Assessment of Glycosylated Hemoglobin (HbA1c) Level in Age and Blood Glucose Matched Nondiabetic Hypothyroid and Euthyroid Females in an Urban Population of Eastern India- An Observational Cross Sectional Study

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ABSTRACT

Background: Spuriously high level of glycosylated hemoglobin has been observed in non-diabetic hypothyroid patients in recent studies.
Aims: To investigate the impact of thyroid hormones on Serum HbA1c levels in an urban female hypothyroid nondiabetic population in Eastern India.
Materials & methods: This present observational cross sectional study was conducted on 200 hypothyroid subjects in Burdwan Medical College in a period of 12 months after taking Institutional Ethical Clearance and informed consent of the subjects. 100 controls were also included for the study. Serum TSH, FT4, HbA1c and Fasting plasma glucose levels were estimated. The subjects were age and blood glucose matched. The computer software SPSS, version 16.0 was used for analyzing data.
Results: Significant difference was observed between control and hypothyroid subjects for mean TSH (P<0.0001), mean FT4(P<0.0001) and mean HbA1c (P<0.0001). No significant difference was observed for mean age (Age in years: 30.82±6.55 vs. 31.54 ±6.68; P value: 0.376) and fasting plasma glucose (83.97± 6.24 vs 84.65±6.00, p=0.362) between control and hypothyroid subjects. Serum HbA1c level was positively correlated with serum TSH (r=0.684, P<0.00001) and was negatively correlated with Sr. FT4 (r= -0.495, P<0.00001).
Conclusion: Serum HbA1c levels were significantly higher in Hypothyroid nondiabetic females compared to controls. There is a positive correlation between HbA1c and TSH. In hypothyroid individual assessment of HbA1c levels may be considered as an important parameter for diagnosis of diabetic or prediabetes state.

Keywords: Hypothyroidism, Indian female population, FT4, TSH, HbA1c,

INTRODUCTION

Thyroid disorders like diabetes are some of the most common endocrine disorders in the world and second to diabetes. [1-4] Among the general population rate of diabetes is persistently increasing and has already reached a state of challenge. [2] Reduced synthesis of thyroid hormone is the key feature of hypothyroidism. [5] In diabetic subjects, prevalence of thyroid disorder is known to...
increases by more than 10%. [6,7] Glycation of valine present in β-chain of hemoglobin forms Glycosylated hemoglobin (HbA1c). [7,8] Depletion of iron store may elevate HbA1c concentration by altering the rate of glycation of hemoglobin, independent of glucose levels. [7] American Diabetic Association (ADA) has recommended HbA1c as a diagnostic test for diabetes mellitus. [9]

In liver, thyroid hormone counteracts the action of insulin and causes increase in gluconeogenesis and glycogenolysis. But they have synergistic action with insulin in adipose tissue and skeletal muscle. [6] Thyroid hormone plays an important role in regulation of overall metabolic rate of the body and also influences the glucose level in blood. [10]

In 1973 the association between diabetes and thyroid dysfunction was first reported [11] In Diabetic individuals, there is increased release of TSH by a central mechanism and at peripheral tissue there is early conversion of T4 to T3. [1,11]

It has been shown in various studies that glycosylated hemoglobin (HbA1c) is increased in nondiabetic hypothyroid patients. [5,7,8,10,12-22]

Altered life span of erythrocyte in hypothyroidism might be responsible partially for spurious rise of HbA1c levels in these individuals. [8] In these subjects, hyperglycemia due to insulin resistance and mitochondrial dysfunction; decreased metabolism causing decreased turnover of proteins; increased oxidative stress leading to increased glycation of protein; tendency of glycated proteins to accumulate in the tissues also contribute to elevated HbA1c level in hypothyroidism. [14]

The present study was conducted to investigate the impact of hypothyroid state on Serum HbA1c levels in an urban female hypothyroid nondiabetic population of reproductive age group in Eastern India. Hypothyroid condition is more common in females as compared to males. So, they were selected as the study population and thyroid disorders are very common endocrine problems found in India and worldwide. So, the female subjects were included our present observational cross sectional study.

MATERIALS AND METHODS

This cross sectional study was conducted on 200 newly diagnosed hypothyroid nondiabetic female subjects in Burdwan Medical College for a span of 12 months after taking approval from institutional ethics committee and informed consent from the participating subjects. 100 controls (age and blood glucose matched) were taken for the present study. The formula used to calculate the size of the required sample was \( n = \frac{z^2 \times p \times (1-p)}{d^2} \), \( n = \) sample size, \( z = \) z statistic for a level of confidence (95% level of confidence used, so \( z \) value is 1.96), \( p = \) expected prevalence of proportion, \( d = \) desired precision taken as 6% and previous studies were taken into consideration.

