thyroid peroxidase, pregnant women

## 1. Introduction

Improved hygiene and technological advances in several developed and developing countries have implications for health improvements marked by a decrease in the population's infectious diseases. However, on the other hand, the tendency of autoimmune disease [1] and cancer [2] is increasingly being found with the availability of early detection screening tools. Genetic susceptibility, nutrition, and environmental factors are risk factors for the increasing prevalence and incidence of autoimmune diseases in the population [3, 4].

Autoimmune cases in specific thyroid organs are the second-highest after rheumatoid arthritis [1], with an incidence rate of about 2–5% affecting the world population [5]. Based on screening data from US and European populations, it was reported that autoimmune thyroid diseases such as Graves' Disease were more dominant in women than men with a ratio of 5: 1 [6, 7]. The cause of the predominant tendency of women to develop autoimmune disorders is still being debated. Several hypotheses are associated with the structure of the X chromosome and hormonal changes. Morphologically, the X chromosome is larger and contains more genes (800–900 genes) than Y chromosomes (50–60 genes). Most of the genes related to immune system response and regulators are present on the X chromosome so that women have a higher risk of developing autoimmune disorders than men who only have one X chromosome [8, 9].

## 2. Graves' Disease in pregnancy

Graves' Disease (GD) is a form of specific autoimmune disorder in the thyroid organ characterized by the formation of thyroid-stimulating antibodies (TSAb) and increased thyroid hormone secretion (hyperthyroidism) [10]. This disorder was first introduced by Robert James Graves in 1835, who has clinical features such as goiter, palpitations, and orbitopathy [11]. About 60–80% of hyperthyroid disorders in the population are due to GD [12], with an incidence of 1–2 cases occurring in 1000 pregnancies [13]. GD disorders can affect all age groups, both children [14], reproductive age [15], and the elderly [16], but the most incidence occurs in women aged 20–49 years [6, 17].

Pregnant women are the most susceptible to GD, which is thought to be due to decreased immune tolerance during pregnancy [18] and hormonal changes [19]. Approximately 0.4%–1.0% of women of reproductive age have GD before pregnancy, and 0.2% have it during pregnancy [13]. The American Thyroid Association (ATA) has issued recommendations for routine thyroid health screening in pregnant women, especially the first trimester of pregnancy and postpartum [20]. Besides, all women of reproductive age who are suffering from GD or have a previous history of GD are encouraged to seek counseling when planning pregnancy as an effort to improve fetomaternal health [19].

The high titer of maternal TSAb that can cross the placental barrier will manifest in impaired fetal thyroid function, increasing fetomaternal morbidity, and mortality [18, 21]. Preterm birth (prematurity) [22], low birth weight (LBW) [21, 22], and risk of neonatal thyrotoxicosis [23] are some of the frequently reported fetomaternal complications. Apart from TSAb testing, the ATA also recommends that pregnant women with a positive TPOAb be advised to evaluate serum TSH levels every four weeks during the second trimester [20].

## 3. TSHR and TPO as autoantigen

Genetically, polymorphisms in the thyroid-stimulating hormone receptor (TSHR) gene found on chromosome 14q31 [24–26] and the thyroid peroxidase gene on chromosome 2p25 (TPO) [27, 28] are closely related to susceptibility and severity of GD disease in various populations. Both thyroid-specific genes can act as autoantigens and are potential genetic biomarkers for GD [29, 30]. The term autoantigen indicates that a protein originates within the individual's own body, has a highly conserved structure, and is coded for genes with a low mutation rate. Thus, autoantigen is not an abnormal molecule but is coded only for genes that undergo

polymorphisms in the population. Polymorphisms cause variations in protein structure and function so that they are sometimes recognized as foreign antigens that can interact with T lymphocytes and antibodies [31, 32].

Thyroid-stimulating hormone receptor (TSHR) is a protein molecule that plays a vital role in the growth and differentiation of the thyroid gland and is directly involved in signal transduction and regulation of thyroid hormone biosynthesis [33, 34]. TSHR protein is the primary autoantigen that triggers GD and is a target that is attacked by TSAb [35]. T lymphocyte immunotolerance's failure to the TSHR antigen triggers the infiltration of lymphocytes, dendritic cells, and macrophages into the thyroid follicle. Furthermore, lymphocyte infiltration triggers the secretion of several pro-inflammatory cytokines such as interleukin 1 $\beta$ , IL-6, IL-12 interferon- $\gamma$ , ligand CD40, and tumor necrosis factor- $\alpha$ . Presentation of TSHR peptides by dendritic cells on MHC-II molecules will activate B cells and differentiate plasma cells to synthesize and secrete TSAb into the circulation [10, 36]. TSAb protein, which mimics the action of TSH on the surface of the thyroid follicle cells, is the leading cause of thyroid hyperplasia and hyperfunctioning of T3 and T4 secretion becomes uncontrolled [37].

