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Research Article

ESTIMATION OF SIALIC ACID, L-FUCOSE AND ADENOSINE DEAMINASE LEVEL IN HYPO AND HYPERTHYROIDISM

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ABSTRACT

Background:Enhanced sialylation of glycoproteins occurs during hypo and hyperthyroidism. The role of sialic acid (SA) in cell membranes is well-standing, but its role in intracellular structures is still under analysis. Aims: The objectives of the study was to estimate the levels and role played by sialic acid, L-Fucose and Adenosine deaminase in hypo and hyperthyroidism. Study design: This study was conducted on total 60 subjects, 20 hypothyroidism patients 20 hyperthyroidism patients and the control group consisted of 20 healthy peoples with no complications, attending the central research laboratory, A.B.Shetty Memorial Institute of Dental Sciences, Nitte University. Methods and Materials: Blood samples was taken, centrifuged and serum was collected for subsequent analysis. Serum Sialic acid was estimated by the Diphenylamine method. Serum L-Fucose was estimated by the Winzler method. Serum Adenosine deaminase was estimated by Giusti (331) method. Results: Data was collected and statistically analyzed by "Students 't' test". The sialic acid level increases in hypothyroidism and decreases in hyperthyroidism when compared to normal. The serum L-Fucose level increases significantly in hypothyroidism and decreases in hyperthyroidism when compared to the normal. The adenosine deaminase level is approximately similar to that of normal and decreases in hyperthyroidism when compared to the normal.

Keywords: Hypothyroidism, Hyperthyroidism, Sialic Acid, L-Fucose, Adenosine Deaminase

INTRODUCTION

Hypothyroidism is the disease state in humans and other animals caused by insufficient production of thyroid hormone by the thyroid gland.[1] Factors such as iodine deficiency or exposure to Iodine-131 (I-131) can increase the risk of Hypothyroidism. Iodine deficiency is the most common cause of hypothyroidism worldwide. Hypothyroidism can result from postpartum thyroiditis, a condition that affects about 5% of all women within a year after giving birth. The first phase is typically hyperthyroidism. Then, the thyroid either returns to normal or a woman develops hypothyroidism. Although iodine is substrate for thyroid hormones, high levels prompt the thyroid gland to take in less of the iodine that is eaten, reducing hormone production. Hypothyroidism is often classified by the organ of origin- Primary (thyroid gland origin), Secondary (Pituitary gland origin) and tertiary (hypothalamus origin).[2,3] Some of the symptoms of hypothyroidism are fatigue, cold intolerance, goiter, drowsiness, puffy face, constipation and dry brittle hair etc. Hypothyroidism is treated with the levorotatory forms of thyroxine (L-T_4) and triiodothyronine (L-T_3). Both synthetic and animal derived thyroid tablets are available and can be prescribed for patients in need of additional thyroid hormone.

Hyperthyroidism is the term for overactive tissue within the thyroid gland, resulting in overproduction of thyroid hormones and thus an excess of circulating free thyroid hormones: thyroxine (T_4), triiodothyronine (T_3), or both. It is sometimes called as thyrotoxicosis which is the technical term for too much thyroid hormone in the blood.[4] Thyroid hormone is important at a cellular level, affecting nearly every type of tissue in the body. It functions as a stimulus to metabolism, and is critical to normal function of the cell. Some of the symptoms of hyperthyroidism are fatigue (muscle weakness), irritability (nervousness), weight loss, palpitations, sweating, heat intolerance, goiter etc. The most common cause of hyperthyroidism is Graves disease which is an autoimmune disease wherein the body's immune system acts aginst its own healthy cells and tissues. In this disease, the immune system makes an antibody called thyroid stimulating immunoglobulin (TSI) and causes the thyroid to make too much thyroid hormone. Radioactive iodine – 131 is a common and effective treatment for hyperthyroidism. Thyroid gland collects iodine to make thyroid hormone, it will collect the radioactive iodine in the same way. The radioactive iodine will gradually destroy the cells that make up the thyroid gland but will not affect other tissues in the body. The least used treatment is surgery to remove part or most part of the thyroid gland.[5-8]