Inclusion criteria:

200 newly diagnosed hypothyroid nondiabetic female subjects in the reproductive age group attending in the Department of Biochemistry, Burdwan Medical College and 100 control subjects were taken for the study.

Exclusion criteria:

- Liver dysfunction
- Renal disorder
- Patients with diabetes mellitus
- Anemia
- Hemoglobinopathy
- Hypothyroid subjects on thyroid hormone replacement therapy
- Hemolytic disorder
- Recent history of blood transfusion (<3 months)
- Pregnancy

Parameters studied:

- Age
- TSH
- FT4
- Glycosylated hemoglobin (HbA1c) level
- Fasting plasma glucose
Methods:
Approval from the Institutional ethics committee was taken before conduction of the study and Informed consent was taken from the participants. Subjects were recruited by random sampling using an online randomizer. Detailed history was taken from each subject as per case record format. Participants were also screened based on the inclusion and exclusion criteria.

Fasting Blood samples (5 ml) were drawn from subjects by sterile needle and syringes and sent to biochemical laboratory in sterile vials for analysis.

Estimation of Serum TSH was done by Quantitative determination of TSH concentration by Microplate immunoenzymometric assay using Monobind Inc. USA manufactured TSH AccuBind ELISA Kit (Normal value:0.39-6.16 micro IU/ml).

Estimation of Serum FT4 level was done by Quantitative determination of FT4 concentration by Microplate Enzyme Immuno assay using Monobind Inc. USA manufactured FreeT4 AccuBind ELISA Kit (Normal value:0.8-2.0 ng/dl).

The HbA1c levels were measured by Immunoturbidimetric method (Normal value: Non diabetic: 4-6%) and plasma FBS (Normal value:74-100 mg/dl) was measured by GOD POD method in automated analyser using reagents supplied by Erba Mannheim, Germany.

Estimation of TSH:
Measurement of serum TSH is generally regarded as the most sensitive indicator for the diagnosis of Hypothyroidism.

Test principle: (Method-Immunoenzymometric assay)

The immobilization occurs at the surface of microplate well between the interaction of streptavidin coated on the well and biotinylated monoclonal anti-TSH antibody. By mixing the monoclonal biotinylated antibody, the enzyme labelled antibody and a serum containing native antigen, reaction occurs between native antigen and the antibodies to form a soluble sandwich complex. Then the complex is deposited to the well. After equilibrium is achieved, the antibody bound fraction is separated from unbound antigen by aspiration or decantation. The enzymatic activity of the antibody bound fraction which is directly proportional to the native free antigen concentration is measured by adding substrate. By utilizing calibrators of known antigen concentrations, a dose response curve can be generated from which the antigen concentration of an unknown sample can be ascertained.

Kit content:
1. Streptavidin Coated Microplates-96 wells coated with streptavidin and packaged in an aluminium bag with a drying agent.
2. TSH Enzyme Reagent- 13 ml/vial containing enzyme labelled polyclonal antibody, biotinylated monoclonal IgG in buffer, dye and preservative.
3. Thyrotropin Calibrators-seven vials(0.5ml/vial) of references for TSH Antigen at levels of 0,0.5,2.5,5.0,10,20 and 40 μIU/ml
4. Substrate A (7ml/vial) -one bottle containing tetramethylbenzidine (TMB) in buffer.
5. Substrate B (7ml/vial)-one bottle containing hydrogen peroxide (H2O2) in buffer.
6. Stop Solution (8ml/vial)- one bottle containing a strong acid (1 N HCL)
7. Concentrate Wash Solution (20 ml)-single vial contains a surfactant in buffered saline. A preservative has been added.

Calculation of results:
(1) Calculation of the mean absorbance value of calibrator and samples was done at 450 nm.
(2) A point to point curve was plotted by plotting the absorbance of each calibrator on the vertical Y-axis against concentration of each calibrator on the horizontal or X-axis.
(3) Using the absorbance value for each sample the corresponding concentration of TSH was determined in micro IU/ml. and the standard curve is used.
Estimation of FT4: -
Thyroxine circulates in blood almost bound to carrier proteins. Thyroxine binding globulin (TBG) is the main carrier protein. Only the free (unbound) fraction of thyroxine is biologically active. Concentrations of the carrier proteins are altered in different clinical conditions. So the free thyroxine (FT4) concentration remains constant. The measurement of FT4 concentration correlates better than total thyroxine level.

