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Insulin Resistance Among Hypothyroid Patients in India

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ABSTRACT

Hypothyroidism and Diabetes mellitus are among the most common endocrine disorders in India. Oxidative stress has been proposed to be one of the causative factors associated with insulin resistance. In our previous study, we reported the presence of oxidative stress in hypothyroidism. However, the link between oxidative stress and the status of insulin resistance in hypothyroidism has not been addressed yet especially among the Indians. We recruited sixty seven untreated hypothyroid patients consecutively from the out patient department and analyzed their homeostatic model assessment of insulin resistance (HOMA-IR) and oxidative stress parameters. Oxidative stress parameters and HOMA-IR were significantly higher compared to euthyroid controls. The severity of disease, body mass index and altered lipid profile, glycated hemoglobin and MDA levels were significantly associated with insulin resistance. However, only glycated hemoglobin and MDA levels were found to be independent predictors of HOMA-IR in these patients. Increased OS could be one of the mechanisms for mild insulin resistance as reported in our study in untreated hypothyroid patients.

Key words: Insulin resistance, hypothyroidism, India

INTRODUCTION

Insulin Resistance (IR) is a pathologic state in which the target cell fails to respond to ordinary levels of circulating insulin resulting in failure to maintain normal glucose and lipid levels in circulation. This leads to increased insulin secretion in order to maintain normoglycaemia (Eckel *et al*., 2005; Wang *et al*., 2004). Several lines of evidence suggest that hyperglycemia and hyperinsulinemia, accumulation of advanced glycosylation end products in various tissues and dyslipidemia may be important risk factors for the development of atherosclerosis (Libby, 2005).

The principle action of insulin after a meal is to reduce circulating glucose levels, by stimulating the uptake of glucose into insulin sensitive tissues (primarily skeletal muscle) and reducing hepatic glucose output. Normal insulin sensitivity is usually seen in hypothyroid subjects (Gimenez-Palop *et al*., 2005). However, studies related to glucose tolerance in these patients are not extensive. Glucose intolerance has been reported in hypothyroidism as early as 1970 (Andreani *et al*., 1970). The 6-propyl-2-thiouracil (PTU) induced hypothyroidism in sheep resulted in insulin resistance when tested by euglycaemic-hyperinsulinaemic clamp test (Achmadi and Terashima, 1995). Experimental hypothyroidism was found to decrease glucose

utilization in one study (Cettour-Rose *et al*., 2005) and in another animal study, induction of hypothyroidism was associated with insulin resistance in terms of decreased glucose uptake in muscles and adipose tissue (Dimitriadis *et al*., 2006). Due to these controversial findings insulin resistance in hypothyroidism is a much ignored issue.

On the other hand, hypothyroidism is the most common endocrine disorder in India second to Diabetes mellitus. Hypothyroidism is the most common thyroid diseases today. Because of uncertain symptoms, it is very difficult to diagnose the disease. Particularly in rural part of India, the correct diagnosis of the disease for female patients takes place at a very later stage and hence the patient suffers physically and economically. An accuracy of 88% is achieved in diagnosis of hypothyroidism (Khanale and Ambilwade, 2011). Hence, any study related to insulin resistance and thyroid disorder is deemed pertinent especially in the Indian subcontinent.

Insulin resistant DM is the commonest form which accounts for 90% of the diabetic population in the world (Wild *et al*., 2004). It develops following long standing episodes of abnormal glucose homeostasis. Moreover, a recent report in India shows that the number of hypothyroids is more among patients with metabolic syndrome (Shantha *et al*., 2009). In our previous study we reported the presence of oxidative stress in hypothyroidism (Nanda *et al*., 2008). This is corroborated by other studies too (Marjani *et al*., 2008). Therefore, the present study assessed the insulin resistance in untreated hypothyroid patients and analyzed the influence of various biochemicals, hormonal and oxidative stress parameters on insulin resistance status.

MATERIALS AND METHODS

Subjects: Sixty seven newly diagnosed primary hypothyroidism patients with TSH level greater than 10 μIU mL^{-1}, were recruited consecutively from Medicine OPD prior to initiation of therapy. Sixty eight gender matched healthy euthyroid volunteers of age similar to that of study group were selected by clinician and enrolled as controls for the study. This study was approved by the research council and human ethics committee of JIPMER. Written consent was obtained from all the subjects.

Blood collection: The subjects were asked to fast overnight following which fasting blood samples were collected. EDTA whole blood was used for the estimation of whole blood reduced glutathione. Serum used for the estimations of glucose and lipid profile, thyroid profile, protein carbonyls and malondialdehyde (MDA).

Glucose, lipid profile, thyroid profile: Serum glucose, lipid profile and thyroid profile were estimated using commercial kits. The estimation procedures are described in detail in our previous report (Nanda *et al*., 2008).

