



ASSESSMENT OF THYROID HORMONES IN ASCIDIANS IN THE TUTICORIN COASTAL AREA (TAMIL NADU)

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ABSTRACT

Ascidiacea is found to be commonly known as the ascidians or sea squirt. It is a paraphyletic class in the subphylum Tunicata of sac-like marine invertebrate filter feeders.[2] Ascidians are characterized by a tough outer "tunic" made of the polysaccharide cellulose. Literatures showed that 2,300 species of ascidians and three main types: solitary ascidians, social ascidians that form clumped communities by attaching at their bases, and compound ascidians that consist of many small forming colonies up to several meters in diameter. Ascidians are also consumed as food in the form of various preparations in many parts of the world including Chile, France, Korea, Italy, and Japan. Thus the study focused on level of trace elements and thyroid hormone in Ascidians. Ascidians were collected with the help of Scuba divers from Tuticorin coastal area three times for a period of 3 months. The Powdered samples were used for the analysis of T₄, T₃ and TSH. The levels were determined by using automated analyzer—Immunochemiluminometric analyzer (ICMA) (ADVIA Centaur), Radioimmuno assay (RIA), Beckman, Beckman Coulter, Access-2 in Dr. K. Kannan Endocrinology clinic, Madurai. The observed results indicate clearly that the selected species of tunicates such as, *Phallusia arabica*, *Styela canopus*, *Microcosmus exasperates* and *Herdmania pallida*. The study found that the presence of significantly (at p<0.0001) increased level of T₃ hormone in *Phallusia arabica*, *Microcosmus exasperates* and *Herdmania pallida*. Hence the present study could suggest that the Ascidians used as a dietary source as supplementation of thyroid hormone for the hypothyroidism.

KEYWORDS

Tunicates, Disease, Trace elements, Thyroid hormone, Ascidians and Thyroidism.

Introduction:

Thyroid is a butterfly-shaped gland composed of 2 encapsulated lobes, located on either side of the trachea, and just below the cricoid's cartilage. This is connected by thin isthmus and is composed of spherical thyroid follicles, which contain the hormone colloidal form. T₃ and T₄ are active hormones secreted under the control of TSH. T₃ is 3-4 fold more potent than T₄. It is involved in normal growth and development in children, temperature regulation, metabolism, energy production and intelligence in both children and adults. It ensures normal growth and development of nervous system (Guyton. 1991). In homeotherms, thyroid hormones regulate Basal metabolic rate (BMR) and responsible for the maintenance of high and constant body temperature. The thyroid hormone circulating in the blood is bound to transport proteins such as TBG (Thyroxine binding globulin), Thyroxine binding prealbumin TBPA (0-15%) and Albumin (15-20%) (Darras et al., 2004).

The normal range of T₄ is 77-155 nmol/L (6-12 µg/dl), T₃ is 1.2-2.8 nmol/L (78 - 182 ng/dl), and TSH is 0.3-4.0 mU/L. If the hormone levels are above or below the normal range, it leads to Hyperthyroidism or hypothyroidism. (Kinjo et al., (2002). Most common hypothyroidisms are Hashimoto's thyroiditis and congenital hypothyroidism. Graves' disease is found to be most common hyperthyroidism and other forms of are hyperpostpartum thyroiditis and thyrotoxicosis factitia. Hyperthyroidism also leads to a number of complications like heart problems, brittle bones (Osteoporosis), eye problems (Graves' ophthalmopathy), red, swollen skin (Graves' disease) and thyrotoxic crisis.

The treatment of hyperthyroidism depends on the cause and severity of the disease, as well as on the patient's age, goiter size, comorbid conditions, and treatment desires. The goal of therapy is to correct the hypermetabolic state with the fewest side effects

and the lowest incidence of hypothyroidism. Beta-blockers and iodides are used as treatment adjuncts. Antithyroid drugs, radioactive iodine, and surgery are the main treatment options for persistent hyperthyroidism each therapy can produce satisfactory outcomes if properly used. Mengel and Schwiebert, (2001). Hence the study planned to desire the natural sources as the supplementation of thyroid hormone to reduce the effect of hypothyroidism.

Iodine is found to be critical for formation of thyroid hormones T₃, or triiodothyronine, and T₄, or thyroxine. Inadequate production of thyroid hormones can cause enlargement of the thyroid gland, also known as goiter, while its deficiency during pregnancy could cause irreversible brain damage in newborns. Potassium, sodium, calcium, magnesium, copper and zinc minerals are found to be crucial for human thyroid health. All these minerals have been reported to be involved in the production, degradation or cellular response to thyroid hormone which is proved in Graves hyperthyroidism patients among South Indian population (Madurai) (Veeramuthumari, 2007) and suggested that the level of severity of the disease and its complications could be reduced through diet pattern. Above observation the work insight to focus on tunicates and the study aimed to select the species of ascidians to estimate the level of elements and thyroid hormone in order to fulfill the demand for local food. Veeramuthumari and Packiyalakshmi (2016) analyzed and reported that the significant (at p<0.0001) presence of trace elements like Calcium (Ca), Sodium (Na), Potassium (K), Iron (Fe), Manganese (Mn) and Magnesium (Mg) in four tunicates such as *Phallusia arabica*, *Styela canopus*, *Microcosmus exasperates* and *Herdmania pallida*. Their study also proved that the ascidians could be used as a dietary source for the thyroid disease, polycystic kidney disease, diabetic mellitus patients and would reduce the risk factors like hypertension, osteoporosis, cardiovascular problems, osteodystrophy, anemia etc.

