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ORIGINAL ARTICLE

EVALUATION OF ISCHEMIA MODIFIED ALBUMIN AND NITRIC OXIDE LEVELS AND THEIR INTER-RELATIONSHIP IN HYPERTHYROIDISM

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ABSTRACT: BACKGROUND: Ischemia Modified Albumin (IMA) is an ischemia/reperfusion injury marker which has been considered to be formed under oxidative stress conditions. Endothelial L-Arginine/NO pathway dysfunction can lead to oxidative stress which has deleterious effects seen on the vascular wall causing ischemia. **AIM:** 1) The study was conducted to estimate the levels of IMA and NO in hyperthyroidism patients. 2) To evaluate the relationship between IMA and NO levels in hyperthyroidism. **MATERIALS AND METHODS:** A cross sectional study was done with 30 newly diagnosed hyperthyroid patients as cases and 30 age and sex matched healthy controls. Serum levels of IMA and NO were estimated by colorimetric methods and thyroid profile was done by ELIFA methodology. **STATISTICAL ANALYSIS:** Data were analyzed using SPSS. Values were expressed as Mean±SD. **RESULTS:** Nitric Oxide levels were significantly decreased in hyperthyroid patients (7.08±1.57µmol/L) as compared to healthy controls (39.76±4.98µmol/L) (p=0.001). Ischemia Modified Albumin levels were found to be significantly increased in hyperthyroid patients (0.73±0.10 ODU) when compared to healthy controls (0.28±0.01 ODU) (p=0.001). **CONCLUSION:** In our study there was increase in IMA levels with decreased NO levels which could be due to the consequence of oxidative stress and ischemia which is present in hyperthyroidism.

KEYWORDS: Ischemia modified albumin, Nitric oxide, hyperthyroidism, oxidative stress.

INTRODUCTION: Hyperthyroidism is a clinical condition characterized by excess secretion of thyroid hormones by thyroid glands which leads to hypermetabolic state – thyrotoxicosis[1]. Hyperthyroidism is more common in females than males with sex ratio of up to 5:1[2].

Nitric oxide is synthesized from L – arginine in the presence of enzyme Nitric oxide synthase (NOS). Through its vasodilator property it helps in modulation of vascular tone, regulation of cell growth, protection of the vessel from injurious consequences of platelets and cells circulating in blood and also inhibits proatherogenic process[3, 4]. Dysfunction of this arginine/NO pathway is a common mechanism by which they cause deleterious effects on the vascular wall. NOS is inhibited by ADMA (asymmetric dimethyl arginine), its concentration is increased in hyperthyroidism [5]. NO is involved in regulation of thyroid function, oxidation reactions yielding free radicals, ROS which damage cells by lipid peroxidation or by oxidizing DNA or proteins [6].

Ischemia modified albumin (IMA) is considered as one of the marker of ischemia/reperfusion injury in clinical conditions which include ischemic events in their pathophysiology. The human serum albumin has the ability to bind to certain metal ions particularly cobalt and copper at the N-terminus. On exposure to ischemic environment, structure of albumin N-terminus is changed such that it can no longer bind to cobalt. It also acts as a mortality predictor in renal disorder and myocardial ischemia [7, 8, 9]. Studies have shown that hyperthyroidism can aggravate neurological damage due to cerebral ischemia and modulates the outcome of ischemic reperfusion injury. Free

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thyroid hormone levels are found to be elevated in ischemic stroke patients [10]. Sheu et al., found that the complications of ischemic stroke was 1.44 times greater in hyperthyroidism patients [11, 12].

This study was done to know the interrelationship between the oxidative stress and ischemia/reperfusion in the pathogenesis of hyperthyroidism. IMA levels are found to be affected by ischemic changes that occur in hyperthyroidism and not many studies are found in literature hence we undertook this study. Here we evaluated the levels of NO and IMA, as both are markers of ischemia and oxidative stress.

MATERIALS AND METHODS: The study was conducted with 2 groups. Group-1, 30 newly diagnosed hyperthyroid patients and Group-2, 30 healthy controls. Patients with history of chronic smoking, alcoholism, diabetes mellitus, liver, kidney, cardiac, endocrinal and immunological diseases were excluded in both the groups. Informed consent was taken from all subjects involved in the study which was approved by institutional ethics committee.