Insulin, HOMA-IR and HbA1: Fasting plasma insulin was estimated using IRMA kit for human insulin following manufacture's (Immunotech, Beckman coulter Co, Czech Republic) instructions. From the fasting glucose and insulin values the homeostatic model assessment-insulin resistant (HOMA-IR) was calculated using the following formula; HOMA-IR = fasting insulin (μU mL^{-1}) X fasting glucose (mM/L)/22.5 (Pickavance and Wilding, 2007). Glycated hemoglobin was estimated by using haemoglobin A_1 microcolumns (Biocon, Vohl-Marienhagen, Germany).

Reduced glutathione: Reduced glutathione (GSH) in whole blood was estimated with 5, 5'-bis-dithionitrobenzoic acid reagent (Fairbanks and Klee, 1999).

Lipid peroxide and protein carbonyl: Malondialdehyde, the end product of lipid peroxidation was estimated by thiobarbituric acid method modified by K. Satoh (Satoh, 1978). Protein carbonyl (PCO) was estimated by the method of Levine (Levine *et al*., 1994).

Statistical analyses: All parameters were expressed as Mean±SD. Significance of the differences between control and test groups was analyzed by student't' test. For parameters without normal distribution, we used Mann Whitney-U test for analyzing the significance of differences. The correlations were assessed by Pearson correlation followed by stepwise regression analyses. All the statistical analyses were performed using the SPSS software.

RESULTS

The clinical characteristics and biochemical parameters are depicted in Table 1. The subjects of test group had significantly higher TSH and lower T_3 and T_4 levels in comparison to the subjects of the control group. There was a significant rise in the Body Weight (BW) and Body Mass Index

Table 1: Comparison of various parameters of hypothyroid patients with controls

Parameter	Control (n = 68)	Cases (n = 67)
General parameters		
Age (years)	34.19±11.92	35.32±11.23
Body weight (kg)	58.83±10.38	66.93±9.80 ***
BMI (kg m^{-2})	23.38±3.55	27.98±4.05 **
Thyroid profile		
T_3 (ng dL^{-1})	27.50±28.31	63.39±30.15 ***
T_4 (µg dL^{-1})	8.90±1.80	3.85±2.26 ***
TSH (µIU mL^{-1})	2.24±1.10	64.43±36.01###
Lipid profile		
TC (mM L^{-1})	4.50±0.80	6.52±1.54###
HDL-C (mM L^{-1})	1.29±0.29	1.20±0.30
LDL-C (mM L^{-1})	2.64 ± 0.76	4.16±1.49***
TG (mM L^{-1})	1.24±0.43	2.52±0.83###
VLDL-C (mM L^{-1})	0.24±0.08	0.50±0.16###
OS parameters		
Blood GSH (µmol g^{-1} Hb)	9.44 ±2.72	7.32±2.57 ***
Serum MDA (µMl L^{-1})	1.56±0.55	3.25±1.44***
PCO (nM mg^{-1} protein)	1.26±0.44	2.36±1.01###
Other biochemical parameters		
Fasting glucose (mM L^{-1})	4.27±0.71	4.46±0.58
Total protein (g L^{-1})	72.39±5.07	72.16±3.57
Hb A1	7.56±0.51	8.11±0.84***
Insulin (µU mL^{-1})	6.47±5.07	9.91±8.96#
HOMA-IR	1.24±1.00	1.99±1.86#

Data expressed as Mean±SD. **p<0.01, ***p<0.001, when significance was checked by student's t test; #p<0.05, ### p<0.001 when significance was checked by Mann Whitney-U test. BMI: Body mass index, LDL-C: Low density cholesterol, HDL-C: High density cholesterol, TC: Total cholesterol, TG: Triglyceride, VLDL-C: Very low density cholesterol, TSH: Thyroid stimulating hormone, PCO: Protein carbonyl, GSH: Glutathione, MDA: Malondialdehyde, HOMA-IR: Homeostatic model assessment of insulin resistance

Table 2: Pearson correlation of HOMA-IR with other biochemical parameters in hypothyroidism

Parameter	r	p
Age	0.168	0.173
BMI	0.259	0.034
TSH	0.269	0.028
HbA1	0.352	0.005
MDA	0.409	0.001
TC	0.322	0.008
TG	0.298	0.014
HDL-C	-0.309	0.011
LDL-C	0.319	0.008

$p<0.05$ was considered significant

Table 3: Stepwise linear regression analysis of various factors correlating with HOMA-IR in hypothyroid patients (n = 67)

	Standardized coefficient beta	t	p
Model 1			
Constant			0.049
HbA1	0.352	2.935	0.035
Model 2			
Constant			0.006
HbA1	0.372	2.372	0.002
MDA	0.329	2.329	0.005

Independent variable: HOMA-IR, Excluded variables: TSH, BMI, TC, LDL-C, HDL-C and TG

(BMI) of the hypothyroid subjects compared to controls. Lipid profile showed a significant rise in TC, LDL-C, TG and VLDL-C in the test group. There was no difference in the level of serum glucose and HDL-C levels.