Test principle: (Competitive Enzyme Immunoassay-EIA)

In competitive EIA, a competitive reaction results between the native free antigen and enzyme-antigen conjugate for limited number of insolubilized binding sites on antibody coated on the micro well. After the equilibrium is attained the antibody-bound fraction is separated from unbound antigen by aspiration or decantation. The enzymatic activity of the antibody bound fraction which is inversely proportional to the native free antigen concentration is measured by adding substrate. By utilizing calibrators of known antigen concentrations, a dose response curve can be generated from which the antigen concentration of an unknown sample can be found out.

Kit contents:
1. FT4 Antibody coated microplate (96 wells)-one96 well microplate coated with anti-thyroxine serum.
2. Enzyme reagent (13ml/vial)-one vial of thyroxine-horseradish peroxidase(HRP) conjugate in a protein stabilized matrix.
3. FT4 calibrators (0.5 ml/vial)- six vials of human serum based reference calibrators for free thyroxine.
4. Substrate A (7 ml/vial)-one bottle containing tetramethylbenzidine(TMB) in acetate buffer.
5. Substrate B (7 ml/vial)-one bottle containing hydrogen peroxide(H2O2) in acetate buffer.
6. Wash solution concentrate (20 ml)-one vial containing a surfactant in buffered saline.
7. Stop solution (8 ml/vial)-one bottle containing a strong acid (1 N HCL).

Calculation: -
1. Calculation of absorbance value was done at 450 nm.
2. A point to point curve was plotted by plotting the absorbance of each calibrator on Y axis against concentration of each calibrator on X axis.
3. Using the absorbance value for each sample the corresponding concentration of FT4 was determined in ng/dl. and the standard curve is used

Hypothyroidism was defined as an elevated TSH (>6.16micro IU/ml) with a decreased (<0.8ng/dl) or normal serum FT4 level (range:0.8-2.0 ng/dl) as per kit values.

Estimation of serum HbA1c: (Particle enhanced Immuno turbimetric method)

Principal: In hemolysed blood total Hb and HbA1c bind to Latex particles in R1 reagent with the same affinity. The amount of such binding is proportional to the concentration (relative) of both substances present in the blood. Particles bound HbA1c bind with Mouse anti-human HbA1c monoclonal antibody (R2a). Goat anti-mouse IgG polyclonal antibody (R2b) reacts with monoclonal mouse anti-human HbA1c antibody. The absorbance measured is proportional to HbA1c bound particles which in order is proportional to the HbA1c percentage of the sample.

Reagent composition:
R1-Buffer, Latex
R2a-Buffer, Mouse anti-human HbA1c monoclonal Ab.
R2b- Buffer, Goat anti-mouse IgG polyclonal antibody, stabilizers
R3 – Hemolsing solution

Estimation of plasma glucose: (GOD POD method)

Principal: Alpha-D glucose present in the sample is converted to Beta-D glucose by Mutarotase which in turn is oxidized to gluconic acid and H2O2 by the action of glucose oxidase. The Peroxidase enzyme
calyses the oxidative coupling between 4 amino antipyrine and phenol to produce a coloured quinonemine complex. The absorbance of such complex is proportional to the concentration of glucose in sample.

Reagents:
- Triphosphate buffer
- Glucose oxidase
- Peroxidase
- Mutarotase
- Phenol

**Statistical data analysis:**
The computer software “Statistical Package for the Social Sciences (SPSS) version 16 (SPSS Inc. Released 2007. SPSS for Windows, Version 16.0, SPSS Inc. Chicago)” was used to analyze the data, P<0.05 was considered as significant and P<0.01 was considered as highly significant.

**RESULTS**
Newly diagnosed 200 hypothyroid nondiabetic female subjects and 100 controls were enrolled for our present study. Significant difference was observed between control and hypothyroid subjects for mean TSH (P<0.0001), mean FT4 (P<0.0001), mean HbA1c level (P<0.0001). No significant difference was observed for mean age (Age in years: 30.82±6.55 vs. 31.54 ±6.68; P value: 0.376) and fasting plasma glucose (83.97± 6.24 vs 84.65±6.00, p=0.362) between control and hypothyroid subjects (Table 1, Figure 1).

Serum HbA1c level was positively correlated with serum TSH (r=0.684, P<0.00001) and was negatively correlated with Sr. FT4 (r= - 0.495, P<0.00001).