In the majority of people with GD, other autoantibodies can also be found, such as thyroid peroxidase antibody (TPOAb) [38, 39]. Thyroid peroxidase (TPO) is the main enzyme that assists in the biosynthesis of thyroid hormones. The TPO enzyme catalyzes the organization of iodine (iodination) and the coupling process of iodothyrosine residues in thyroglobulin [40]. In GD, the persistent lymphocyte infiltration of the thyroid follicular cells can also trigger a failure to tolerate the TPO autoantigen's immune response. About 80% of people with GD have positive TPOAb, which can activate the complement cascade, causing thyroid gland damage and dysfunction [38, 41]. Physiologically, the presence of TPOAb can also be found in normal populations around 10%-15% [42, 43], and in thyroid malignancies around 10%-20% [41].

Although both autoantibodies cross the placental barrier, only maternal TSAb titer can interfere with fetal and neonatal thyroid function. In contrast, the presence of TPOAb does not significantly affect neonatal thyroid function [20, 44]. However, monitoring of thyroid antibody titer and regular counseling is necessary during pregnancy due to complications of morbidity in the mother and infant [20, 45].

#### **4. The role of TSAb and TPOAb in early-onset and relapse investigation**

The early diagnosis of thyrotoxicosis is a challenge for clinicians because of the atypical clinical features and parallels the physiological changes in normal pregnancy [18]. Total T3, free T4, and TSH levels established during pregnancy also have different parameters or reference values from non-gravidas [46, 47]. The cause of thyrotoxicosis during pregnancy must be identified immediately, and must be able to differentiate between GD and other non-autoimmune hyperthyroidism such as gestational transient thyrotoxicosis (GTT) [13]. Although serum human chorionic gonadotropin (hCG) levels were higher in GTT patients compared to GD, this parameter is not typical in the early phase, so TSAb examination can be indicated to differentiate the cause of thyrotoxicosis [20].

The presence of TSAb in serum is a hallmark or the primary marker in the diagnosis of GD. More than 95% of the presence of TSAb can be found in serum with GD [31, 48]. The presence of TSAb can be detected in the early phase of GD before causing characteristic clinical symptoms (asymptomatic), and the titer will continue to increase if not handled adequately [44]. Apart from the diagnostic

screening for GD, TSAb measurement is also useful for predicting cases of GD relapse after stopping treatment [37, 49]. The research of Kwon et al. (2016) found that only TSAb measurements could predict cases of GD relapse, while the thyrotropin-binding inhibitory immunoglobulin (TBII) examination was insensitive to relapsing GD cases [49]. Although the use of thionamide anti-thyroid drugs such as propylthiouracil (PTU) and methimazole (MMI) has an immunomodulatory effect of lowering TSAb titers [50], many reports of remission after treatment [51–54]. Carella et al. reported that TSAb titers remained positive in the majority of GD patients after 18 months of treatment with Methimazole (MMI) [51].

Increased TSAb titer also affects extrathyroid clinical manifestations, such as orbitopathy [55] and dermopathy (pretibial myxedema) [56], which can increase morbidity and decrease the quality of life for Graves sufferers. Bahn stated that TSAb titers have diagnostic value in euthyroid patients with exophthalmic [57].

In addition, TSAb measurement can also be used to determine treatment response (monitoring) and prognosis of Graves' Disease patients who have been treated with oral anti-thyroid [52, 58].

TSAb examination in pregnant women suffering from GD or post anti-thyroid treatment can predict the likelihood of neonatal thyrotoxicosis [59, 60]. Pregnant women who have high TSAb titers and persist until the third trimester require special monitoring of neonates and mothers because of their increased risk of thyrotoxicosis [37]. The results of research by Hamada et al. showed that patients with GD with a history of radioactive iodine therapy (I-131) had a higher TSAb titer during pregnancy and were at risk of delivering babies with thyrotoxicosis [61].

Positive TPO autoantibodies can also be found in GD abnormalities, even though a change in titer can predict recurrence of GD after anti-thyroid treatment [27]. TPOAb titers are less specific than TSAb in determining the diagnosis of GD. This is because, in certain levels, TPOAb titer can be found in the serum of normal individuals (euthyroid) and pregnant women without autoimmune thyroid disorders. The prevalence of both reaches 15% in the average population and 14% in pregnant women [42, 43]. This percentage affects the clinical specificity of TPOAb as a diagnostic indicator for the detection of AITD.

Interestingly, TPOAb titers were also found in people with GD, which suggests an association with the course of thyroiditis. Umar et al. found that 15–20% of Grave's patients had spontaneous hypothyroidism as a result of chronic thyroiditis (Hashimoto), and suspected that the widespread immune response in Grave's episode would trigger an increase in TPO and Tg autoantibodies, causing marked thyroiditis. With lymphocyte infiltration in thyrocytes cells [62].