Sialic acid is a generic term for the N- or O-substituted derivatives of neuraminic acid, a monosaccharide with a nine-carbon backbone.[9] It is also the name for the most common member of this group, N-acetyl neuraminic acid (Neu5Ac or NANA). Sialic acids are found widely distributed in animal tissues and to a lesser extent in other species ranging from plants and fungi to yeasts and bacteria, mostly in glycoproteins and gangliosides. The amino group generally bears either an acetyl or glycolyl group but other modifications have been described. The hydroxyl substituents may vary considerably: acetyl, lactyl, methyl, sulfate, and phosphate groups have been found.[10] Sialic acid rich glycoprotein binds selectin in humans and other organisms. Cancer cells that can metastasize often have a lot of sialic acid rich glycoprotein. This helps these late stage cancer cells enter the blood stream. Sialic acid rich oligosaccharides on the glycoconjugates found on surface membranes help keep water at the surface of cells. The sialic acid rich regions contribute to creating a negative charge on the cells surface. Since water is a polar molecule with partial positive charges on both hydrogen atoms, it is attracted to cell surfaces and membranes. This also contributes to cellular fluid uptake. Normal level of the sialic acid in the blood of males is 60 to 70 mg/dL.

Fucose is a hexose deoxy sugar with the chemical formula $C_6H_{12}O_5$. It is found on N-linked glycans on the mammalian, insect and plant cell surface, and is the fundamental sub-unit of the fucoidan polysaccharide. Alpha1→3 linked core fucose is a suspected carbohydrate antigen for IgE-mediated allergy.[11] Two structural features distinguish fucose from other six-carbon sugars present in mammals: the lack of a hydroxyl group on the carbon at the 6-position (C-6) and the L-configuration. It is equivalent to 6-deoxy-L-galactose. In the fucose-containing glycan structures, fucosylated glycans, fucose can exist as a terminal modification or serve as an attachment point for adding other sugars.[12] In human N-linked glycans, fucose is most commonly linked α-1,6 to the reducing terminal beta-N-acetylglucosamine. However, fucose at the non-reducing termini linked α-1, 2 to galactose forms the H antigen, the substructure of the A and B blood group antigens. Fucose is metabolized by an enzyme called alpha-fucosidase.[13] Normal level of L-Fucose in the blood is 3.9 to 16.9 mg/dL.

Adenosine deaminase (ADA) is an enzyme (EC 3.5.4.4) involved in purine metabolism. It is needed for the breakdown of adenosine from food and for the turnover of nucleic acids in tissues. ADA irreversibly deaminates adenosine, converting it to the related nucleoside inosine by the removal of an amino group. Inosine can

then be deribosylated (removed from ribose) by another enzyme called purine nucleoside phosphorylase (PNP), converting it to hypoxanthine. Some mutations in the gene for adenosine deaminase cause it to be not expressed. The resulting deficiency is one cause of severe combined immunodeficiency (SCID). [14] Conversely, mutations causing this enzyme to be overexpressed are one cause of hemolytic anemia. [15] There is some evidence that a different allele (ADA2) may lead to autism.[16] There are 2 isoforms of ADA: ADA1 and ADA2. ADA1 is found in most body cells, particularly lymphocytes and macrophages, where it is present not only in the cytosol and nucleus but also as the ecto- form on the cell membrane attached to dipeptidyl peptidase-4 (aka, CD26). ADA2 was first identified in human spleen. [17] It was subsequently found in other tissues including the macrophage where it co-exists with ADA1. The two isoforms regulate the ratio of adenosine to deoxyadenosine potentiating the killing of parasites. ADAR is an RNA-specific ADA.[18]ADAT (ADAT1, ADAT2, and ADAT3) is a tRNA-specific ADA, changing the tRNA to allow for a wobble base pairing. ADA2 is the predominant form present in human blood plasma and is increased in many diseases, particularly those associated with the immune system: for example rheumatoid arthritis, psoriasis and sarcoidosis. The plasma ADA2 isoform is also increased in most cancers. ADA2 is not ubiquitous but co-exists with ADA1 only in monocytes-macrophages. Total plasma ADA can be measured using high performance liquid chromatography, enzymatic or colorimetric techniques. Perhaps the simplest system is the measurement of the ammonia released from adenosine when broken down to inosine. After incubation of plasma with a buffered solution of adenosine the ammonia is reacted with a Berthelot reagent to form a blue colour which is proportionate to the amount of enzyme activity. To measure ADA2, erythro-9-(2-hydroxy-3-nonyl) adenine (EHNA) is added prior to incubation so as to inhibit the enzymatic activity of ADA1. It is the absence of ADA1 that causes SCID. Adenosine deaminase deficiency, also called ADA deficiency or ADA-SCID, is an autosomal recessive metabolic disorder that causes immunodeficiency. [19] It accounts for about 15% of all cases of severe combined immunodeficiency (SCID). ADA deficiency may be present in infancy, childhood, adolescense, or adulthood. Age of onset and severity is related to some 29 known genotypes associated with the disorder. Normal level of adenosine deaminase in the blood is 10 to 15 µ/L.

