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EXPERIMENTAL ANIMAL MODELS USED IN HYPOTHYROIDISM - OVERVIEW

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ABSTRACT

Thyroid hormone has an effect practically on every tissue and organ system in the body. Iodine absorption is as essential in the body as TSH balance and the persistent imbalance in iodine with TSH causes an increased risk of disorders. Deficiency of iodine and abnormal serum TSH results in hypothyroidism and hyperthyroidism. Hypothyroidism is a common clinical and pathological condition which arises from the deficiency of thyroid hormones produced insufficiently by thyroid gland. Several animal models of hypothyroidism are performed to determine the pathophysiological conditions and level of thyroid hormones in blood. The main objective of this article is to explain the details of hypothyroidism including pathophysiology, signs and symptoms, diagnosis and different principles of different experimental animal models and the main focus is on methods to induce hypothyroidism using laboratory animals.

Keywords: Hypothyroidism, Triiodothyronine, Carbimazole, Drug induced hypothyroidism

INTRODUCTION

Thyroid hormone has an impact on practically every tissue and organ system in the body by energy supply in the human thyroid cell which is necessary for many activities like synthesis of proteins, nuclear acids, nucleotides, lipids, transport functions and other activities like lysosome, phagocytosis movement etc. and boosting

basal metabolic rate and tissue thermogenesis and up-regulating alpha-adrenergic receptors, which results in an increase in sympathetic activity. As the two thyroid hormones secreted by follicular cells of thyroid gland i.e., Triiodothyronine (T3) and thyroxin (T4) are essential for involvement in many biological activities like metabolism, growth regulation, expanding energy with other aspects, these hormones are controlled mainly by Thyroid-stimulating hormone (TSH) which is synthesized in the pituitary gland. The release of TSH is mainly possible by the stimulation of Thyroid releasing hormone (TRH), secreted by hypothalamus **[1-2]**. Due to the activation of TSH receptors, there is an increased cellular uptake of iodine from blood and so causes secretion of T3 and T4 hormones into the blood stream **[3]**.

Iodine absorption is as essential in the body as TSH balance and the persistent imbalance in iodine with TSH causes an increased risk of disorders like colloid goiter **[4]**, hypothyroidism, Grave's disease, hyperthyroidism, Hashimoto thyroiditis. All these diseases occur by the development of risk factors **[5]** such as ethnic (based on population analysis data) **[6]** and genetic **[7]** susceptibility, alcohol intake, smoking **[8]**, infections, using different drugs with others and can be diagnosed biochemically.

Abnormal serum TSH results in subclinical as well as overt thyroid dysfunction hypothyroidism and hyperthyroidism. The subclinical hyperthyroidism is defined as low or suppressed serum TSH with normal serum-free T4 and T3 **[9]**, while subclinical hypothyroidism is defined as raised serum TSH with normal circulating T4. Without enough thyroid hormones, many of your body's functions slow down. Both diseases are quite opposite and consist of different causes **[10-11]**.

HYPOTHYROIDISM

It is a common clinical and pathological condition which arises from the deficiency of thyroid hormones produced insufficiently by thyroid gland. The incidence of hypothyroidism varies between 4 and 10% depending upon the age, gender and population data studied **[12]**. The majority of adult hypothyroid individuals (frequently in less than 65 years of age) and women have acquired the condition, which can start in the thyroid gland, pituitary, or hypothalamus **[13]**. Due to the trace element iodine is necessary for the manufacture of thyroid hormones, it affects the normal function of thyroid hormones and so severe iodine deprivation can also result in hypothyroidism **[14]**. It is diagnosed by clinical features such as a typical facial appearance, hoarse slow

speech, and dry skin and by low levels of thyroid hormones **[15]**.

Pathophysiology- T3 is produced from the thyroid and from deiodination of T4 in extrathyroidal tissues. Due to environmental triggers (iodine, infection, cytokine therapy, in pregnancy), there is a breakdown of immune tolerance with biochemical and clinical symptoms of hypothyroidism are brought on by T3 depletion. As a result, fundamental intracellular processes including the slowed down of mitochondria's oxygen consumption and calorigenesis occurs. Reduced hunger, cold intolerance, a low basal metabolic rate, and a slightly lower baseline body temperature are all symptoms of a decline in energy metabolism and heat production **[16]**. Because of different signs and severity, the disease is classified with respective pathophysiology.

Classification- The disease is classified into congenital and acquired conditions based on when symptoms first appear, second it is on primary and secondary (which is also called central hypothyroidism) stage on the basis of endocrine dysfunction and according to how severe the condition is, overt (clinical) and subclinical type is considered **[17-18]**. In overt condition, the TSH level increases from 97.5th percentile (max limit reference range) and free T4 (fT4) decreases from 2.5th percentile (min limit reference range) whereas in subclinical condition, the level of TSH are increased and fT4 levels remain in the reference range **[19]**. Other types are based on signs and symptoms like Postpartum thyroiditis, Subacute granulomatous thyroiditis and genetic hypothyroidism.