Ascidians are known as Tunicates or sea-squirts which are marine invertebrates belonging to the phylum Chordata, sub-phylum Urochordata. Chordata is the same phylum that humans are in. Even though tunicates look like sacks of water or sheets of colorful jelly, they are under the invertebrate group but most closely related to us. During their tadpole-like larval phase they possess critical chordate characteristics such as a notochord, a dorsal tubular nerve cord, and pharyngeal slits. Although not all of these traits are retained in the adult stage, in their larval stage they look very much like other developing chordate embryos, including humans (Simkanin and Noble, 2011). Shenkar and Swalla (2011) reported that the Tunicates are diverse and abundant members of benthic marine invertebrate communities and studies on their biology and ecology are providing critical insights into evolution and development, natural products chemistry, physiology, biogeography, the impacts of climate change and species invasions. Hence the present study also to be focused on Tunicates and human physiology.

Minerals, iodine, vitamins, fat and digestible nature of proteins are present in ascidians (Millar (1971). The seasonal variations found to the level of carbohydrates, proteins and lipids are commonly noted in tunicates at two stations in Tuticorin coast (Tamilselvi (2008). The present study will provide the additional steps to minimize the malnutrition, awareness regarding the Tunicate (Ascidians) as natural food in India. It also prove the medicinal significances for certain recently found common metabolic and genetic diseases like hypo and hyper thyroidism, diabetes mellitus, obesity, kidney disease and cardiovascular problems.

Methodology:

Study subjects:

Ascidians were collected with the help of Scuba divers from Tuticorin coastal area situated along the South east coast of India for a period of every 3 months. The collected tunicates were washed with sea water to remove the sand and silt. Then the samples were dried in an oven at 110°C for about 24 hours. The dried samples were grind into fine powder (Radhalakshmi et al., 2014). The Powdered samples were stored at 4°C. 10 grams of powdered sample of each species was subjected to acid digestion using a mixture of 3ml of concentrated sulphuric acid and 60% perchloric acid. The digested sample was dried and it was dissolved in 20ml of 2N hydrochloric acid was subjected to centrifugation and filtered. (Radhalakshmi et al., 2014). The powdered sample also mixed with saline and used for thyroid hormone analysis.

Biochemical analysis:

Most clinical laboratories with an automated immunoassay system offer a menu of free T4, free T3 and thyroid-stimulating hormone (TSH), with a smaller number offering thyroid autoantibody testing. Concentration of T4, T3 and TSH were determined by using automated analyzer—Immunochemiluminometric analyzer (ICMA) (ADVIA Centaur), Radioimmuno assay (RIA), Beckman, Beckman Coulter, Access-2 in Dr. K. Kannan Endocrinology clinic, Madurai.

Statistical analysis:

The significant differences between the levels of thyroid hormones (T3, T4 and TSH) among the ascidians were analyzed by student t test.

Results and Discussion:

The four minerals, sodium, potassium, calcium and magnesium are next to each other in the Periodic Table and form a square on the left side (Edmonds and Smith et. al., 1999). There are strong interactions between these four minerals. The balances between these four minerals seem to be critical to health and are probably very critical for thyroid health. Excess amounts or deficiencies of any one of the four may severely disrupt thyroid function. (Disashi et.al., 1999) Additionally there seem to be interactions between these four minerals and copper and zinc, where are two metallic minerals with critical thyroid functions. It seems that a copper deficiency interferes with the proper functioning of both potassium and magnesium, and zinc seems more related to

sodium and calcium metabolism. Also all of these minerals seem involved in the production, degradation, or cellular response to thyroid hormone. Hence, this was also reported and proved by Veeramuthumari et al., (2007; 2011); Veeramuthumari and Packiyalakshmi (2016) in Graves' disease among the South Indian population.

Cadmium appears to be the largest single contributor to autoimmune atoms combine with cadmium to form thyroid diseases. One of the greatest effects of Cadmium is that it depletes selenium in the body because selenium is essential for cadmium removal. Selenium atoms combine with cadmium atoms and are escorted out of the body via the bile system. (Ghosh et.al., 1992) When selenium is depleted by cadmium, there is less selenium to form the deiodinase enzymes, which convert T4 to T3, resulting in low T3 and hypothyroidism. (Nishijo et.al., 1994). So, to solve this like major problems, the present study suggested that ascidians could be used as cheapest source. In recent years several study reported that the children who die worldwide with nutritional deficiency related disease like malnutrition, anemia, imbalance of thyroid hormone and even renal failure etc. (Silverberg et.al., 2003; Sullivan et al., 2013). Hence there is a need to promote an alternative nutritive food sources to meet the needs of people.