The venous blood sample was collected under aseptic precautions. Thyroid profile was estimated by ELIFA (Enzyme linked immuno fluorescent assay). The NO level was estimated by Griess reagent method. In this method nitrite reacts under acidic conditions with sulfanilic acid to form a diazoniumcation which couples with the aromatic amine 1- napthlylamine to produce red-violet coloured water soluble azo dye [13]. IMA was estimated by Bar- Or et al method. 200µl of serum is incubated with 50µl of 0.1% cobalt chloride in water for 10min at room temperature for adequate cobalt-albumin binding. 50µl of dithiothreitol (DTT) was used for colorizing the reaction for 2min before quenching with 1ml of 0.9% NaCl. The absorbance (Optical density) was measured at 470nm. Colour development with DTT was compared with serum-cobalt blank without DTT and expressed as OD units [14].

Reference range of different parameters T_3 (0.9-2.3nmol/L), T_4 (60-120nmol/L), TSH (0.25-5µIU/ml), FT_3 (4-8.3pmol/L), FT_4 (9-20pmol/L), NO (30-50µmol/L), IMA (0.25-0.32OD units).
Statistical analysis was done by using SPSS (Statistical Package for the Social Sciences). Values are expressed as Mean±SD. p value <0.05 was considered significant.

RESULTS:

GROUP	Control	Cases	p-value
Number(n)	30	30	0.001
T_3 (nmol/L)	1.63±0.38	4.41±0.93	0.001
T_4 (nmol/L)	90.40±15.77	255.70±71.58	0.001
TSH (µIU/ml)	3.25±0.71	0.03±0.01	0.001
FT_3 (pmol/L)	4.57±0.36	8.71±3.29	0.001
FT_4 (pmol/L)	17.35±1.38	24.34±15.41	0.016
NO (µmol/L)	39.76±4.98	7.08±1.57	0.001
IMA (OD units)	0.28±0.01	0.73±0.10	0.001

* p value <0.05 – significant, † Values - Mean±SD.

NO levels were significantly decreased in hyperthyroid patients (7.08±1.57µmol/L) as compared to healthy controls (39.76±4.98µmol/L) (p=0.001). Ischemia Modified Albumin levels were found to be significantly increased in hyperthyroid patients (0.73±0.10 OD units) when compared to healthy controls (0.28±0.01 OD units) (p=0.001).

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DISCUSSION: The present study showed raised levels of IMA and reduced levels of NO in hyperthyroid patients as compared to healthy controls.

NO is increased in oxidative stress. In our study there was decrease in the levels of NO which can be attributed to increased ADMA levels in hyperthyroidism, an endogenous inhibitor of NO synthase. Thyroid hormone up regulates protein methylase 1 activity. The increased activity of these enzymes will methylate internal arginine residues in a variety of polypeptides. Catabolism of these polypeptides will lead to increased synthesis of ADMA. In hyperthyroidism, the activity of DDHA (dimethyl arginine dimethyl amino hydrolase) enzyme which is required for breakdown of ADMA is reduced due to increased oxidative stress. This negative correlation between ADMA and NO leads to increased vascular tone. Also decreased NO synthesis increases the sensitivity of vascular system to contractile responses induced by sympathetic system causing contraction of blood vessels leading to ischemia [15].

There was significant increase in the levels of IMA in hyperthyroid patients. This could be due to reduced NO levels. Diminished NO bioactivity may facilitate vascular inflammation that leads to oxidation of lipoproteins, foam cell formation, precursor of atherosclerotic plaque [16]. Reduced nitric oxide levels auto stimulate synthesis and release of endothelin which increases vasoconstrictor tone. These factors may lead to ischemia which leads to oxidative stress with release of free radicals which in turn modify the metal binding regions of albumin. This modification is by the degradation of specific transition metal binding site of albumin accounting for decreased cobalt binding observed during ischemic events [17].

It has been seen in a study that during the reperfusion period after ischemia, endothelial NOS is down regulated which may further lead to decreased levels of NO. Thus, increased IMA levels may be associated with a decrease in NO level [3].

CONCLUSION: From the study we can conclude, increase in IMA levels with decreased NO levels may be due to the consequence of oxidative stress and ischemia which is prevailing in hyperthyroidism status. Major limitation of the study is with respect to the small sample size, studies with larger sample size should be done for further evaluation.

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