MATERIALS AND METHODS

The study was conducted in central research laboratory of Nitte University after obtaining the approval from the institutional ethical committee. The study group consists of 60 subjects, 20 hypothyroidism patients, 20 hyperthyroidism patients and the control group consisted of 20 healthy peoples with no complications.

5 ml of blood samples was collected, centrifuged and serum was separated for subsequent analysis. Serum Sialic acid was estimated by the Diphenylamine method. Serum L-Fucose was estimated by the Winzler method. Serum Adenosine deaminase was estimated by Giusti (331) method. Data was collected and statistically analyzed by ***"Students't' test".***

RESULTS

In the present study, we estimated the levels of Sialic Acid, L-Fucose and Adenosine Deaminase in hypothyroidism and hyperthyroidism and compared with healthy subjects. In this study we found that, the sialic acid level increases in hypothyroidism (102.8 ± 15.50mg/dl) and decreases in hyperthyroidism (54.17 ± 4.93mg/dl) when compared to normal (68.34 ± 4.51mg/dl) is shown in figure 1.

The serum L-Fucose level increases significantly in hypothyroidism (16.74 ± 2.34mg/dl) and decreases in hyperthyroidism (4.63 ± 0.81mg/dl) when compared to the normal (8.478 ± 1.30mg/dl) is given in figure 2.

The adenosine deaminase level is approximately similar to that of normal (13.57 ± 1.72u/l) and decreases in hyperthyroidism (7.36 ± 1.31u/l) when compared to normal is shown in Figure 3.

