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Mild hypothyroidism improves glucose tolerance in experimental type 2 diabetes

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ABSTRACT

Background: Co-existence of type 2 diabetes and hypothyroidism is an emerging trend observed in clinical practice. Although the effects of isolated type 2 diabetes and hypothyroidism are well known, there are limited studies addressing the metabolic complications when these two conditions co-exist. The aim of the present study was to assess the interaction between type 2 diabetes and hypothyroidism with respect to glucose tolerance, dyslipidemia and redox balance in a state were these two diseases coexist. *Methods:* Sixty male Wistar Albino rats were randomised into six groups. Group 1: control, Group 2: overt hypothyroidism, Group 3: mild hypothyroidism, Group 4: type 2 diabetes, Group 5: mild hypothyroidism + type 2 diabetes, Group 6: overt hypothyroidism + type 2 diabetes. Experimental hypothyroidism was created by the administration of propyl-2-thiouracil and type 2 diabetes by feeding rats with 60% fructose (w/w). The duration of the study was 6 weeks. All the parameters were estimated at the start (basal) and the end of the study. Intraperitoneal glucose tolerance test was carried out and area under curve (AUC) calculated to assess the glucose tolerance. Thyroid profile, lipid profile and oxidative stress parameters were also measured. *Results:* Plasma TSH level was elevated 3-fold in the mild hypothyroid group and 15.2-fold in the overt hypothyroid group in comparison to the control group. Thyroid profile was found to be normal in type 2 diabetic group. There was no significant difference between hypothyroidism and hypothyroidism + diabetes groups with respect to thyroid profile. Among the six groups the degree of glucose intolerance was found to be maximum for overt hypothyroidism + diabetes group, followed by diabetes group and overt hypothyroidism group. An interesting finding was that glucose intolerance was significantly reduced in mild hypothyroidism + diabetes group (increase in AUC: 48.04%) in comparison with isolated diabetes group (increase in AUC: 71.63%). Similar results were obtained with parameters of oxidative stress. Oxidative stress was observed in overt hypothyroidism + diabetes, diabetes, overt hypothyroidism groups with severity decreasing in that order. Coexistence of mild hypothyroidism with diabetes decreased oxidative stress in comparison with isolated diabetes group. There was no statistical difference in lipid profile between mild hypothyroidism + diabetes and isolated diabetes group. *Conclusion:* Presence of mild hypothyroidism in type 2 diabetes confers a protective effect with respect to glucose tolerance and redox balance whereas presence of overt hypothyroidism in type 2 diabetes has a deleterious effect. The increased incidence of hypothyroidism in diabetes, especially subclinical hypothyroidism, could be a reflection of a physiological attempt by the body to mitigate damage wrought by diabetes.

1. Introduction

Diabetes mellitus is the most common endocrinopathy encountered in clinical practice. It encompasses a spectrum of disorders which share the common feature of hyperglycemia. The number of people suffering from diabetes has seen a significant increase over the last half a century despite rapid advances in our understanding of this metabolic disorder. About 8% of the world adult population, approximately 380 million individuals, were estimated to suffer from diabetes in 2013 [1]. India is home to one in every six adults suffering from diabetes. Type 2 Diabetes is characterised by hyperglycemia in the setting of a peripheral resistance to insulin and a relative decrease in the amount of circulating insulin. It constitutes nearly 90% of all cases of diabetes.

Thyroid disorders constitute another major group of endocrinopathies. Hypothyroidism is the most common form of thyroid

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dysfunction seen in the general population. One in ten adults in India suffer from hypothyroidism [2]. Hypothyroidism is divided into overt and subclinical based on the levels of circulating hormones. Subclinical disease is a mild form of disease in which the peripheral thyroid hormone levels are within the normal range but levels of thyroid stimulating hormone (TSH) are abnormally high. Overt disease on the other hand is characterised by deranged peripheral thyroid hormone levels also.

Co-existence of type 2 diabetes and hypothyroidism is an emerging trend observed in clinical practice. A strong link has been reported between these two diseases in the recent years [3]. Epidemiological studies reveal that type 2 diabetic patients are at a greater risk for development of hypothyroidism in comparison with normal subjects [4,5]. The Fremantle Diabetes Study found subclinical hypothyroidism to be a common incidental finding in women with type 2 diabetes [6].

Thyroid hormones play a major role in regulation of energy metabolism. Abnormalities in levels of thyroid hormones can lead to a dysregulation of glucose homeostasis thus complicating the management of diabetes. Patients with coexisting hypothyroidism and diabetes have been found to require earlier insulin therapy and need more endocrine attention [7]. Hypothyroidism is known to play a causal role in some of the classical risk factors of diabetes such as dyslipidaemia and hypertension. Conversely, abnormalities in thyroid hormone profile have been found in patients with diabetes. The nocturnal peak in levels of TSH is blunted and levels of serum T3 is reduced in patients with poorly controlled diabetes [8].