There was a significant increase in the level of serum protein carbonyls and serum MDA in hypothyroid patients. GSH was significantly reduced in the test group. The simple correlations among HOMA-IR with various parameters are shown in Table 2. The stepwise regression analysis of parameters (which significantly correlated with HOMA-IR in Table 2 is depicted in Table 3.

DISCUSSION

According to a recent report from India, the number of hypothyroid patients was more among patients with metabolic syndrome (Shantha *et al*., 2009). Oxidative stress has been shown to be one of the causative factors associated with insulin resistance (Evans *et al*., 2003). In our previous studies we have reported the presence of OS in hypothyroid patients (Nanda *et al*., 2008) in a smaller sample size. Therefore, we hypothesized that despite being a hypometabolic state, the presence of underlying OS in hypothyroidism may predispose them to insulin resistance in the long run.

The gold standard for evaluating IR is hyperinsulinaemic euglycaemic clamp where insulin is infused at a constant rate and glucose is held at basal levels by glucose infusion. The rate of glucose infusion is a measure of insulin mediated glucose disposal (DeFronzo and Beckles, 1979). However, this method is invasive, time taking, expensive and can not be employed for a large number of cases. Therefore, there are alternative measures of IR used for the assessment of insulin resistance such as fasting plasma insulin concentration (Granberry and Fonseca, 1999), Home Ostasis Model Assessment (HOMA) index (Matthews *et al*., 1985).

A significant increase was found in fasting insulin and HOMA-IR levels in hypothyroid patients, compared to euthyroid controls, though there was no difference in fasting glucose level

between the two groups (Table 1). HOMA-IR correlated with the degree of hypothyroidism (with TSH), BMI, lipid profile (TC, LDL-C, HDL-C and TG) and OS parameter (MDA) which is depicted in Table 2. Even though the fasting insulin (normal range: 5 to 30 μU L^{-1}) and HOMA-IR (normal range: upto 7.4) were higher than the control group, nevertheless they were within normal range (calculated from normal levels of glucose and insulin in their upper reference range). Hence, this rise does not imply a frank insulin resistance in our study group. Nevertheless the significant rise in HOMA-IR compared to control cannot be overlooked. The exact mechanism of the underlying insulin resistance with normal fasting plasma glucose concentration in hypothyroidism is not known. This could be due to the effect of OS *per se*, as OS is reported to induce glucose intolerance (Kaneto *et al*., 2004).

Moreover, previous reports suggest that the proportion of diabetes is more in women than men (King *et al*., 1998). Certain studies showed that 5 year old girls and adolescents show higher insulin resistance than boys (Hoffman *et al*., 2000; Murphy *et al*., 2004). As hypothyroidism is a disease more prevalent in women, the sexual dimorphism in insulin sensitivity may make hypothyroid women more susceptible towards insulin resistance than the euthyroid women.

Among various factors influencing HOMA-IR level in our study, the Hb A1 and MDA levels were found to be the independent predictors of insulin resistance in hypothyroidism. However it is to be noted that glycation of hemoglobin itself is influenced by non traditional factors such as increased lipid peroxides or reduced glutathione activity (Jain and Palmer, 1997; Huby and Harding, 1988) etc. Hence increased HbA1 level not only indicates an alteration in carbohydrate metabolism but also indicates towards an impairment of oxidative balance in hypothyroid patients.

As glycation of body protein is associated with increased risk of micro-vascular complications (Taghi *et al*., 2005), the increased level of HbA1 observed in our study should not be ignored irrespective of the mechanism involved in it.

Therefore, this study suggested that despite the normoglycemia usually observed in hypothyroid patients, the fasting glucose and insulin levels should also be monitored from time to time and compared with the values in their first hospital visits, in order to rule out development of forthright glucose intolerance or insulin resistance. Also in patients where fasting blood can not be obtained in case of tertiary health care centers or patients who report to OPD at a later time of the day, the simple assessment of glycated hemoglobin assay at four to five months interval also can predict the alterations in carbohydrate metabolism in hypothyroidism.

As OS per se can promote glucose intolerance, we suggest that the association of sustained OS in chronic hypothyroidism may predispose a hypothyroid individual to metabolic syndrome on a later stage of this disease. In fact, a recent report on primary hypothyroidism from our country indicates that number of hypothyroids is more among patients with metabolic syndrome (Shantha *et al*., 2009). Keeping this picture in mind we advocate inclusion of timely monitoring of glucose, insulin and glycated haemoglobin levels for the long term prognosis of hypothyroidism.

BMI: Body mass index, LDL-C: Low density lipoprotein cholesterol, HDL-C: High density lipoprotein cholesterol, TC: Total cholesterol, TG: Triglyceride, TSH: Thyroid stimulating hormone, PCO: Protein carbonyl, GSH: Glutathione, VLDL-C: Very low density lipoprotein-cholesterol, MDA: Malondialdehyde, HOMA-IR: Homeostatic model assessment of insulin resistance

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