Journal of Investigational Biochemistry
www.scopemed.org
DOI: 10.5455/jib.20130707090209

Original Research

GESDAV

Comparative study of ischemia modified albumin and nitric oxide in hyperthyroidism

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Received: May 23, 2013
Accepted: July 07, 2013
Published: July 16, 2013

ABSTRACT

Objective: Ischemia modified albumin (IMA) is an altered type of serum albumin that forms under conditions of oxidative stress. Increased IMA has been described as a marker of ischemia reperfusion injury and dysfunction of the endothelial L-arginine/nitric oxide (NO) pathway (affecting NO levels) is a common mechanism by which several cardiovascular risk factors mediate their deleterious effects on the vascular wall. No reports are available in literature to comment on the simultaneous measurement of IMA and NO in hyperthyroidism. Therefore, this study was planned to evaluate these levels in newly diagnosed patients of hyperthyroidism. **Materials and Methods:** This study was conducted on 50 newly diagnosed hyperthyroid patients and the results were compared with 50 age and sex matched healthy controls. IMA levels and NO were estimated by standard colorimetric methods. **Results:** NO concentration was found to be significantly low in hyperthyroid patients (6.4 ± 3.8 μmol/L) as compared with control subjects (36.24 ± 7.61 μmol/L) ($P < 0.05$), while IMA levels were found to be higher in hyperthyroid group (0.662 ± 0.17 ABU) than healthy controls (0.290 ± 0.09 ABU) ($P < 0.05$). IMA levels were negatively correlated with NO ($r = -0.761$, $P < 0.001$) and correlation was highly significant. **Conclusion:** Increased ischemia modified albumin levels may be a consequence of and cause for decreased nitric oxide levels in hyperthyroidism and this study may help to establish the role of oxidative stress and ischemia reperfusion in the pathogenesis of hyperthyroidism.

KEY WORDS: Hyperthyroidism, ischemia modified albumin, nitric oxide, total triiodothyronine, total thyroxine, thyroid stimulating hormone

INTRODUCTION

Hyperthyroidism is a set of disorders that involve excess synthesis and secretion of thyroid hormones by the thyroid gland. The resulting elevation in levels of free thyroxine (FT_4), free triiodothyronine (FT_3), or both leads to the hypermetabolic condition of thyrotoxicosis [1]. Hyperthyroidism is more common in females (2-5%) as compared to males, with sex ratio of up to 5:1 [2].

Nitric oxide (NO) is synthesized from L-arginine by a family of isoformic enzymes known as nitric oxide synthase (NOS). Endothelium-derived NO, through its vasodilator properties, participates in the modulation of vascular tone and inhibits a number of proatherogenic processes [3]. Dysfunction of the endothelial L-arginine/NO pathway is a common mechanism by which several cardiovascular risk factors mediate their deleterious effects on the vascular wall [4]. NO participates in the regulation of thyroid function. NO brings about oxidation reactions which will produce free radicals, and can start chain reactions that damage cells. This leads to the production of reactive oxygen species (ROS). These oxidants can damage cells by starting chemical chain reactions such as lipid peroxidation, or the oxidizing deoxyribonucleic acid or proteins [5]. NOS is inhibited by asymmetric dimethylarginine (ADMA) whose production is increased in hyperthyroidism [4].

Increased ischemia modified albumin (IMA) levels have been described as a marker of ischemia reperfusion injury in cardiovascular and other disorders, which include ischemic element

in their pathophysiology. The mechanism whereby IMA represents a marker of ischemia is based upon the fact that human serum albumin (HSA) has the ability to bind certain transition metal ions, particularly cobalt and copper, at the N-terminus. Exposure of albumin to ischemic tissue changes the structure of HSA N-terminus such that it can no longer bind cobalt. It also acts as a mortality predictor in renal disorders and myocardial ischemia [6-8].

It has been seen that hyperthyroidism aggravates neurological damage subsequent to cerebral ischemia. It modulates the outcome of ischemic-reperfusion injury and free thyroid hormones have been found to be increased in ischemic stroke patients [9]. In their study Sheu *et al*. they found that the hazard of ischemic stroke during the 5-year follow-up period was 1.44 times greater (95% confidence interval, 1.02-2.12; $P = 0.038$) for patients with hyperthyroidism than for patients in the comparison cohort [10,11].

This study was planned to establish the role of oxidative stress and ischemia reperfusion in the pathogenesis of hyperthyroidism. Modified albumin levels might be affected by ischemia occurring in hyperthyroidism. Therefore, we evaluated the levels of IMA and NO as both are markers of ischemia and oxidative stress.