• **Primary hypothyroidism** mainly arises in the thyroid gland where deficiency of iodine and Hashimoto thyroiditis occurs as the principal causes and other causes

- Drugs—e.g., amiodarone, monoclonal antibodies (e.g., ipilimumab), antiepileptic drugs (eg, valproate), drugs used in multidrug-resistant tuberculosis.
- Iatrogenic—radioiodine treatment (e.g., to treat Graves' disease or toxic nodular disease), radiotherapy, hemithyroidectomy or surgery in the neck or head region.
- Transient thyroiditis—viral (De Quervain's syndrome), post-partum, silent and destructive thyroiditis.
- Chronic autoimmune response i.e., Hashimoto thyroiditis
- Thyroid gland infiltration—infectious (e.g., mycoplasma), malignant, autoimmune (e.g., sarcoidosis), inflammatory.
- Genetic—autoimmunity-related genes (e.g., HLA class I region, PTPN22, SH2B3, and

VAV3), general and thyroid-specific genes (e.g., FOXE1, ATXN2, and PDE8B) **[20]**.

• **Central hypothyroidism** is a rare disorder of the hypothalamus-pituitary axis which affects adulthood of man and woman. It results in lesions in hypothalamic or pituitary region, iron overload reduced or normal TSH secretion and, as a result, decreased thyroid hormone (fT4) synthesis and release. The common causes in pituitary area are Pituitary adenomas, History of pituitary surgery or radiotherapy, head trauma and pituitary apoplexy **[21-22]**, whereas in hypothalamus area is suprasellar tumors and History of hypothalamic surgery or radiotherapy.

Signs and symptoms- The majority of hypothyroidism symptoms is widespread in the general population and is non-specific but common are like weakness, skin changes, decreased sweat, cold intolerance, fatigue, constipation, and vocal changes. Less common hypothyroid symptoms include dry skin (when severe, called as myxoedema), hoarseness, anaemia (normally normochromic and normocytic but occasionally macrocytic), increased thrombosis risk (due to impaired coagulation and fibrinolysis), cardiac changes involve hyponatremia various neurological (carpal tunnel syndrome and encephalopathy), musculoskeletal (myalgia and increased serum creatine kinase levels) and metabolic symptoms like lipid abnormalities (low LDL) **[23]**.

Diagnosis- All the symptoms help to assess the examination of the disease. As the serum T4 and T3 are bound with plasma proteins in which thyroxine binding globuline, albumin and transthyretin are also included, the bound do not often measured rather fT4 can be considered to determine the thyroid function status. The measurement is done by fT4 assay after ultrafiltration and then equilibrium dialysis followed by addition of an anti-T4 antibody to the serum; or FT4 index, which is a calculation based on the total T4 and the T3 uptake. For Hashimoto thyroiditis, measurement of TPO antibodies can be helpful. Rest all the blood tests confirms the disease **[24-25]**.

Monitoring of treatment for hypothyroidism:- Treatment can decrease the tissue manifestations and also decreases other risks as well. If the patient is >50 years of age and having any risk of cardiac disease then levothyroxine with 0.025-0.05mg per day should be administered. But if patient is not at a risk for any cardiac disease then levothyroxine should be given at a dose of 0.075mg per day. There is a change in TSH level or free T4 level to be monitored every 6-8 weeks and according to that record,

adjust the dosage of levothyroxine until tests are normal. After getting improper reports and the patient feels lethargy then continue the therapy with triiodothyronine at a dose of 0.0125mg per day till the normal condition. In case of pregnant women, the fixed dose of LT4 is administered where the TSH level should be <2.5 mlU/L **[26]**.

Animal models for development of Hypothyroidism

For determining the signs and symptoms of any disease at preclinical or on research stage, some experimental models are often performed. Suitable animals having morphology similar to humans like rodents are being employed for the study of diseases. Here, by using some animal models of hypothyroidism, one can study the abnormalities at morphological and functional parameters of hypothyroidism and to undertake preclinical evaluations. Though the methods were conducted relatively years ago but are still applicable. And the main purpose of this review is to highlight the principles, procedures and advantages with disadvantages of each method. The various methods to induce hypothyroidism in animals are a) Surgical method, b) Dietetic method, c) Radioactive method, and d) Drug induced method respectively **[27]**.

a) Drug induced method

This method is the most convenient way of causing thyroid hypofunction due to variety of drugs can be possibly used on experimental animals. Depending on the method of administration, animals are selected for the study. Some antithyroid drugs (mercazolil, propylthiouracil, potassium thiocyanate) and their analogues (carbimazole, metisol, thiamazole, methimazole) are used as thyreostatic agent for experimental modeling **[28]**.

