International Journal of Pharmacy and Biological Sciences-IJPBS™ (2020) 10 (4): 113-117
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Review Article | Biological Sciences | OA Journal | MCI Approved | Index Copernicus

# Biomarkers and Experimental Models of Hyperthyroidism

**Kashaf M Shaikh* and Shivalinge Gowda KP**
**Dept of Pharmacology, FPS, PES University, Bengaluru, Karnataka, India**

Received: 16 Jul 2020/ Accepted: 18 Aug 2020 / Published online: 1 Oct 2020
***Corresponding Author Email:** shaikhkashaf@gmail.com

## Abstract
Hyperthyroidism is excessive functional activity of the thyroid gland, characterized by increased basal metabolism and disturbances in the autonomic nervous system because of excess thyroid hormone production. The incidence is higher in women (2%) than in men (0.02%). Several conditions can lead to hyperthyroidism: diffuse toxic goiter or Grave's disease, toxic nodular goiter, toxic adenoma, therapy-induced hyperthyroidism (eg, excess T 4 or T 3 substitution), excess iodine intake, thyroiditis, follicular carcinoma, and TSH-producing tumor of the pituitary. Biomarker, or biological marker is a measurable indicator of some biological state or condition. Biomarkers are often measured and evaluated to examine normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention. Various biomarkers of hyperthyroidism are; T3, T4, TSH, Tg, Anti-Tg, TPO, Anti-TPO, Synapsin 1 and Transthyretin. Experimental models are essential to evolving the complexity and pathogenetic mechanisms of the disease and in the design of specific and effective treatments. Various models have been developed for the design of experimental models of Hyperthyroidism.

**Keywords**
Hyperthyroidism, Biomarkers, Experimental models

*****

**INTRODUCTION:**
Hyperthyroidism is the condition that occurs due to excessive production of thyroid hormones by the thyroid gland. Thyrotoxicosis is the condition that occurs due to excessive thyroid hormone of any cause and therefore includes hyperthyroidism. Some, however, use the terms interchangeably. Signs and symptoms vary between people and may include irritability, muscle weakness, sleeping problems, a fast heartbeat, heat intolerance, diarrhea, enlargement of the thyroid, hand tremor, and weight loss. Symptoms are typically less severe in the elderly and during pregnancy. An uncommon complication is thyroid storm in which an event such as an infection results in worsening symptoms such as confusion and a high temperature and often results in death. The opposite is hypothyroidism, when the thyroid gland does not make enough thyroid hormone. Graves' disease is the cause of about 50% to 80% of the cases of hyperthyroidism in the United States. Other causes include multinodular goiter, toxic adenoma, inflammation of the thyroid, eating too much iodine, and too much synthetic thyroid hormone. A less common cause is a pituitary adenoma. The diagnosis may be suspected based on signs and symptoms and then confirmed with blood tests. Typically blood tests show a low thyroid stimulating hormone (TSH) and raised T3 or T4. Radioiodine uptake by the thyroid, thyroid scan, and TSI antibodies may help determine the cause. Treatment depends partly on the cause and severity of disease. There are three main treatment options: radioiodine therapy, medications, and thyroid surgery. Radioiodine therapy involves taking iodine-

131 by mouth which is then concentrated in and destroys the thyroid over weeks to months. The resulting hypothyroidism is treated with synthetic thyroid hormone. Medications such as beta blockers may control the symptoms, and antithyroid medications such as methimazole may temporarily help people while other treatments are having effect. Surgery to remove the thyroid is another option. This may be used in those with very large thyroids or when cancer is a concern. In the United States hyperthyroidism affects about 1.2% of the population. It occurs between two and ten times more often in women. Onset is commonly between 20 and 50 years of age. Over all the disease is more common in those over the age of 60 years.[1-6]

**BIOMARKERS:**

A bio-marker, or biological marker is a measurable indicator of some biological state or condition. Biomarkers are often measured and evaluated to examine normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.[7]

