Research article

Altered levels of fructosamine and glycated haemoglobin (HbA1c) in thyroid disorder patients without diabetes mellitus-A cross sectional study

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ABSTRACT

Introduction and Aim: Co-existence of thyroid disorder and Diabetes Mellitus is no more a coincidence. The cause and impact of thyroid disorder on glucose levels or vice versa is a well-established fact. Hence in this study we wanted to know the glycemic status by estimating fructosamine and glycated hemoglobin of the newly diagnosed thyroid patients without diabetes mellitus. The aim of the study was to estimate fructosamine and glycated hemoglobin levels in newly diagnosed subclinical hypothyroid, clinical hypothyroid and hyper thyroid patients without diabetes mellitus.

Material and Methods: Twenty cases of subclinical hypothyroid, 30 cases of hypothyroid, 30 cases of hyperthyroid and 30 healthy participants were included in the study. Fasting plasma glucose and thyroid profile was estimated in suspected cases of thyroid disorder and participants with fasting plasma glucose (FPG) more than 110 mg/dL were excluded from the study. The participants who were eligible for an inclusion criterion were estimated for fructosamine by nitro blue tetrazolium, (NBT) method and anion-exchange high performance liquid chromatography was for glycated hemoglobin.

Results: In subclinical hypothyroid group there was a statistically significant increase in the mean fasting plasma glucose, fructosamine and glycated hemoglobin levels when compared with the controls. There was a significant increase in the mean fasting plasma glucose, fructosamine and glycated hemoglobin (HbA1c) levels in clinical hypothyroid group when compared with the controls. Pairwise comparison of FPG (p=0.001), fructosamine (p=0.001) and HbA1c (p=0.001) levels with controls showed a statistically significant difference. In clinical hypothyroid group the mean FPG and HbA1c levels were high and low fructosamine levels when compared with the controls by one way ANOVA. Pairwise comparison of FPG (p=0.001), fructosamine levels (p=0.001) and HbA1c (p=0.001) levels (p=0.001) with controls showed a statistically significant difference.

Conclusion: Unidentified hyperglycemia could have an impact on thyroid disorder leading to its complication. Hence a systematic approach to fructosamine testing (monitor the plasma glucose concentration over 2-3 weeks) as a routine test in thyroid disorder patients, needs to be considered. Also the management of hyperglycemia in thyroid patients without diabetes mellitus may prove to be beneficial.

Keywords: Fructosamine; glycated haemoglobin; thyroid disorder.

INTRODUCTION

Hypothyroidism, hyperthyroidism and subclinical hypothyroidism are sequel of altered biochemical function of thyroid gland (1). Thyroid hormones have a major impact on levels of glucose, insulin levels etc. (2). Hence, altered function of thyroid gland disrupts the delicate balance leading to altered glucose levels (3). Impact of increased blood glucose along with alteration in the thyroid gland function leads to inflammatory process, leading to mitochondrial dysfunction (4). Increased glucose levels cause glycation of proteins (5). Examples of these glycated proteins are Glycated Haemoglobin, Fructosamine and Glycated Albumin. These all are produced by non-enzymatic glycation (6). Glycated Hemoglobin assessment (3 month's glucose status) is still the corner stone of assessing glycemic control (7). While Fructosamine is a measure of glycemic index over a short period i.e., 2-3 weeks (8). However, thyroid diseases affect Glycated Hemoglobin levels and hematological facts and Glycated albumin levels are affected by albumin turn over but Fructosamine levels are not affected by RBC life span (9).

Keeping in this view the following study was undertaken to know the levels of fructosamine in comparison to glycated hemoglobin (ideal glycated status indicator), in non-diabetic newly diagnosed cases of hyperthyroid, clinical hypo and subclinical hypothyroid patients in comparison with normal individuals. This study may help clinician to consider Fructosamine as a marker of glycemic status in the thyroid disorder patients. Estimation and comparison of serum levels of fructosamine and glycated hemoglobin levels in newly diagnosed subclinical hypothyroid, clinical hypothyroid, clinical hyper thyroid patients without diabetes mellitus and healthy participants.

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MATERIALS AND METHODS
This observational cross-sectional study was conducted between January to December 2016. Patients attending medicine outpatient department at KLE’S Dr Prabhakar Kore Hospital and Medical Research Centre, Belagavi, were study participants.