MATERIALS AND METHODS

This study was conducted on 50 newly diagnosed hyperthyroid patients and 50 healthy controls with thyroid profile in normal range. To eliminate the factors which might affect free radical antioxidant activity, we excluded all chronic smoking and alcoholic subjects. All individuals suffering from chronic diseases, such as diabetes mellitus, diseases of the liver, kidney, cardiac, and other endocrine and immunological disorders were also excluded from both patient groups and healthy controls with the help of suitable investigations.

After obtaining informed consent from the subjects venous blood was collected from median cubital vein aseptically. Serum was separated and stored at −20°C until analysis. The serum total triiodothyronine (TT_3) and total thyroxine (TT_4) were estimated by radioimmunoassay and thyroid stimulating hormone (TSH) levels were estimated by immunoradiometric assay to group them as normal subjects and hyperthyroid patients. The NO level (measured as nitrite-plus-nitrate (NO(x)) concentration) was estimated by Griess reagent method. In this method, nitrite reacts under acidic conditions with sulfanilic acid ($HO_3\,SC_6\,H_4\,NH_2$) to form a diazonium cation ($HO_3\,SC_6\,H_4$–$N \equiv N^+$) which subsequently couples to the aromatic amine 1-naphthylamine ($C_{10}\,H_7\,NH_2$) to produce a red-violet colored ($\lambda_{max} \approx 540$ nm), water-soluble azo dye ($HO_3\,SC_6\,H_4$–$N = N$–$C_{10}\,H_6\,NH_2$) [12]. Serum-FT_3 and -FT_4 were assayed by a chemiluminescent assay method using Advia centaur CP analyzer with original kits obtained from Siemens Healthcare Diagnostics Ltd. (Bayswater Victoria, Australia). IMA was estimated by colorimetric method developed from Bar-Or *et al*. 200 μL of human serum were incubated with 50 μL of 0.1% cobalt chloride (Sigma, $CoCl_2{\cdot}6H_2O$) in H_2O for 10 min at room temperature for adequate cobalt-albumin binding. 50 μL of dithiothreitol (DTT) (Sigma, 1.5 mg/ml H_2O) was added for colorizing the reaction for 2 min before quenching with 1.0 ml of 0.9% NaCl. Then, the absorbance were measured at 470 nm (Shimadzu, model UV 160U), color development with DTT was compared to a serum-cobalt blank without DTT and reported in absorbance units [13].

Normal ranges of different parameters used in the study are as following: TSH (0.3-5.0 μIU/mL), TT_3 (70-200 ng/dL), TT_4 (5.5-13.5 μg/dL), FT_3 (2.3-4.2 pg/mL), and FT_4 (0.89-1.76 ng/dL).

All statistical analysis was performed using the IBM SPSS 20 (Statistical Package for the Social Sciences version 20). Values shown in the text, tables and figures are mean ± standard deviation. Student's *t*-test was applied for comparison of means of study groups. $P < 0.05$ were considered to be significant. Correlations between groups were analyzed using Pearson correlation coefficient (r) formula.

RESULTS

The mean age of the patients in hyperthyroid group was 44.40 ± 14.48 (18-68) years, while in the control group was 38.16 ± 11.8 (20-57) years. Of 50 patients, six were males and 44 were females in hyperthyroid group, while there were four males and 46 females in the control group. This shows that hyperthyroidism is more common in females. Body mass index was found to be slightly lower in hyperthyroid patients (27.34 ± 0.78 kg/m^2) as compared to controls (31.25 ± 0.84 kg/m^2; $P < 0.001$). The biochemical parameters are shown in Table 1. NO concentration was significantly lower in hyperthyroid patients (6.4 ± 3.8 μmol/L) than in control subjects (36.24 ± 7.61 μmol/L) ($P < 0.05$). NO was found to be positively correlated with TSH ($r = 0.109$) and inversely correlated with TT_3 ($r = -0.302$), TT_4 ($r = -0.268$), FT_3 ($r = -0.307$) and FT_4 ($r = -0.353$), but it was not significant statistically.

IMA levels were found to be higher in hyperthyroid group (0.662 ± 0.17 ABU) as compared to healthy controls (0.290 ± 0.09 ABU) ($P < 0.05$). IMA has positive and non-significant correlation with TT_4 ($r = 0.210$), FT_4 ($r = 0.145$), TT_3 ($r = 0.036$) and FT_3 ($r = 0.022$) and negative non-significant correlation with TSH ($r = -20.108$). However, a highly significant negative correlation was found with NO ($r = -0.761$, $P < 0.001$) [Figure 1].