**Serum T3**; triiodothyronine (T3) is a hormone secreted by thyroid gland. Serum levels of triiodothyronine (T3) is analyzed by colorimetric competitive enzyme immunoassay using individual ELISA Kit. In detail, microtiter wells coated with antibody are prepared and 100 µl of samples and standard T3 solution is applied, then followed by 50 µl of HRP conjugate, 50 µl of color solution, and 50 µl of stop solution. The absorbance is measured by an ELISA reader at 450 nm.[8] typically, normal results range from 100 to 200 Nano grams per deciliter (ng/dL). Most of the T3 in your body binds to protein. The T3 that doesn't bind to protein is called free T3 and circulates unbound in your blood. The most common kind of T3 test, known as the T3 total test, measures both kinds of T3 in your blood.[9]

**Serum T4**; Thyroid produces a hormone called thyroxine, which is known as T4. This hormone plays a role in several body's functions, including growth and metabolism.

Some of the T4 exists as free T4. This means it hasn't bonded to protein in blood. This is the type available for use by the body and tissues. However, most of the T4 in blood stream is bonded to protein.

There are two kinds of T4 tests: a total T4 test and a free T4 test.

A total T4 test measures the T4 that's bonded to protein along with any free T4. A free T4 test measures only the free T4 in blood.

Typical results for the total T4 test in adults generally range from 5.0 to 12.0 micrograms per deciliter (µg/dL). Results for children vary based on age.

Typical results in adults for the free T4 test generally range from 0.8 to 1.8 Nano grams per deciliter (ng/dL). Like total T4 in adults, free T4 also varies in children according to age.[10]

Blood samples are collected, and serum is separated by centrifugation at 3000 rpm for 10 min at 4°C. Serum levels of T4 were analyzed by colorimetric competitive enzyme immunoassay using individual ELISA Kit and the absorbance is measured by an ELISA reader at 450 nm.[8]

**Serum TSH;** TSH stands for thyroid stimulating hormone. A TSH test is a blood test that measures this hormone. TSH levels that are too high or too low can indicate your thyroid isn't working correctly.

It is also known as thyrotropin, is a 28-kDa glycoprotein, released by thyrotrophs. TSH is the physiologic controller of T4 and T3. TSH normal values are 0.5 to 5.0 mIU/L. [11, 12]

Blood samples are collected, and serum is separated by centrifugation at 3000 rpm for 10 min at 4°C. Serum levels of thyroid-stimulating hormone (TSH) is analyzed by colorimetric competitive enzyme immunoassay using individual ELISA Kit. In detail, microtiter wells coated with antibody are prepared and 100 µl of samples and standard T3, T4, or TSH solution is applied, then followed by 50 µl of HRP conjugate, 50 µl of color solution, and 50 µl of stop solution. The absorbance is measured by an ELISA reader at 450 [8]

**Tg;** Thyroglobulin (Tg) is dimeric protein delivered by the follicular cells of the thyroid and utilized totally inside the thyroid organ, thyroglobulin protein represents around half of the protein substance of the thyroid organ. The combination of the protein antecedent for Tg is the initial phase in the development of T4 and T3. This substance is a660-kDa glycoprotein made out of two comparative 330-kDa subunits held together by disulfide spans.[12]

Tg is measured using ELISA kit. The indirect microplate ELISA procedure:

(i) Thyroglobulin was dissolved in carbonate/ bicarbonate buffer (1.59 g $Na_2$ $CO_3$, 2.93 g $Na_2HCO_3$, made up to 1 liter with distilled water and adjusted to pH 9.6). The solution (200 pl) was added to each well of a polyvinyl microtitration plate and incubated in a humid chamber overnight at 4°C to allow passive adsorption of the thyroglobulin to the polyvinyl surface.

(ii) Unreacted material was removed by washing. This was achieved by emptying the plates, refilling the wells with PBS Tween (phosphate buffered saline, 0.15 M, pH 7.4, containing 0.05% Tween 20) and leaving for 3 min. The procedure was repeated three times and then

the plate was shaken dry before the next reagent was added.