Sample size calculation
Following formula was used to calculate the sample size:

\[
\text{Sample size (n)} = 4 \times \text{S.D/d}^2
\]

S.D- Standard Deviation, d – error

Considering the Mean ± S.D. values from previous studies taking 95% confidence limit and 5% tolerance level along with error as 3. Sample size in subclinical thyroid patients were 19, clinical hypothyroid and hyperthyroid patients were 26. However for our convenience we took 20 cases of subclinical hypothyroid, 30 cases of clinical hypo and hyperthyroid patients and 30 normal subjects. This sample size was achieved by simple random sampling.

Inclusion criteria
- Newly diagnosed subclinical hypothyroid patients
- Newly diagnosed hypo and hyperthyroid patients
- Age 20-60 years

Exclusion criteria
- Diagnosed cases of diabetes mellitus
- Diagnosed cases of thyroid disorder who are on anti thyroid treatment
- Hemolyzed samples

Study protocol
Ethical committee clearance was obtained (Ref:MDC/DOME/437 dated on 18th November 2016) Suspected cases of thyroid disorders aged 20-60 years, coming to Medicine outpatient Department of KLE’S Dr Prabhakar Kore Hospital and Medical Research Centre, Belagavi, were explained about the study in detail and requested to fill the written consent form and proforma (n=152).

5ml of Fasting blood was collected from the participants (n=152) taking aseptic precautionary measures using disposable syringe. Then the blood was transferred into two plasma separating test tubes. 3ml of blood was used for estimating TSH, T₃ and T₄ levels by Chemiluminescence Immunoassay method (10) and 2ml was for estimating glucose by GOD-PAP Trenders kit method (11). All the estimations were done at calibrated instruments of KLE’S Dr Prabhakar Kore Hospital and Medical Research Centre, Belagavi at NABL accredited Hi-Tech laboratory.

Hyperbilirubinemic and hemolyzed samples (n=6), Subjects on antithyroid treatment (n=6) and with normal thyroid profile and FPG Levels ≥110mg/dl were excluded from the study (n=12).

Included participants details are in the form of consort (Fig. 1).

In participants, FPG and thyroid profile was done who attended the OPD of Medicine Department belonging to KLE’S Dr Prabhakar Kore Hospital and Medical Research Centre, of this 12 were excluded as they Diabetic (FPG ≥110) and normal thyroid profile. Rest of the participants were categorized into 4 groups.

1. Controls (n=30)
2. Subclinical hypothyroidism (n=20)
3. Clinical hypothyroidism (n=30)
4. Clinical hyperthyroidism (n=30)

Then from the included participants 3ml blood was collected under aseptic precautionary measures using disposable syringe in EDTA tube for HbA1c and 2ml blood in plain tubes for fructosamine estimation.
Methodology for the Estimation of HbA1c and Fructosamine

HbA1c estimation

The estimation of Glycated Hemoglobin was done by ion-exchange high performance liquid chromatography (HPLC; 12).

Fructosamine estimation

The estimation of serum fructosamine was done by nitro blue tetrazolium NBT method (13).

Statistical analysis

The data was entered into excel sheet

Data analysis was done by using software package of social Sciences (SPSS) trial version 20. All the numerical values were summarised as Mean± SD with p<0.05 considered as level of significance.

Results

110 eligible participants aged between 20-60 years were enrolled in our study.

Graph 1 and 2 show the details of eligible participants.

Table 1: Comparison of mean thyroid profile, fasting plasma glucose, fructosamine and HbA1c levels in our study participants

<table>
<thead>
<tr>
<th>Variables</th>
<th>Healthy Controls n=30</th>
<th>Subclinical Hypothyroid n=30</th>
<th>Clinical Hypothyroid n=30</th>
<th>Clinical hyperthyroid n=30</th>
<th>F Value</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSH(µIU/ml)</td>
<td>2.32±0.70</td>
<td>13.85±8.70</td>
<td>56.81±47.05</td>
<td>0.19±0.15</td>
<td>33.56</td>
<td>0.001</td>
</tr>
<tr>
<td>T3(µg/ml)</td>
<td>1.05±0.36</td>
<td>0.91±0.29</td>
<td>0.71±0.82</td>
<td>10.76±7.53</td>
<td>45.00</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Graph 1 represents that most of the participants were females and Graph 2 shows most of our participants were aged 21-30 years, followed by 31-40 years.