## **5. Development of TSAb and TPOAb measurement methods**

In recent decades, methods of measuring thyroid antibodies have continued to evolve, ranging from semiquantitative testing via agglutination and complement fixation tests [63, 64], to ligand-specific testing using recombinant antigens and cultured cells transfected with human TSHR [65, 66]. The hemagglutination method is rarely used and has many shortcomings in terms of specificity, sensitivity and depending on operator skills (subjective). Current examination methods have better precision because they directly measure autoantigen and autoantibody interactions with high sensitivity and specificity [67].

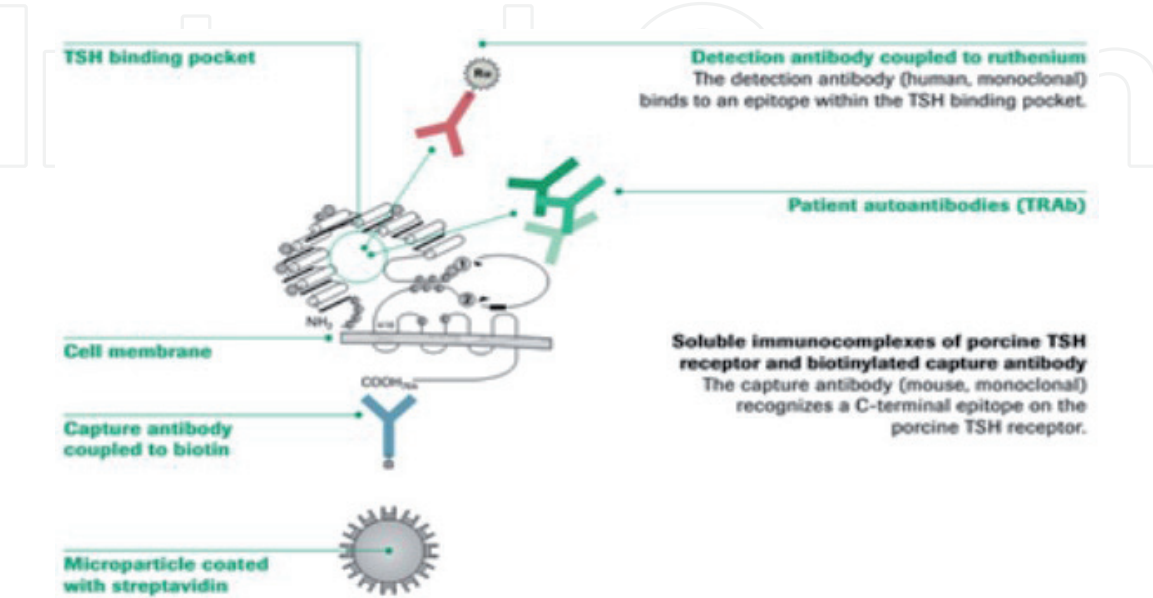
Currently, there are two methods that are often used to detect the presence of TSHR autoantibodies, namely (i) the TBII test (TSH Binding Inhibition Immunoglobulin), also known as the TRAb test, which is a test to assess the capacity of a patient's serum or IgG to inhibit TSH receptor binding with TSH labeled I125

or against recombinant TSHR protein expressed on CHO (Chinese Hamster Ovary) cells [37, 66]. (ii) A functional test (bioassay) to detect the presence of TSAb (stimulation) or TBAb (inhibition), using intact cells that are transfected with chimeric or human TSH receptors, which will then produce a biological response in the form of an increase in cAMP or bioreporter (luciferase) genes as markers. Biological activity against TSAb or TBAb activity in patient serum [68].