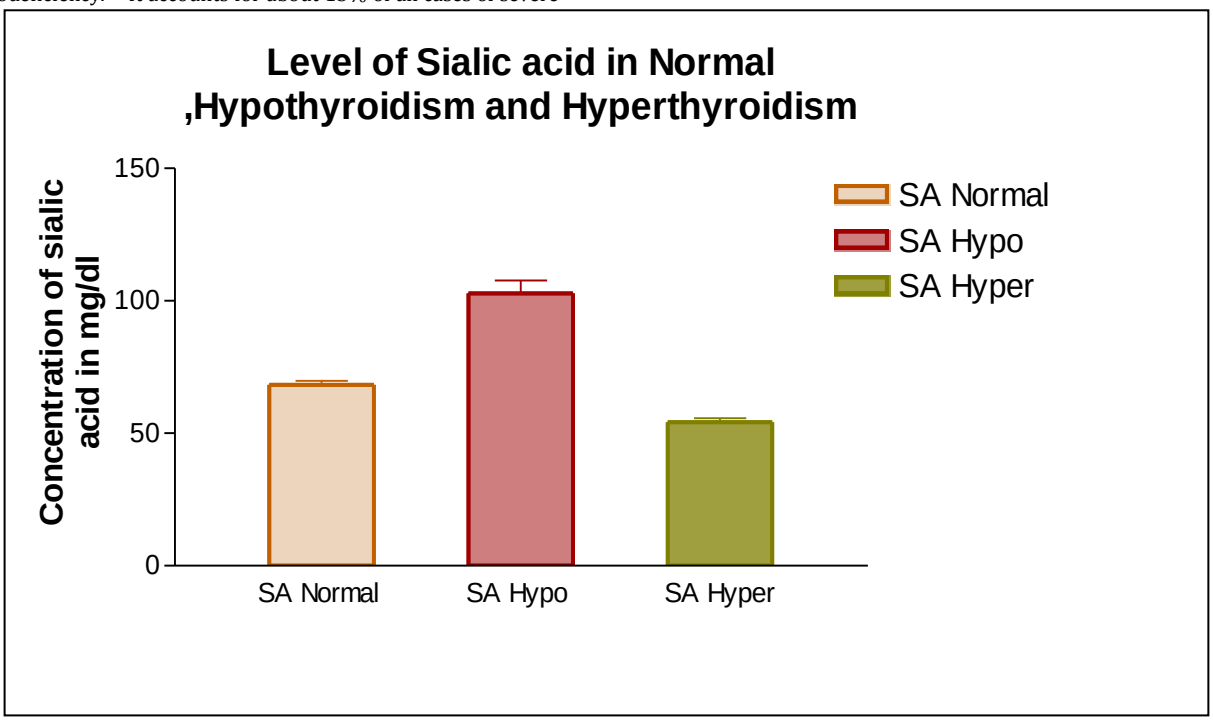

Fig 1: shows serum Sialic acid increases in hypothyroidism and decreases in hyperthyroidism when compared to normal.

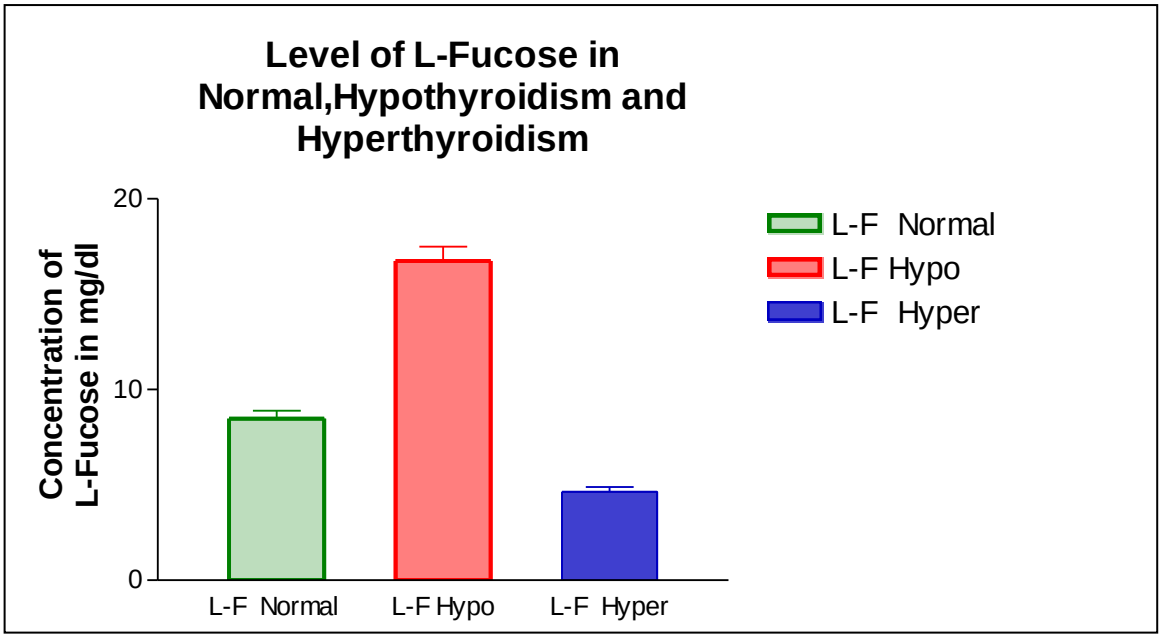

Fig 2: shows serum L-Fucose increases significantly in hypothyroidism and decreases in hyperthyroidism when compared to the normal