(iii) The test serum or plasma specimens were diluted 1:100 in PBS Tween, and 200-4 samples were added to two wells in the plate, permitting duplicate tests on each sample.

(iv) The plates were then incubated 4 hr at 37°C in a humid chamber.

(v) Washing (step 2) was repeated.

(vi) Conjugate (200 pl) consisting of sheep anti-human globulin labelled with alkaline phosphatase standardized and diluted in PBS Tween was added to each well and the plate incubated overnight at 4°C.

(vii) Washing (step 2) was repeated.

(viii) The enzyme substrate (200 pl) consisting of p-nitrophenyl phosphate, 1 mg/ml in 10% diethanolamine buffer (97 ml diethanolamine, 800 ml H20, 1 M HCl, added to give pH 9.8, made up to 1 liter with H20), was added to each well and incubated for 20 min at room temperature.

(ix) NaOH (50 pl of 3 M NaOH) was added to each well to stop the enzyme substrate reaction.

(x) The contents of each well were removed and the absorbance read in a spectrophotometer.[13]

The serum level TG is proportional to the volume of thyroid tissue in the body at a rate 1 ng/mL per 1 g of thyroid mass. Since the size of the normal thyroid gland is 20-25 g, the reference range has to be generally about 20 to 25 ng/mL.[14]

**Anti-Tg;** Antithyroglobulin antibody testing is used in the evaluation for thyroid problems.

Antithyroglobulin is not normally found in the blood stream. However, 10-20% of healthy individuals have detectable anti-thyroglobulin levels. The estimation is done using ELISA. The measured serum level should be less than 4 IU/mL.[15, 16].

**TPO;** Thyroid peroxidase (TPO) or iodide peroxidase, is a protein communicated principally in the thyroid where it is discharged into colloid. The estimation is done using ELISA.[15]

**Anti-TPO;** In immune system ailments, in any case, the resistant framework glitches, erroneously assaulting solid organs and tissues as if they were outside trespassers. In individuals with a thyroid-related immune system condition, the blood level of TPO antibodies may rise, against TPO antibodies are the most widely recognized hostile to thyroid autoantibody, show in roughly 90% of Hashimoto's thyroiditis, 75% of Graves' malady and 10-20% of nodular goiter or thyroid carcinoma. Additionally, 10-15% of ordinary people can have abnormal state hostile to TPO counter acting agent titers.

The measured serum level should be less than 9 IU/mL. It is measured by a micro-ELISA method.[12, 16, 17]

**Synapsin 1;** The Synapsin 1 (SYN 1) protein is from the Synapsin family that are neuronal phosphoprotein, which interface with the cytoplasmic surface of synaptic vesicles.

This phosphoprotein is as an endogenous substrate bound to the vesicular film. It is phosphorylated by four known classes of protein kinases including those actuated by cAMP, calcium/calmodulin mitogen, and cyclin.

It is estimated using ELISA, this assay is based on the sandwich ELISA principle; each well of the supplied microtiter plate has been pre-coated with a target specific capture antibody. Standards or samples are added to the wells and the target antigen binds to the capture antibody. Unbound Standard or sample is washed away. A biotin-conjugated detection antibody is then added which binds to the captured antigen. Unbound detection antibody is washed away. An Avidin-Horseradish Peroxidase (HRP) conjugate is then added which binds to the biotin. Unbound Avidin-HRP conjugate is washed away. A TMB substrate is then added which reacts with the HRP enzyme resulting in color development.

A sulfuric acid stop solution is added to terminate color development reaction and then the optical density (OD) of the well is measured at a wavelength of 450 nm ± 2 nm. The OD of an unknown sample can then be compared to an OD standard curve generated using known antigen concentrations in order to determine its antigen concentration.[18]

**Transthyretin;**

Human Transthyretin (TTR) is a 55kDa homo-tetramer with a dimer of dimers quaternary structure that is integrated in the liver, choroid plexus and retinal shade epithelium for emission into the circulatory system, cerebrospinal liquid and the eye.