Table 1: Comparison of mean thyroid profile, fasting plasma glucose, fructosamine and HbA1c levels in our study participants

One way ANOVA and pairwise comparisons of FPG, fructosamine and HbA1c levels by Tukey’s multiple Post-hoc Bonferroni test

Comparison of means of several groups.

To know the significant difference in the means of variables:

- Between controls and subclinical hypothyroid group
- Between controls and clinical hypothyroid group
- Between controls and clinical hyperthyroid group
It has been well documented that the effects of thyroid hormones can lead to oxidative stress and increased glycaemia due to hypometabolic state as the half-life of thyroid hormone (T3) is short. In previous studies reported, higher levels of FPG in hyperthyroid group as compared to normal control groups was due to increased hepatic glucose output. Increased all of these lead to increased output of glucose as thyroid hormones have an impact on epinephrine and glucagon (4).

In clinical hypothyroidism raised fructosamine values could be due to hypometabolic state as the half-life of plasma proteins are prolonged (23). It is an inflammatory process, causes creation of free radicals that leads to increased oxidative stress and increased glycation of proteins. Subnormal function of the thyroid gland and insufficient iodination, becomes a major site of a damaging free radical generation. Free radical production depletes the defence mechanisms like glutathione peroxidise. Reduced production of glutathione peroxidise worsens the functioning of the thyroid gland and increases the glycation of proteins (15).

The study done in 2014, in Tamil Nadu, India, proved that in hypothyroidism there is hyperinsulinemia and insulin resistance in peripheral tissue. They observed altered glucose homeostasis thereby causing increased protein glycation. The inclination of glycated proteins in the tissues cause easy proteolysis and being further source of free radicals (16).

**Clinical hyperthyroidism**

Higher levels of FPG in clinical hypothyroidism than the control group, however mean fructosamine levels were lower than the control group and were statistically significant. These findings were in accordance with previous studies done in South Karnataka and North India (14,15).

Hyperthyroidism is a hypermetabolic state with increased muscle protein breakdown. There is increased metabolic activity with increased protein turnover, however the fructosamine concentrations were significantly lower in clinical hyperthyroid patients as compared to controls. Also there is a state of oxidative stress which increases the possibility of proteins getting glycated. The higher levels of FPG in hyperthyroid group as compared to normal control groups was due to change in carbohydrate metabolism (14).

The study done in North Indian population showed that the effect of thyroid hormone (T3) cause glyco genesis, gluconeogenesis, increased hepatic expression of glucose transporter (GLUT2) and hepatic glucose output. Increased all of these lead to increased output of glucose as thyroid hormones have an impact on epinephrine and glucagon (4).

In previous studies reported, marked decrease in serum fructosamine concentrations in hypothyroidism patients as compared to controls. They unveiled, instead of decreased concentration of fructosamine and albumin levels there is an increase in HbA1c level which corresponds to raised glucose metabolic disorders and diabetes mellitus. There are lack of studies to correlate the effects of thyroid hormones on glycation. Fructosamine indicates short term glycemcic control. Hence this study was undertaken to estimate the glycated haemoglobin and fructosamine levels in clinical hyper and hypothyroid patients without diabetes mellitus along with apparently healthy controls.

**Subclinical hypothyroidism**

In the present study the mean FPG, HbA1c and Fructosamine levels were statistically significant when compared with the controls. These findings are in accordance with one of the study done in south Karnataka (14).

**Clinical hypothyroidism**

Higher levels of FPG in clinical hypothyroid cases when compared with the controls and were statistically significant (p<0.05). However the levels were in the normoglycemic range. The fructosamine levels were greatly increased in clinical hypothyroid patients when compared with the controls which was statistically significant. This is in accordance with many previous studies done in south Karnataka and North India (14,4).