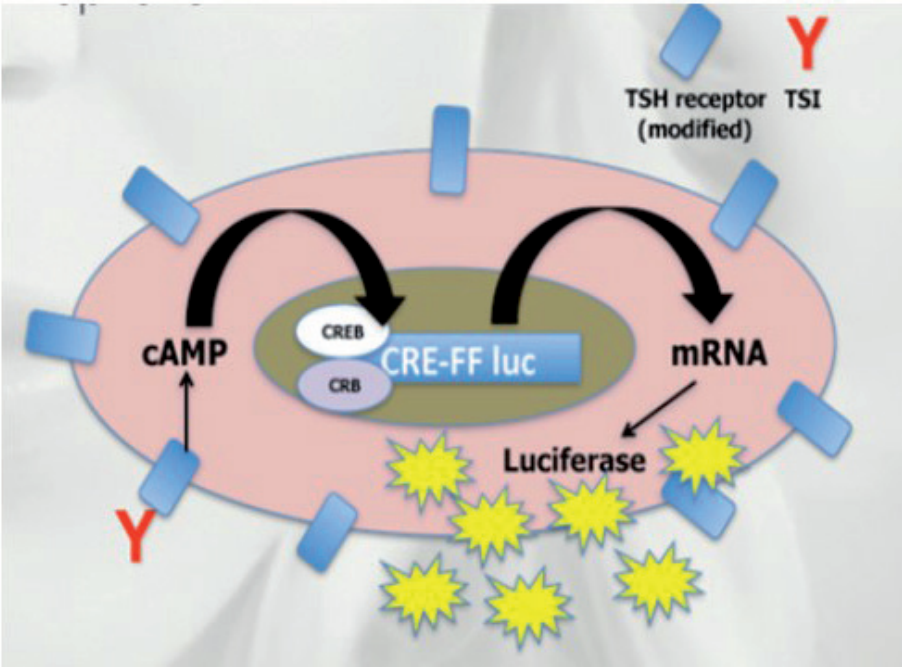
The TRAb method can detect TSHR autoantibodies that interact with the TSH receptor, regardless of their functional character (stimulation or inhibition). This method works based on competitive binding, i.e., the immunoglobulin in the patient's serum will compete with either porcine (porcine) or bovine TSH that is a radiolabeled or human monoclonal antibody (code M22) which binds to the TSH receptor (recombinant human TSHR) (**Figure 1**) [66, 67]. In its development, the third generation TRAb test uses a monoclonal antibody that binds to TSH (M22). In the research of Zöphel et al. proved that the sensitivity of using human TSHR monoclonal antibody M22 (90.3%, positive cutoff 0.32 IU / I) was better than that of bovine TSH (62.9%, positive cutoff 1.64 IU/I) [66]. The results of a meta-analysis study conducted by Tozzoli, et al. showed that the sensitivity and specificity of the third generation TRAb test (98.3% and 99.2%) were higher than the second generation (97.1% and 97.4%) [67].

The second method is bioassay (TSAb or TBAb), which uses intact cells transfected with chimeric or human TSH receptors, which will produce a biological response in the form of an increase in cAMP or a bioreporter gene (luciferase) as a biological marker of stimulating or inhibiting TSHR antibody activity in serum patient [68]. This method is a functional examination, which can technically be modified to detect the presence of TSAb or TBAb that are present together in a patient's serum. The development of a second-generation bioassay method using a mouse/human chimeric TSHR-LH receptor (MC4) can effectively eliminate the effect of TBAb. This approach demonstrates sufficient specificity and sensitivity for the diagnosis of GD and is clinically useful for monitoring the effect of anti-thyroid treatment (relapse and remission) [69].

One of the modalities of the bioassay method introduced recently and widely used in research is Thyretain, a method of measuring TSAb activity with a luciferase bioreporter using chimeric TSH-LH receptors (MC4) expressed on the surface of CHO (MC4-CHO-Luc) cells (**Figure 2**). In MC4, C-terminal TSHR



**Figure 1.** Schematic illustration of TSHR autoantibodies detection with competitive binding of TSH receptor with radiolabelled or human monoclonal antibody (code: M22) [68].



**Figure 2.**  
*Schematic illustration of measuring TSAb activity with a luciferase bioreporter chimeric TSH-LH receptor [69].*

(amino acid 262–335) is substituted with amino acids 261–329 derived from the mouse LH-hCG (luteinizing hormone-choriogonadotropin) receptor. The substituted TSHR C-terminal area contains the epitope TBAb. The MC4 receptor was designed to reduce TBAb interactions when TSAb was measured, by eliminating the epitope area of TSHR using TBAb. When TSAb binds to the MC4 receptor on CHO-MC4 cells, it produces a cascade signal that stimulates increased intracellular cAMP production. Furthermore, cAMP induces activation of a promoter containing the luciferase gene CRE-luc (cAMP response element-luciferase). Luciferase activity is measured as a relative light unit, determined in cell-lysate through a luminometer [69–71].

Initially, TPO autoantibodies were detected as antibodies to thyroid microsome or AMA (anti microsomal antibody), using the semiquantitative method of erythrocyte hemagglutination and complement fixation [63]. The development of more specific detection methods is done by immunoassay or immunometric, using recombinant or purified TPO [72]. A recent study by D’Aurizio et al. evaluating and developing the diagnostic performance of third-generation immunometric methods, obtained increased sensitivity and specificity that are better than before [73].

Although many benefits can be obtained from regular TSAb and TPOAb titer checks in pregnant women who are suffering from GD or who have a history of GD before pregnancy. However, in reality, most clinicians have not routinely performed thyroid antibody tests, of course, with various considerations such as the high cost of examinations. Not all laboratory facilities can carry out TSAb examinations.

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