Principle:- The animal model is designed to induce hypothyroidism by using Carbimazole drug for a particular period of time and investigate the effect of drug **[29]**. Carbimazole is an antithyroid drug that inhibits thyroid inorganic iodine absorption and concentration, as well as the synthesis of di-iodotyrosine and thyroxine. When converted to its active form i.e., methimazole stops the thyroid peroxidase enzyme from coupling and iodinating the tyrosine residues on thyroglobulin, lowering thyroid hormone synthesis T3 and T4 **[30]**.

Procedure:- For study, 20 adult male Wistar rats of 180-220gm in weight were required and housed in 2 cages with maintained temperature (22°C), relative humidity and natural light cycle. Food and water supply was adequately available. The rats were separated in groups like each group consist of

equal number of rats and the groups were like control group receiving water and standard food whereas treated group of carbimazole. The treated group was administered carbimazole at the dose of 0.05 mg/kg by oral route for six weeks. All the animals were at chow diet. After six weeks of drug administration, some determinations were followed such as serum T3, TSH and serum total antioxidant capacity (TAC) on blood collection **[31]**.

Another model of propylthiouracil can be used to induce hypothyroidism in which the principle is based on reduction in concentration of thyroid hormone and observe the effect on nerve cells **[32]**. Propylthiouracil hinders the conversion of iodide to iodine by binding to thyroid peroxidase. Thyroid peroxidase converts iodide to iodine (with hydrogen peroxide as a cofactor) and catalyses the integration of the resultant iodide molecule onto the 3 and/or 5 positions of the phenol ring of tyrosines present in thyroglobulin. Thyroglobulin is degraded to create thyroxine (T4) and tri-iodothyronine (T3), the thyroid gland's major hormones. As a result, propylthiouracil efficiently limits thyroid hormone synthesis. In this model, adult male Wistar rats (6 weeks old) were used. Their body weight should be between 180-200gm. Animals were divided into groups with equal number of animals i.e., control group having animals were administered 0.9% sodium chloride at 1ml/day and hypothyroidism group having animals were administered 0.05% propylthiouracil saline solution at 1ml/day. Blood samples were collected before intervention for 8 weeks for centrifugation process and measure tT_3, tT_4 and TSH. After taking animal weight, all were euthanized after 8 weeks **[33]**.

Advantage- This model aids to determine frequent consequences regarding thyroid hormone, clear manifestation reproducibility, requirement of drug is less and outcome is easy to get by daily record.

Disadvantage- Carbimazole causes heartburn and cardiac arrest, drugs can produce other side effects and also can harm the experiment conductors due to cutaneous symptoms.

b) Dietary method

As a crucial component, iodine is frequently used by thyroid hormones and found in thyroid gland. It is first stored in the form of iodide and used in its oxidized form i.e., iodine which is essential for almost every vital organ in the body **[34]**. When iodine consumption is adequate, the proportion of ingested iodine removed from the blood by the thyroid ranges from 10% to 80%. In

pregnancy, there is an increase in demand of iodine and so thyroid hormones increases in production **[35]**. For ensuring the sufficient intake of dietary iodine for adults is about 150µg and for pregnant or lactating women is about 250µg **[36]**. Iodine deficiency can be one of the main causes of developing hypothyroidism including metabolic disorders and mental disorders as well. In pregnant women, it causes adverse effects in growth development of fetus, and leads to maternal hypothyroidism **[37]**.

Principle:- On the basis of this information, an animal model was performed considering iodine lack diet or pellets used to determine the effect of thyroid hormone deficiency in pregnant women, for brain activities and for metabolism.

The procedure was started with around 207-208 young (age of 40-60 days) adult female Long Evans rats were paired and housed according to the standard conditions with temperature maintained at 22°C and relative humidity should be 40-60%. Groups were divided after 2-3 weeks and were given five casein based diet having adjusted potassium iodate in the base AIN-76A (purified rodent diet) ranging from base I = 0.975 µg/gm (group 1) to deficient base I = 0.025 µg/gm (group 5). On the basis of food consumption (starting from gestational period to lactating period) and body weight, the calculation was possibly done. Then body weight of pups and dams were recorded. It should be noted that after the sperm presence in vagina was found, animals were separately housed. Next, dams from control group and the 2 from last 4 and 5 group were euthanized on gestational day 16 or gestational day 20. Samples were taken from each group to calculate the mean.

Measurements:- Chow verification, serum TH measures, quantifying iodine in urine, serum T4 analysis of fetus by collecting blood, analysis of brain hormone and toxicity measures.

Observation:- Brain activities, Thyroidal T4 reduction, metabolites disturbances **[38]**.