It is a transport protein in the serum and cerebrospinal fluid that carries the thyroid hormone thyroxine (T4) and retinol-binding protein bound to retinol. It can be estimated using ELISA.[12, 19]

The normal serum transthyretin concentration is 250 mg/L, corresponding to maximal binding capacity of 2mg T4/L. Transthyretin binds approximately 10% of T4 and 10% of T3. In addition, transthyretin also binds retinol-binding protein, thereby being involved in vitamin A transport.[20]

**EXPERIMENTAL MODELS:**

Experimental models are essential to evolving the complexity and pathogenetic mechanisms of the disease and in the design of specific and effective treatments. Various models have been developed for the design of experimental models of Hyperthyroidism.

**Induction of thyroiditis;** Thyroiditis is induced in rabbits by injecting rabbit thyroid extracts together with complete Freund's adjuvant (CFA) into the footpads. Studies of mice immunized with human or murine Tg demonstrated a role for MHC antigens and cytotoxic T cells (but not antibodies) in the development of thyroiditis.[21]

**Shimojo model (transfected fibroblasts);** Graves' disease was induced by injecting fibroblasts transfected with MHC class II molecules (RT4.15HP cells) as well as the cDNA for human TSHR. For Two weeks in female AKR/N mice, that developed TSHR antibodies with TBI activity. Mice became thyrotoxic, with elevated serum T4 and T3 levels as well as detectable TSAb activity. An important aspect to the Shimojo approach is that it is restricted to H2-k mouse strains (AKR/N and H-2k congenics).[22]

**Adenovirus encoding the TSHR**; Intramuscular injection of a replication-deficient adenovirus vector encoding the human TSHR-cDNA is an efficient approach for inducing Graves'-like hyperthyroidism in mice. After three injections [3-wk intervals] of TSHR-adenovirus, most BALB/c mice developed TSHR antibodies detectable by TBI, and 50% had TSAb activity and became thyrotoxic. In contrast, only 25% of C57BL/6 mice became hyperthyroid and DBA/1J, DBA/2J, CBA/J, and SJL/J mice remained euthyroid.[23]

**Mice transgenic for a monoclonal TSAb (mTSAb);** TSAb-transgenic mice in which a patient-derived TSAb is expressed in B cells. Expression of the human TSAb in mice resulted in various manifestations of hyperthyroidism including increased free thyroxine levels with concomitantly decreased TSH levels, increased thyroid uptake of technetium pertechnetate, hyperthermia and thyroid hyperplasia. Two human monoclonal antibodies (B6B7 and 101–2) isolated by Epstein-Barr virus transformation of Graves' peripheral blood lymphocytes had weak TSAb activity when used at high concentrations (23 µg IgG/ml of B6B7).

**Hamster Shimojo model;** Outbred hamsters repeatedly injected with TSHR-expressing Chinese hamster ovary (CHO) cells together with the adjuvants alum and pertussis toxin developed TSHR antibodies including TBI activity. Moreover, 30% of animals had elevated T4 levels and goiters as well as thyroid lymphocytic infiltration.[24]

