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Original Research article

Title: Glycation of Hemoglobin Among Individuals With Subclinical Hypothyroidism – An Urban Cross-Sectional Study

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ABSTRACT

Background: HbA1c provides a retrospective index of integrated plasma glucose values over an extended period of time and is not subjected to the wide fluctuations. Still, the life span of the erythrocytes and so, the conditions, like thyroid hormonal derangement, which affect the erythrocyte turnover or survival may lead to elevated or lowered HbA1C levels, unrelated to the causes of hyperglycemia. An association between hypothyroidism and hyperglycemia is well recognized but the effect of thyroid disorders on glycation of Hemoglobin levels in normoglycemic patient is still an area to be explored. In this study, the possible effects of subclinical hypothyroidism on HbA1c values in normoglycemic healthy adult urban population, have been explored in a cross-sectional manner.

Methodology: The study has been conducted among 177 healthy adult population attending NABL accredited corporate laboratories in West Bengal for one year duration, and their TSH, FBS, PPBS and HbA1c values have been documented for further analysis.

Results: Using appropriate statistical methods, it has been found that there is significant differences between the mean values of HBA1C, FBS, PPBS level in subjects having TSH <4.2 uIU/ml & TSH ≥ 4.2 uIU/ml. (p value < 0.01). Whereas by Correlation study, no significant concordance found between Serum TSH level and FBS, PPBS levels but a positive correlation found between serum TSH and HBA1c. Also, strong association has been found between subclinical hypothyroidism (TSH ≥4.2uIU/ml) and increased HBA1C value (HBA1C > 5.7) in this normoglycemic study population. (p value- <0.01).

Conclusion: These findings point towards an independent association of subclinical hypothyroidism and baseline elevation of HBA1c value even in Normoglycemic otherwise healthy individuals, and it should be taken into consideration by practitioners while interpreting the glycemic status of a subclinical hypothyroid patient.

INTRODUCTION

Measurement of glycated proteins, primarily glycated hemoglobin is effective in monitoring long term glycemic control. It provides a retrospective index of integrated plasma glucose values over an extended period of time and is not subjected to the wide fluctuations observed when blood glucose concentrations are assayed. So it has been recommended for the diagnosis of diabetes

and monitoring the long-term glucose control in diabetic patients. [1]

Normal range (ADA 2010 recommendations):

<5.6%: Non diabetic.

5.7–6.4% : Increased risk for diabetes.

>6.5% : Diabetic range.

Glucose combines with Hb continuously and nearly irreversibly during the life span of RBC (120 days). Therefore, glycosylated Hb (GHb) will be proportional to mean plasma glucose level during previous 6–12 weeks. [2]

The two major factors determining the HbA1c concentration are the persistent blood glucose level and the life span of the erythrocytes. Hence, factors leading to alterations of the rate of the erythrocyte turnover or survival are potential candidates for raising or lowering HbA1C levels. [3, 4, 5]

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It is already established that thyroid status of patients affects their blood glucose level in several way. TSH in vitro is capable of stimulating glucose oxidation, whereas Thyroid hormone affects glucose homeostasis in various way including increasing hepatic glucose output, increasing futile cycling of glucose , decreasing glycogen stores in the liver and skeletal muscle, altering oxidative and non-oxidative glucose metabolism, decreasing active insulin secretion and increasing clearance. **[6, 7]** An association between hypothyroidism and hyperglycemia is well recognized but the effect of thyroid disorders on glycation of Hemoglobin levels in normoglycemic patient is still an area to be explored. RBC turnover is increased in thyrotoxic states whereas hypothyroidism has the opposite effect, resulting in altered HBA1C value. **[8]** Based on this research question, we would like to explore, the possible effects of subclinical hypothyroidism on HbA1c values in normoglycemic healthy adult urban population.

MATERIALS AND METHODS:

The present study is an observational study with Cross-sectional model for one-year duration. Study population includes healthy adult populations (20-55 years) who are coming for annual health checkup as per their corporate company policy in NABL accredited corporate laboratories from different places of South Bengal. Approval was taken by research and ethics committee and an informed consent was obtained from individuals who are willing to take part in this study.

Exclusion criteria for the selection are the followings:

1. Persons with history of Habitual alcohol intake, Smoking, any other addiction or drug abuse like cannabis, opioids, sedatives etc.
2. Any medication like lipid lowering drugs, methotrexate, barbiturates, oral contraceptives etc.
3. Diabetes was excluded by doing fasting and post prandial blood sugar levels or from past and family history.
4. Bilirubin concentrations > 20 mg/dL, Hemoglobin F concentrations >10% , Labile A1c (LA1c/CHb-1)> 4%,Carbamylated hemoglobin (LA1c/CHb-2) > 3.5% interfere with the assay. So subjects having these conditions are excluded from the study.
5. Pregnant ladies,
6. Patients having haemolytic anaemia, other hemoglobinopathies, anaemia due to other chronic illnesses, renal disease that shorten erythrocyte survival may proportionately decrease Hba1C levels and are also associated with iron deficiency, but the degree of renal impairment at which anemia occurs is unclear. CKD patients were also excluded, defined as a glomerular filtration rate (GFR) < 60 ml/min/1.73 m^2 or GFR from 60 to 90 ml/min/1.73 m2 with microalbuminuria from the primary analysis.
7. Acute or chronic hepatic, cardiovascular pathologies, acute or chronic inflammatory conditions.
8. Overt hypothyroidism (TSH >10 uIU/ml) and patients are on Thyroid supplementation for previously diagnosed hypothyroidism.