Advantage:- Model is comparatively easy to perform without any surgery and all the findings are clear and similar with clinical conditions.

Disadvantage:- Time consuming process, calculation process makes this model slightly complex and totally based on diet preparation.

c) Surgical method

The invasive method of hypothyroidism always involves the deficiency of thyroid hormone directly by removing thyroid gland and the purpose is very controversial because it causes postoperative hypothyroidism.

Principle- The major principle is based on surgically removal of thyroid gland completely or partially in animals used in laboratory which results in thyroidectomy with the inhibition of thyroid hormones to circulate throughout the blood **[39]**. Usually animals like mice, rats, rabbits, sheep and other can be involved as experimental objects. Different animal is employed for different procedure to show the signs of hypothyroidism.

Procedure- In case of rats, animals required were 7 weeks old female wistar and 170-200g in weight. Groups were divided such that rats for Thyrodectomy (TD) and rats under control group. TD group were given water with 1% $CaCl_2$ to preserve calcium homeostasis due to parathyroid gland removal after surgery. Sample collection of skin is done in weeks at 4, 8, 12, 16 and 32 under anesthesia. Repeat the sampling at interval of 4 weeks and proceed by histologic analysis but suture after every collection. The blood collection for assay from heart under anesthesia was done and centrifuged for 15 min so that serum T4 and FT4 concentration can be determined.

Observation:- Hairs were seen after 12 weeks of surgery in control group but not in TD group, epidermis thick in control group but thin in TD group after 16 and 32 weeks, epidermopoiesis changes on peripheral action of thyroid hormone **[40]** and hair growth were inhibited. The deficiency of thyroid hormone promotes the telogen phase and delay anagen phase **[41]**.

Advantage- Possibly induce hypothyroidism to study the severity developed in disease

Disadvantage- One should have knowledge to perform surgery, other disturbances may occur with TD and there is a loss of C cells.

d) Radioisotope method

Somewhere the radiations are used in the treatment purpose but somewhere it causes high level of cell damage. Radioisotope ^{131}I is generally used for treatment of thyroid disorder and so the correct dose is given. If high doses of ^{131}I are administered and if dose is continued for years **[42]**, it causes follicular call damage and results in hypothyroidism **[43]**.

Principle:- the animal model here mainly explains the use or administration of Radioisotpe ^{131}I for inducing hypothyroidism in animal and calculating different changes on different parameters.

Procedure:- 55 days old mice of C57B1/S strain were required for the study and around 30 mice were obtained for grouping into 15 mice for treated(A group) and 15 for control group (B group). Further, 3 cages having 5 mice were maintained in each group. The

treated group was set on iodine deficient diet from starting to 14th day and control group was on standard diet only. On 15th day, the A group was injected Na ^{131}I intraperitonially with 3.66 MB_q and the diet was replaced with normal diet. Then animals were sacrificed in the days interval of 10, 20, and 40 just like on 10th day of administration of ^{131}I only the treated 5 mice of A group was sacrificed but on 20th day 5 mice from each group was killed for the comparison. At last 40th day, all 15 mice were sacrificed. On each killing, the blood was collected for centrifugation at 3000rpm for 10mins.

Measurements:- Thyroid gland weight, serum T4 level was determined and changes observed in cell size reduction.

Observation:- Thyroid gland size was normal in B group but reduced in A group, different days interval showed level of colloid and change in follicle, C cell depletion and fall in serum T_4 level **[44]**.

Advantage:- with small dose of radioisotope iodine, there can be development of continue and long term hypothyroidism. Observations and findings is amplified by using radioisotope.

Disadvantage:- one should know the perfect handling of radioactive isotope, risks of harm and this model should have more findings.

Focus on research

Apart from drugs, surgical and other popular methods to induce hypothyroidism, a considerable cause of hypothyroidism can be a growing environmental exposure to endocrine disrupting chemicals (EDCs). These substances show disruption in male and female reproductive system, neuroendocrine function and thyroid hormone levels **[45]** through abnormal nuclear receptors signaling. Mainly by affecting the endocrine system, it participates in causing thyroid dysfunction and called as Organotin compounds as well. Tributylin chloride (TBC) is one of the chemical that causes reproductive toxicity in vertibrates leading to abnormalities, also affects human and considered as obesogenic chemicals in some cases. TBC also exerts toxicological action on Hypothalamus-pituitary axis thus results in thyroid gland **[46]**.

CONCLUSION

Wide range of findings and researches of thyroid dysfunction hypothyroidism with the aid of animal models have cleared the vision of treatment therapies. The essentiality of these experimental models since decades give the reason to enhance the knowledge about every disease and so the above mentioned different models have been performed with different principles and criteria which will contribute in

discontinuing he causes of hypothyroidism. By continuing the studies of experimental animal models, one can get more information which will help the health professionals to deal with diseases.

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