**Table 1:** Shows Age, TSH, FT4, serum HbA1c level and fasting plasma glucose values of control and hypothyroid subjects

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (Mean ± SD)</th>
<th>Hypothyroidism (Mean ± SD)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>30.82 ± 6.55</td>
<td>31.54 ± 6.68</td>
<td>0.376</td>
</tr>
<tr>
<td>TSH(micro IU/ml)</td>
<td>1.90 ± 0.91</td>
<td>18.93± 9.50</td>
<td>&lt;0.0001**</td>
</tr>
<tr>
<td>FT4(ng/dl)</td>
<td>1.32 ± 0.24</td>
<td>0.78±0.36</td>
<td>&lt;0.0001**</td>
</tr>
<tr>
<td>HbA1c(%)</td>
<td>4.63±0.39</td>
<td>5.00±0.7</td>
<td>&lt;0.0001**</td>
</tr>
<tr>
<td>Fasting plasma glucose(mg/dl)</td>
<td>83.97±6.24</td>
<td>84.65±6.00</td>
<td>0.362</td>
</tr>
</tbody>
</table>

P<0.05 *significant, <0.01 ** highly significant

Figure 1: shows the difference of Age, TSH, FT4, serum HbA1c and Fasting plasma glucose values between control and hypothyroid subjects

Figure 2: Shows serum HbA1c was positively correlated with serum TSH (r=0.684, P<0.0001)

Figure 3: Shows Serum HbA1c level was negatively correlated with Sr. FT4 (r= - 0.495, P<0.0001)
DISCUSSIONS

Autoimmunity is the main cause of thyroid dysfunction in diabetes. [23] HbA1c is the major form of glycohemoglobin and is an important marker for monitoring glycaemia in diabetes. [24] HbA1c reflects glycemic control over the preceding 8-12 weeks [25-31] and also correlates with the diabetic complications. [25]

In thyroid subjects different glycated proteins may be used to determine the degree of glycaemia. [32] Increased HbA1c in nondiabetic is associated with increased risk of cardiovascular events. [33,34] Many factors influence HbA1c but glucose is one of the most important to affect the HbA1c. [10]

The present study was conducted to investigate the impact of hypothyroid state on Serum HbA1c levels in an urban female hypothyroid nondiabetic population in Eastern India. We had enrolled 200 newly diagnosed hypothyroid nondiabetic female subjects and 100 controls for our present study. Factors interfering with glycation of hemoglobin were excluded from our study.

Significant difference was observed between control and hypothyroid subjects for mean TSH (P<0.0001), mean FT4 (P<0.0001), mean HbA1c level(P<0.0001). No significant difference was observed for mean age (Age in years: 30.82±6.55 vs. 31.54 ±6.68; P value: 0.376) and fasting plasma glucose (83.97± 6.24 vs 84.65±6.00, p=0.362) between control and hypothyroid subjects

Insulin resistance is associated with subclinical hypothyroidism and linked to risk of metabolic syndrome and impaired lipid balance. [10] Concentration of HbA1c not only depends on glycaemia but life span of erythrocyte also. [5,15] Reduced erythropoiesis in hypothyroidism may cause rise of HbA1c and lead to erroneous diagnosis of prediabetes or diabetes. [12,13]

Lediju et al. [17] noticed a significant difference of HbA1c between subclinical hypothyroidism and Euthyroid subjects (6.5±1.5 vs 5.4±1.7, p<0.021) in their study. Kim et al. [18] studied the influence of thyroid hormone on glycated hemoglobin and glycated albumin and found increased level of HbA1c in hypothyroid subjects compared to controls (5.54 ± 0.43 vs. 5.34 ± 0.31%, P < 0.001). In another studies by Christy et al. [20] observed a statistically significant difference of HbA1c between hypothyroid non-diabetic subjects and Euthyroid subjects (6.32±0.75% vs 5.87±0.46%) and Makadia et al. [15] found a significant higher level of HbA1c in non-diabetic hypothyroid subjects than controls (5.70±35% vs 5.26 ±0.17% ,p<0.0001)

In the present study we also found a significant difference of HbA1c between hypothyroid subjects and Euthyroid control subjects (5.00±0.78 vs4.63± 0.39, p<0.0001).

Billic-Komarica et al. [35] reported a significant positive correlation between TSH level and HbA1c level (r=0.46, p<0.05). Acharya et al. [2] in their study observed a positive correlation between TSH and HbA1c(r=0.397) and negative correlation between FT3 and HbA1c (r=-0.508). Positive correlation between TSH and HbA1c has also been observed in other studies. [15,36]

In the present study, we noticed that HbA1c level was positively correlated with serum TSH (r=0.684, P<0.00001) and was negatively correlated with Sr. FT4 (r= -0.495, P< 0.00001).

CONCLUSION

Serum HbA1c levels were significantly higher in Hypothyroid nondiabetic females compared to controls. There is a positive correlation between HbA1c and TSH. In hypothyroid individual assessment of HbA1c levels may be considered as an important parameter for diagnosis of diabetic or prediabetes state.

Conflict of interest: Declared none.
Source of funding: Nil

REFERENCES

1. Mukherjee S, Datta S, Datta P, Mukherjee AK, Maisnam I. A study of prevalence of primary hypothyroidism in recently


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