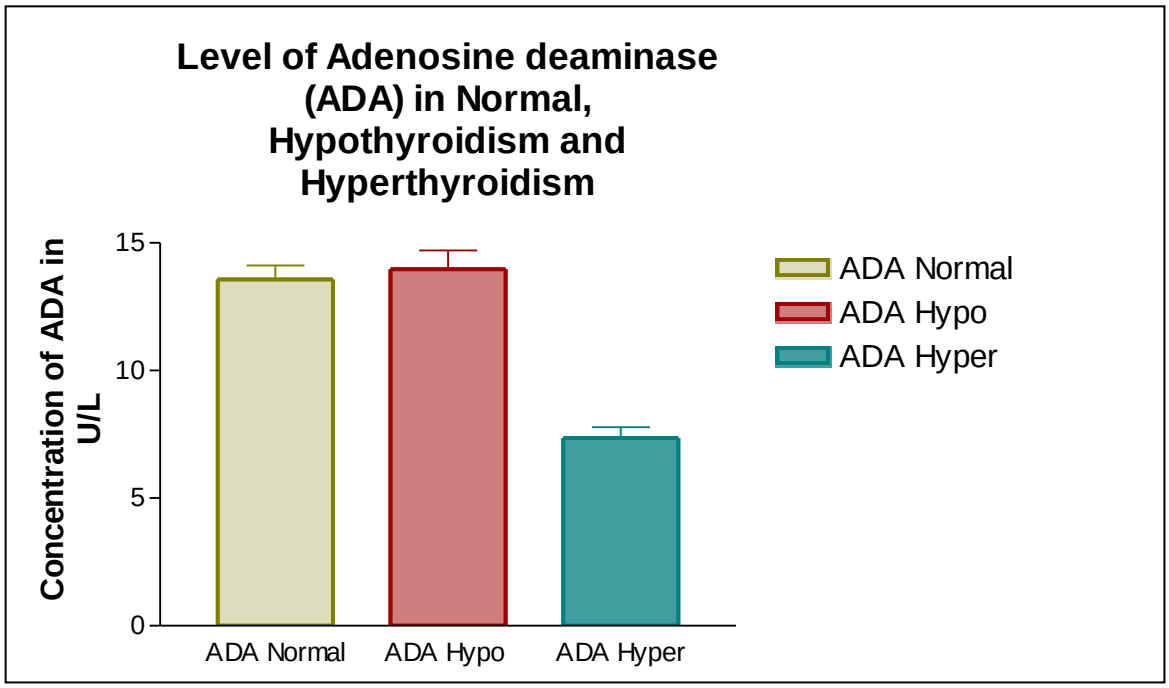

Fig 3: shows adenosine deaminase approximately similar to that of normal and decreases in hyperthyroidism compared to normal.

DISCUSSION

When comparison is done between normal, hypo & hyperthyroidism, in this study we found that there is an increase in the sialic acid level in hypothyroidism and decrease in hyperthyroidism when compared to normal. The increased content of serum glycoproteins in hypothyroid might possibly have come from disruptions of membranes. Depleted glycoproteins in hyperthyroid might be due to increase in depolymerisations of glycoproteins and accelerated secretion of glycoproteins. The serum L-Fucose level increases significantly in hypothyroidism and decreases in hyperthyroidism when compared to the normal. The increased content of serum glycoproteins in hypothyroid might possibly have come from disruptions of membranes. Depleted glycoproteins in hyperthyroid might be due to increase in depolymerisations of glycoproteins and accelerated secretion of glycoproteins. The adenosine deaminase level is approximately similar to that of normal and decreases in hyperthyroidism when compared to normal.

The hypothyroidism increased oxidative stress, whereas hypothyroidism induced by methimazole reduced oxidative stress and significant changes in the T3 and T4 and TSH level during thyroid state with all the employed stressors [17].In hyperthyroidism, serum proteins undergo increased levels of oxidative changes leading to high turnover rate of blood proteins. A significant negative correlation between carbonylation and sialic acid content of serum proteins in hyperthyroidism and enhanced desialylation and carbonylation of serum proteins by in vitro H_2O_2 suggest that oxidative stress can cause desialylation of serum glycoproteins [19].

Hypothyroidism did not change the activity and hyperthyroidism showed 15% decrease in the adenosine deaminase activity [21].The activity of serum adenosine deaminase isoenzymes in patients with

Graves' disease in whom CD26 presented T-cells were in increased. The activities of total ADA and ADA2 were significantly higher in Graves' disease than normals[20].

From this study we conclude that, sialic acid level increases in hypothyroidism and decreases in hyperthyroidism when compared to normal. The serum L-Fucose level increases significantly in hypothyroidism and decreases in hyperthyroidism when compared to the normal. The adenosine deaminase level is approximately similar to that of normal and decreases in hyperthyroidism when compared to the normal. Sialic acid, L-Fucose and Adenosine deaminase can be used as marker for hyper and hypothyroidism.

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