**REFERENCES:**

1. "Hyperthyroidism"(https://web.archive.org/web/20150404183926/http://www.niddk.nih.gov/healthinformation/healthtopics/endocrine/hyperthyroidism/Pages/fact-heet.aspx). www.niddk.nih.gov. July 2012. Archived from the original (http://www.niddk.nih.gov/healthinformation/healthtopics/endocrine/hyperthyroidism/Pages/factsheet.aspx) on 4 April 2015.Retrieved 2 April2015.
2. Devereaux D, Tewelde SZ. Hyperthyroidism and thyrotoxicosis. Emergency Medicine Clinics. 2014; 32(2):277-92.
3. Bahn RS, Burch HB, Cooper DS, Garber JR, Greenlee MC, Klein I, Laurberg P, McDougall IR, Montori VM, Rivkees SA. Hyperthyroidism and other causes of thyrotoxicosis: management guidelines of the American Thyroid Association and American Association of Clinical Endocrinologists. Thyroid. 2011; 21(6):593-46.
4. Al-Ghurabi ME. Diagnosis of hyperthyroidism by using some biomarkers and immune markers. Journal of Pharmaceutical Sciences and Research. 2018; 10(2) :310-5.
5. El-Massry A, AbouSamra A, Iqbal M, Fawzy O, Doheim MF. Prevalence among Keratoconic Patients.
6. Brent GA. Graves' disease. New England Journal of Medicine. 2008; 358(24):2594-605.
7. doi:10.1067/mcp.2001.113989
8. Kim M, Lee BC. Therapeutic effect of Scutellaria baicalensis on L-thyroxine-induced hyperthyroidism rats. Evidence-Based Complementary and Alternative Medicine. 2019;2019
9. https://www.healthline.com/health/t3
10. https://www.healthline.com/health/t4-test
11. https://www.uclahealth.org/endocrine-center/normal-thyroid-hormone-levels
12. Al-Ghurabi ME. Diagnosis of hyperthyroidism by using some biomarkers and immune markers. Journal of Pharmaceutical Sciences and Research. 2018; 10(2):310-5.
13. Voller A, Bidwell DE, Burek CL. An enzyme-linked immunosorbent assay (ELISA) for antibodies to thyroglobulin. Proceedings of the Society for Experimental Biology and Medicine. 1980; 163(3):402-5.
14. doi: 10.4331/wjbc. v8. i1.81
15. Kohno Y, Naito N, Hiyama Y, Yoshio N, Suzuki N, Tarutani O, et al. Thyroglobulin and thyroid peroxidase share common epitopes recognized by autoantibodies in patients with chronic autoimmune thyroiditis. The Journal of Clinical Endocrinology & Metabolism. 1988; 67(5):899-7.
16. https://www.verywellhealth.com/thyroid-disease-diagnosis-4013578
17. Portmann L, Hamada N, Heinrich G, Degroo LJ. Anti-thyroid peroxidase antibody in patients with autoimmune thyroid disease: possible identity with anti-microsomal antibody. The Journal of Clinical Endocrinology & Metabolism. 1985; 61(5):1001-3.
18. Schlaf G, Salje C, Wetter A, Stuertz K, Felgenhauer K, Mäder M. Determination of synapsin I and synaptophysin in body fluids by two-site enzyme-

linked immunosorbent assays. Journal of immunological methods. 1998; 213(2):191-9.
19. Hazenberg BP, Van Schijndel B, Bijzet J, Limburg PC, Bos R, Haagsma EB. Diagnostic performance of transthyretin measurement in fat tissue of patients with ATTR amyloidosis. Amyloid. 2011;18(sup1):79
20. www.sciencedirect.com › topics › neuroscience › transthyretin
21. Kong YM. The mouse model of autoimmune thyroid disease. InImmunology of Endocrine Diseases 1986 (pp. 1-24). Springer, Dordrecht.
22. Shimojo N, Kohno Y, Yamaguchi KI, Kikuoka SI, Hoshioka A, Niimi H, *et al.* Induction of Graves-like disease in mice by immunization with fibroblasts transfected with the thyrotropin receptor and a class II molecule. Proceedings of the National Academy of Sciences. 1996; 93(20):11074-9.
23. Nagayama Y, McLachlan SM, Rapoport B, Niwa M. A major role for non-major histocompatibility complex genes but not for microorganisms in a novel murine model of Graves' hyperthyroidism. Thyroid. 2003; 13(3):233-8.
24. McLachlan SM, Nagayama Y, Rapoport B. Insight into Graves' hyperthyroidism from animal models. Endocrine Reviews. 2005; 26(6):800-32.