In clinical hypothyroidism raised fructosamine values could be due to hypometabolic state as the half-life of plasma proteins are prolonged (due to decreased turnover). It is an inflammatory process, causes creation of free radicals that leads to increased oxidative stress and increased glycation of proteins. Subnormal function of the thyroid gland and insufficient iodination, becomes a major site of a damaging free radical generation. Free radical production depletes the defence mechanisms like glutathione peroxidise. Reduced production of glutathione peroxidise worsens the functioning of the thyroid gland and increases the glycation of proteins (15).

The study done in 2014, in Tamil Nadu, India, proved that in hypothyroidism there is hyperinsulinemia and insulin resistance in peripheral tissue. They observed altered glucose homeostasis thereby causing increased protein glycation. The inclination of glycated proteins in the tissues cause easy proteolysis and being further source of free radicals (16).

**DISCUSSION**

In thyroid dysfunctions the glucose homeostatic balance is broken. Increased concentration of T3 causes protein catabolism and pessimistic nitrogen balance. Circulating sugars mainly glucose and fructose form Advanced Glycation End Products (AGEs) when they come in contact with proteins and lipids. Examples of proteins subject to non-enzymatic glycation are glycated hemoglobin, and Fructosamine. There is a well-recognised positive association between thyroid disorders and diabetes mellitus. There are lack of studies to correlate the effects of thyroid hormones on glycation. Fructosamine indicates short term glycemcic control. Hence this study was undertaken to estimate the glycated haemoglobin and fructosamine levels in clinical hypothyroid and hyperthyroid patients without diabetes mellitus along with apparently healthy controls.

**Table 2:** Pairwise comparisons of FPG, Fructosamine and HbA1c levels by Tukey’s multiple Post-hoc Bonferroni test

<table>
<thead>
<tr>
<th>Variables</th>
<th>Subclinical hypothyroid</th>
<th>Clinical hypothyroid</th>
<th>Clinical hyperthyroid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (FPG)</td>
<td>p=0.001*</td>
<td>p=0.009*</td>
<td>p=0.001*</td>
</tr>
<tr>
<td>Control (Fructosamine)</td>
<td>p=0.001*</td>
<td>p=0.001*</td>
<td>p=0.001*</td>
</tr>
<tr>
<td>Control (HbA1c)</td>
<td>p=0.001*</td>
<td>p=0.001*</td>
<td>p=0.001*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Variables</th>
<th>Subclinical hypothyroid</th>
<th>Clinical hypothyroid</th>
<th>Clinical hyperthyroid</th>
</tr>
</thead>
<tbody>
<tr>
<td>T4 (ng/dl)</td>
<td>7.90±2.18</td>
<td>7.57±1.89</td>
<td>2.18±0.95</td>
</tr>
<tr>
<td>FPG (mg/dl)</td>
<td>85.40±4.84</td>
<td>88.80±2.59</td>
<td>89.23±4.44</td>
</tr>
<tr>
<td>Fructosamine (µmol/L)</td>
<td>361.30±7.88</td>
<td>485.35±40.16</td>
<td>576.77±37.23</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>4.84±0.36</td>
<td>5.32±0.38</td>
<td>5.44±0.14</td>
</tr>
</tbody>
</table>

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concentrations. They inferred that, where one class of proteins resist the glycation and the other corresponded to the plasma glucose concentration (17). Altered glucose homeostasis, increase turnover of proteins, impaired oxidative stress balance in hyperthyroidism, this explains the lower Fructosamine levels (14).

Though our study findings are well in support of previous studies, it has got some limitations. It was single centre case-control study. A limited number of cases and controls are included. With such varied results and discussion we tried to put up findings and probable reason behind these findings in the form of a fig. 2.

**CONCLUSION**

In subclinical and clinical hypothyroid participant’s fructosamine and glycated hemoglobin levels were increased compared to normal participants and in case of clinical hyperthyroid individuals Glycated haemoglobin levels were high but fructosamine levels were low compared to normal participants. Probably as thyroid disorders affect metabolic rate of the individuals and this has an impact on protein turnover leading to altered inflammatory state and oxidative stress causing altered levels of fructosamine and glycated hemoglobin.

Therefore, do patients with thyroid disorders without DM be tested for fructosamine, routinely? This needs to be reconfirmed with a better sample size and later appropriate guidelines should be laid down.

CONFLICT OF INTEREST

Authors declare no conflict of Interest.
REFERENCES