Table 1: Thyroid profile and NO levels in patients with hyperthyroidism, and healthy controls

Groups	Healthy controls	Hyperthyroid group	*P* value
Number	50	50	–
TT_3 (ng/dL)	129.88±32.69	209.04±155.54	0.004*
TT_4 (μg/dL)	8.58±2.49	12.49±5.47	0.019*
TSH (μIU/mL)	1.78±1.67	0.12±0.03	0.000**
FT_3 (pg/mL)	3.01±0.46	5.53±3.93	0.001*
FT_4 (ng/dL)	1.31±0.21	2.17±1.78	0.001*
IMA (ABSU)	0.290±0.09	0.662±0.17	0.022*
NO (μmol/L)	36.24±7.61	6.4±3.8	0.000**

*Significant, **Highly significant, All values are in mean±SD,
TSH: Thyroid stimulating hormone, IMA: Ischemia modified albumin, ABSU: Absorbance units, NO: Nitric oxide, SD: Standard deviation

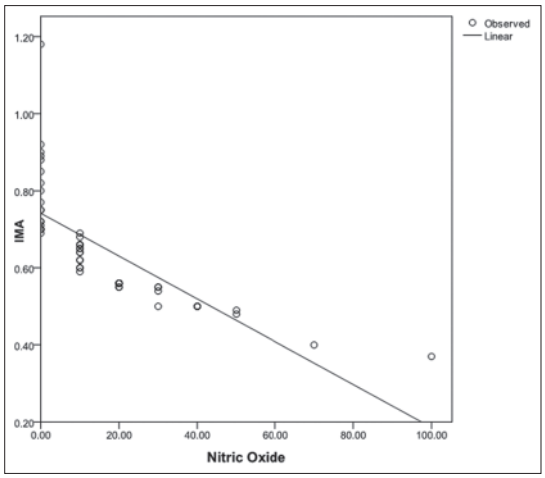

Figure 1: Scatter diagram showing correlation of nitric oxide with ischemia modified albumin ($r = -0.761$, $P < 0.001$)

DISCUSSION

The present study showed increased levels of IMA and decreased levels of NO in hyperthyroid group as compared with healthy controls. No reports are available in literature to comment on the simultaneous measurement of these parameters in hyperthyroidism.

NO is a well-known marker of oxidative stress [14]. Its levels have been found to be increased in hyperthyroidism in various studies [15-18]. However, we have found decreased levels of NO in hyperthyroidism. The reason for decreased levels of NO in hyperthyroidism could be because of increased ADMA levels [19]. ADMA is an endogenous competitive inhibitor of NOS. It inhibits vascular NO production at concentrations found in pathophysiological conditions, and also causes local vasoconstriction when infused intra-arterially [4]. Thyroid hormone up-regulates protein methylase I activity, leading to increased ADMA levels and finally decreased NO associated with hyperthyroidism [20]. In our study also a negative correlation of NO has been found with total and free thyroid hormones.

Reduced NO stimulates the synthesis and release of endothelin, which increases the vasoconstrictor tone [21]. Inhibition of the continuous release of NO markedly reduces myocardial perfusion *in vivo* [22]. It also promotes the release of growth factors, producing smooth muscle cell hyperplasia which migrates into the intima and enhances the synthesis and release of proinflammatory cytokines. In addition, reduced NO could promote platelet adhesion and release of growth factors in the vessel wall [21]. All these factors lead to ischemic changes. Exposure of albumin to ischemic tissue changes the structure of HSA N-terminus such that it can no longer bind cobalt [23] increasing the levels of IMA. We also observed a significant increase in IMA level in hyperthyroid patients as compared to healthy controls. These results are consistent with Ma *et al.* [24], not many studies are available in literature to comment on the levels of IMA in hyperthyroidism.

It has been proved in literature that IMA is a marker of oxidative stress [25]. Hence, increased IMA levels in hyperthyroidism points toward oxidative stress. This oxidative stress like condition could be due to production of ROS as a result of ischemia/reperfusion injury. During ischemia/reperfusion, the structure of the amino terminus of albumin is changed in a way that causes the loss of its Co^{2+} binding capacity leading to the formation of an "ischemia-modified albumin" [26]. 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 [27]. Thus, increased IMA levels may be associated with a decrease in NO level. A significant negative correlation has been found between NO and IMA in the present study also. However, the correlation of IMA with thyroid hormones was not found to be statistically significant.

CONCLUSION

Hyperthyroidism is associated with increased production of ADMA, which acts as an inhibitor of NOS (leading to decreased NO). This may be responsible for ischemic injury (leading to increased IMA) and subsequently increased oxidative stress. After ischemia reperfusion injury endothelial NOS is inhibited, further leading to decreased NO. Thus, we can conclude that increased IMA levels may be a consequence of and cause for decreased NO levels in hyperthyroidism and that this study may help to establish the role of ischemia reperfusion in the pathogenesis of hyperthyroidism.

ACKNOWLEDGMENTS

The authors acknowledge the laboratory technical staff for their cooperation and assistance.

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Source of Support: Nil, Conflict of Interest: None declared.