Although the effects of isolated type 2 diabetes and hypothyroidism are well known, there are few studies addressing the complications of co-existing type 2 diabetes and hypothyroidism. The pathogenic processes in both diabetes and hypothyroidism are involved in an intricate interplay which is yet to be fully elucidated. The present study was designed to assess the interaction between type 2 diabetes and hypothyroidism with respect to glucose tolerance, dyslipidemia and redox balance in a state were these two diseases coexist, using an animal model.

2. Materials and methods

2.1. Chemicals

6-Propyl-2-thiouracil (PTU), 2-thiobarbituric acid (TBA), I-glutathione reduced (GSH), hydrogen peroxide (H_2O_2), 5,5′-dithiobis 2-nitrobenzoicacid (DTNB), sodium sulphate, 2-4-dinitrophenylhydrazine(DNPH) & 2,4,6-tri-(2-pyridyl)-5-triazine (TPTZ) of molecular grade were purchased from Sigma Aldrich (USA). All other chemicals were of analytical grade obtained from SRL (India).

2.2. Animal experiments

The study was conducted in the Department of Biochemistry, Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Puducherry, India after obtaining due approval from Scientific Advisory Committee and the Institutional Animal Ethics Committee. Five month old male Wistar Albino rats were obtained from Institute Central Animal House and maintained in polycarbonate cages under a 12-h light/12-h dark cycle with food and water available ad libitum. Totally sixty rats were used in this study. They were randomised into six groups with ten rats in each group.

The six groups are as follows:

Group 1: control [C]
Group 2: overt hypothyroidism [OH]
Group 3: mild hypothyroidism [MH]
Group 4: type 2 diabetes [D]
Group 5: mild hypothyroidism + diabetes [MH + D]
Group 6: overt hypothyroidism + diabetes [OH + D]

Group 1 did not receive any intervention throughout the experimental period. Group 2 was given 0.05% (w/v) propylthiouracil in drinking water for 6 weeks to induce overt hypothyroidism [9]. Group 3 was given 0.005% (w/v) propylthiouracil in drinking water for 6 weeks to induce mild hypothyroidism. Group 4 was given 60% (w/w) fructose supplemented rat chow to induce type 2 diabetes [10]. Group 5 was given 0.005% (w/v) propylthiouracil in drinking water and 60% (w/w) fructose supplemented rat chow for 6 weeks to induce a state of co-existing mild hypothyroidism and type 2 diabetes. Group 6 was given 0.05% (w/v) propylthiouracil in drinking water and 60% fructose supplemented rat chow for 6 weeks to induce a state of co-existing overt hypothyroidism and type 2 diabetes.

2.3. Sample collection

The duration of the study was 6 weeks. All the parameters were estimated at the start (basal) and at the end of the study. Blood was collected from retro-orbital plexus under mild ether anaesthesia. At the end of the experimental period the animals were sacrificed, liver was excised, snap frozen in liquid nitrogen and stored at −80 °C for subsequent analysis.

2.4. Assessment of glucose tolerance

Intraperitoneal glucose tolerance test (IPGTT) was carried out to assess glucose tolerance. IPGTT was performed as described by Yuan et al. [11]. Blood samples were collected from the rats after they were maintained in a fasting state overnight (15 h). Rats were then injected 2.0 g/kg body weight of glucose (in 1.5 ml saline) intraperitoneally. Blood samples were collected for the estimation of plasma glucose at 30, 60 and 120 min after the glucose injection. Plasma glucose was estimated by glucose oxidase–peroxidase method (Reckon diagnostics, Vadodara, India) in a fully automated clinical chemistry analyser (AU-400, OLYMPUS, Essex, UK).

2.5. Lipid profile

The following biochemical parameters were analysed in fasting plasma sample using a fully automated clinical chemistry analyser (AU-400, OLYMPUS, Essex, UK). Total cholesterol was measured using cholesterol oxidase–peroxidase method (Genuine Bio-systems, Chennai, India), triglycerides (TG) using an enzymatic glycerol phosphate oxidase peroxidase method (Agappe Diagnostics, Kerala, India), and high-density lipoprotein (HDL) cholesterol by the cholesterol oxidase–peroxidase method (Lab-Care Diagnostics, Mumbai, India). Very low density lipoprotein (VLDL) cholesterol and low-density lipoprotein (LDL) cholesterol in plasma were calculated using Friedwald's formula [12].

2.6. Thyroid profile

Plasma rat free T3, free T4 and TSH were measured using ELISA kits from CUSABIO, Japan.

2.7. Oxidative stress

All the oxidative stress parameters were estimated using standardized spectrophotometric methods. Whole blood reduced glutathione was measured by method described by Beutler et al. [13]. Catalase activity in erythrocytes was determined by the