GENERAL

INTRODUCTION
Thyroid dysfunction status with special reference to polymorphism of thyroid peroxidase (TPO) gene

Thyroid is the largest (~25g) of the endocrine glands derived from embryonic endoderm. It is a small butterfly shaped gland located at the base of the throat, just below the Adam’s apple and consists of two lobes joined by the isthmus. The ‘wing’ of the butterfly being the left and right thyroid lobes that wrap around the trachea. The thyroid hugs the trachea on either side at the second and third tracheal ring, opposite of the 5th, 6th and 7th cervical vertebrae.

The thyroid gland is specialized organ for endocrine function in the body. The gland secretes thyroid hormones primarily 3,5,3’5’-1-tetraiodothyronine (T₄) or thyroxine and lesser quantity of 3,5,3’-triiodothyronine (T₃) (Ganong, 2005). The thyroid hormones promote normal growth and development and regulate a number of homeostatic functions, including
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energy and heat production (Fujita, 1988). In addition, the parafollicular C cells of human thyroid gland secrete calcitonin, which is important in calcium homeostasis.

To make thyroid hormone, the thyroid gland uses iodine. When iodine is inadequate in the diet, the thyroid may produce an insufficient amount of hormone. If an inadequate intake continues, the ability to make thyroid hormone is slowly depleted. Many cellular processes occur to keep the thyroid as efficient as possible and the thyroid gland often enlarges in an attempt to maintain function. Subsequently, a goiter may form as the thyroid is stimulated to try to make more thyroid hormone.

Essentially, iodine is converted to its free elemental form, called iodide. Iodide enters the thyroid gland through a special transport mechanism. Iodide then undergoes a process called oxidation and is incorporated into intermediate hormones called MIT (Monoiodotyrosine) and DIT (Diiodotyrosine). These compounds then combine to form the active hormones, tri-iodothyronine ($T_3$) and tetra-iodothyronine or thyroxine ($T_4$). $T_3$ is the most biologically active thyroid hormone. It is formed by combining a MIT with a DIT. $T_4$ is formed in much greater quantity by
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Fig. 2: Chemical structures of thyroid hormones
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Thyroid hormone biosynthesis is mediated by a key enzyme thyroid peroxidase (TPO). It catalyzes both iodination and coupling of iodotyrosine residues in thyroglobulin (Tg). TPO is a membrane bound glycoprotein with a molecular weight of about 102 kDa and a heme compound as a prosthetic group. It is located on the apical surface of thyroid follicular cells as a dimer (Baker et al., 1994). Each monomer of 933 amino acid residues is predicted to contain a peroxidase domain, three additional extracellular domains, a transmembrane helix and a short C-terminal intracellular tail (Banga et al., 1990). The human TPO gene is located on chromosome 2p25, covers approximately 150 kb of DNA and 17 exons (Kimura et al., 1989). Full length of TPO mRNA is 2.8 kb. The mutations in TPO gene may cause the formation of defective enzyme which is directly associated with the level and activity of TPO enzyme.

The thyroid gland operates in concert with the hypothalamus and the pituitary, which is commonly referred to as the 'hypothalamic-pituitary-thyroid axis' in addition to the stimulatory cascade leading to thyroid hormone secretion, the axis is also subject to feedback inhibition by the circulating thyroid hormones.
Iodine deficiency disorder (IDD) affects approximately 200 million people worldwide and widely prevalent in developing countries. An estimated 150 million people in India are at risk of IDD. Although iodine deficiency is primary cause of goiter, the significant role played by food goitrogens in the etiology of IDD is being increasingly recognized (Lal et al., 1996).

The recent research revealed that thyroid abnormality is the source of the many other organ disorders (Dimitriadis et al., 1991, Hoppner and Seitz,
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1989). But it is not very much clear how thyroid dysfunction modulates the function of other organs in the body.

Therefore, further investigation needed to unveil the detailed sequential event of cellular and biochemical changes in rat upon experimentally induced thyroid dysfunction and their relationship with the clinically defined thyroid patients in our population.