Table: 1

Analysis of thyroid hormones in Ascidians collected from Tuticorin Coastal Area (Tamil Nadu):

Ascidians	T3 (ng/dl)	FT4 (ng/dl)	TSH (µgIU/ml)
Phallusia arabica	301.62***	0.58	0.01
Styela canopus	202.53	0.53	0.01
Microcosmus exasperates	293.80***	0.59	0.01
Microcosmus squamiger	226.55	0.53	0.01
Herdmania pallida - Young one	236.42	0.49	00
Herdmania pallida - Adult	249.17***	0.55	0.01

***- Highly significant at $p < 0.0001$

Thyroid hormone (TH) is produced by the thyroid in the form of the biologically inactive precursor thyroxine (T4). The bioactive form of the hormone is triiodothyronine (T3). In humans, only approximately 20% of T3 is secreted by the thyroid. Most circulating T3 is derived from outer ring deiodination (ORD) of T4 in peripheral tissues (Kuijper et al., 2005). Heyland and Moroz (2005) reported that thyroid hormones (THs) are small, lipophilic signaling molecules built from tyrosine and iodine. TH action is well characterized in vertebrates, where these molecules play a fundamental role as regulators of development, metabolism, growth and differentiation. Increasing evidence suggests that THs also function in a variety of invertebrate species. Two alternative sources of hormone for animals are exogenous (from food items) and endogenous synthesis. TH plays a critical role in animals that do not possess a thyroid gland or an endostyle. Moreover, growing evidence suggests that THs generated by food items have physiological effects on the 'consumers'.

The present study analyzed the level of T3, FT4, TSH and (Thyroxine peroxidase antibody TPOAb). The result showed that very minute quantity of T4 and TSH. TPOAb is found to be absence in all species of ascidians and might be need to further study on that. The study also showed that the presence of significantly (at $p < 0.0001$) increased (Table:1) level of T3 hormone in *Phallusia arabica*, *Microcosmus exasperates* and *Herdmania pallida*. This work coincide with the work of Darras and Van Herck (2012). They proved that that the presence of iodothyronine deiodinases in animals as diverse as ascidians and humans suggests that this type of enzyme was already present in the common ancestor of all chordates and possibly even earlier in evolution. The comparative study amongst vertebrates has shown that the structure of each of the three typical types of deiodinases (D1, D2 and D3) has been highly conserved. As a result, the relative contributions of D1 and D2 to circulating T3 levels also show considerable fluctuations. The three deiodinase types have an impact on systemic T3 levels and

they all contribute directly or indirectly to intracellular T3 availability in different tissues

During critical illness, pronounced alterations in plasma thyroid hormone levels occur. Plasma T3 decreases and plasma rT3 increases, and the magnitude of these changes is related to the severity of the disease. Although T4 and FT4 may increase in mild illness, plasma T4 is decreased and FT4 normal or decreased in critically ill patients. Whether the reduction in serum T3 is a beneficial adaptation resulting in a decreased metabolic rate, or that it is a maladaptation contributing to a worsening of the disease, is still a controversial issue that has been discussed extensively by others (Docter et al., 1993; De Groot, 1999). So far, substitution of thyroid hormone in critically ill patients has not been shown to have a positive effect on clinical outcome, but intervention with hypothalamic-releasing factors, which restores pulsatile pituitary hormone secretion and normalizes peripheral hormone levels and it might be a more successful approach (Van den Berghe et al., 1999; 2002; Weeker et al., 2004).

Ascidian, Or Sea squirts, they are often referred to as protochordates because during the larval stage they possess chordate characteristics, most notably the tail contains a notochord and a dorsal hollow nerve cord. After a free-swimming stage, the simple tadpole-like larvae attach to a substrate and undergo metamorphosis that includes tail loss and rearrangement of the internal organs. Subsequently, in the adult form, the similarities to chordates are lost. Although no clear role for thyroid hormones in adult ascidians has been established, studies showing that T4 may be involved in the metamorphosis stage suggest possible functions of thyroid hormones in larval ascidians (Patricolo, 2001). Various studies have described the presence of T4 in ascidian larvae only (Carosa et al., 1998; Dehal et al., (2002)

Hence the study concluded that T3 hormone present in adult of ascidians in *Phallusia arabica*, *Styela canopus*, *Herdmania pallida*, *Microcosmus squamiger* and *Microcosmus exasperates*. Ascidians would be used as supplementation for thyroid dysfunction related disease and addition to do research on clinical study with various patients.

The study also found that limited information available on the effect of thyroid dysfunction on the distribution of HDL subfractions and their metabolism. Tan et al.,(1998) finding showed that the thyroid hormone may have multiple effects on Lipid metabolism. Topping et al.,(1996) have shown that the antithyroid drugs act principally by interfering with the organification of iodine, thereby suppressing thyroid hormone levels. Methimazole (Tapazole) and propylthiouracil (PTU) are the two agents available. So the future focus would be on lipid metabolism and thyroid hormone and Ascidian based drug therapy for the diseases (Hypo and hyper thyroidism, obesity etc..) occurred by thyroid dysfunction

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