During the last few years enormous research has been done in pathophysiology and etiology of thyroid gland related diseases in human as well as in mammalian models. Thyroid hormones affect the functions of several organs like heart, kidney, liver etc. Hypothyroidism causes a depression in contractile and electrical parameters in heart (Capasso et al., 1999). At kidney level thyroid hormones affect proximal tubular sodium transport (Everts, 1996). Hypothyroid patients have a decrease in glomerular filtration rate and renal plasma flow. On the other hand, hyperthyroid subjects exhibit a significant increase in both parameters (Everts, 1996). Moderate hyperthyroidism reduces liver amino nitrogen conversion, muscle nitrogen contents and overall nitrogen balance in rats (Grofte et al., 2003). Thyroid dysfunction may alter serum creatinine, which has been found to be
Thyroid dysfunction status with special reference to polymorphism of thyroid peroxidase (TPO) gene increased in hypothyroidism and decreased in hyperthyroidism (Kreisman and Hennessey, 1999). In contrast, decreased serum creatinine levels may be encountered in patients with hyperthyroidism. Serum cystatin C (CysC) is a novel marker for kidney function that has been claimed to be altered during thyroid dysfunction (Verhelst et al., 1997). In addition to its role in cellular metabolism, thyroid hormone (TH) is critically involved in growth, development and function of the central nervous system (CNS). Thyroid dysfunction in adult is associated with both neurologic and psychiatric abnormalities (Fukui et al., 2001; Mandel et al., 1993; Smith et al., 2002). It has long been known that in vertebrates hyperthyroidism leads to an acceleration of the basal metabolic rate (Paola et al., 2003), which is associated with increased cellular respiration in target tissues such as liver, kidney, heart and skeletal muscle (Schwartz and Oppenheimer, 1978). More recently, it has been suggested that the hypermetabolic state in hyperthyroidism results in oxidative tissue injury, secondary to increased the production of reactive oxygen species (ROS) (Asayama and Kato, 1990). Several clinical investigators have clearly shown that hyperthyroidism, both spontaneous and experimental, results in an unopposed activation of gluconeogenesis (Kreines et al., 1965).
Mutations in the TPO gene of patients with hypothyroidism, resulting in Total or Partial Iodide Organification Defects (TIOD and PIOD) have been reported by several groups. A large volume of work has been performed by countries like Brazil (Antonio et al., 2003), Netherlands (Bikker et al., 1995; Bakker et al., 2000), Germany (Ambrugger et al., 2001), Portugal (Rodrigues et al., 2005), Italy (Fugazzola et al., 2005), Japan (Tajima et al., 2005; Kotani et al., 1999), Argentina (Rivolta et al., 2003) as well as China (Wu et al., 2002) and Taiwan (Niu et al., 2002). These have resulted in identification of population specific mutations, some of which are common and have led to genetic screening programs.

The aim of the research work was to understand the spectrum of biochemical and cellular changes upon the experimentally induced hypo and hyperthyroid conditions in rat. Methimazole, an inhibitor of Thyroid peroxidase (TPO) enzyme (Roy and Mugesh, 2005) was used to induce hypothyroid and thyroxine to induce hyperthyroid conditions in experimental rat. The investigation includes the parameters like T₃ and T₄ hormones, liver glycogen, SGPT, SGOT, OGTT (Oral Glucose Tolerance Test), histology of pancreas, liver and testis.
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The thyroid hormone level may change upon the mutations/polyorphism of TPO gene. Mutations in the thyroid peroxidase (TPO) gene particularly non-synonymous Single Nucleotide Polymorphisms in coding regions (cSNPs) can lead to severe defects in thyroid hormone production, due to total iodide organification defects (TIOD) or partial iodide organification defects (PIOD). Therefore, the investigation was also aimed to understand whether hypothyroid patients are associated with mutations/polyorphism(s) in TPO gene coding sequence.

The results showed significant changes in T₃, T₄ hormones upon experimentally induction of hypothyroid and hyperthyroid. Liver glycogen, serum SGPT and SGOT altered dramatically in both hypothyroid and hyperthyroid rat. Alterations of OGTT curves observed in hypothyroid groups of rat reflected the important role of thyroid hormones in glucose homeostasis.

Tissue samples from liver, pancreas and testes exhibited significant changes in cytomorphology upon experimentation. Sequence analysis of the TPO gene demonstrated a number of mutations in patient samples when compared with the normal individuals. The patients showing mutations in
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TPO gene corroborates the clinical manifestations. Therefore, our results clearly indicate the mutations in TPO gene may be associated with hypothyroidism.