For all the subject, thorough physical examinations including anthropometry was performed. Among laboratory investigations serum thyroid stimulating hormone (TSH) level, FBS, PPBS, HB% and HbA1C levels were done. TSH assayed in Cobas e-411. FBS, PPBS assayed in vitros 5.1 and vitros 250 Dry Chemistry analyser.

Thyroid hormone profile (TSH and Ft4) were measured using Chemiluminiscent Immunoassay (CLIA). HbA1c was measured by HPLC method in BIORAD D-10 analyzer. Quality assurance of the parameters under study was maintained by internal and external quality assurance. The data obtained were analyzed for by standard statistical software and MS-Excel. Results obtained were arranged in tabular and graphical forms as required.

Results and Analysis:

Total 177 subjects have been included in this study matching inclusion and exclusion criteria. Among them 96 were male and 81 were female. The study population was first divided into two groups dependent upon their TSH level (TSH <4.2 uIU/ml and TSH ≥4.2 uIU/ml). Among them 120 persons have TSH level < 4.2 uIU/ml and 57 persons have TSH ≥4.2 uIU/ml but < 10 uIU/ml.

Using Chi-Square (χ^2) test for independence, it was found that there is no significant association between hypothyroidism and sex distribution (**p value-0.109)**. (vide table- 1)

Table no-1: Showing Chi Square (χ^2) test for males and females between subjects having TSH <4.2 uIU/ml & TSH ≥ 4.2 uIU/ml.

Parameters	**TSH<4.2uIU/ml (120) Mean ±SD**	**TSH≥4.2uIU/ml (57) Mean ± SD**	**t value**	**P value**
HBA1C	6.060±0.627	6.830±0.443	8.347	<0.001**
FBS	99.880±12.523	106.140±7.927	2.691	0.008**
PPBS	109.180±18.807	122.700±17.612	3.194	0.002**

	TSH <4.2 uIU/ml	**TSH ≥4.2 uIU/ml**
Male	**60**	**36**
Female	**60**	**21**
'χ^2, Value	**2.696**	
p value	**0.109 ***	

*significance is considered at the level p</= 0.05.

Using unpaired student's t- test it has been found that there is significant differences between the mean values of $HBA1_C$, fasting blood sugar (FBS) and post prandial blood sugar (PPBS) level in subjects having TSH <4.2 uIU/ml & TSH ≥ 4.2 uIU/ml. Difference is significant at the (p < 0.01) level (vide table-2)**.**

Table-2: Comparison of the mean values of HBA1C, FBS, PPBS between subjects having TSH <4.2 uIU/ml & TSH ≥ 4.2 uIU/ml (unpaired Student's t test).

** Difference is significant at the (p < 0.01) level.

By **Correlation test** it is found that there is significant positive concordance between serum TSH level and HBA1C. Correlation is significant at the level of p value<0.01(2 tailed). (vide table-3)

Though in unpaired Student's t test significant differences found between the mean values of FBS and PPBS among the groups of TSH <4.2 uIU/ml & TSH ≥ 4.2 ; no significant correlation found among Serum TSH and FBS , PPBS levels.

		HBA1C	**FBS**	**PPBS**
Serum TSH	**Correlation coefficient (r)**	0.491**	0.126	0.127
	Significance (p) 2- tailed	<0.001	0.083	0.093

**. Correlation is significant at the 0.01 level.

Table-3: Showing correlation between Serum TSH & HBA1C, FBS and PPBS

In further analysis we again subdivided the HBA1C data of the whole study population into two groups ie. **HBA1C ≤5.7 (normal range) & HBA1C >5.7 (impaired glucose tolerance)** and formulate a 2*2 cross tab to find out if there is any strong association between hypothyroidism (TSH ≥**4.2uIU/ml)** and increased HBA1C value (**HBA1C > 5.7)** in this normoglycemic study population. p value <0.001, so, the difference is insignificant at 95% confidence interval and it is clearly stated that there is strong association between hypothyroidism (TSH ≥**4.2uIU/ml)** and increased HBA1C value (**HBA1C > 5.7)** in this normoglycemic study population.

	HBA1C ≤5.7	**HBA1C >5.7**
TSH<4.2 uIU/ml	**90**	**30**
TSH≥4.2 uIU/ml	**14**	**43**
'χ^2' Value	**40.569**	
p value	**0.001 ****	

** Significance is considered at the level p < 0.01.

Table no-4: Showing Chi Square (χ^2) test HBA1C ≤ 5.7 (normal range) & HBA1C >5.7 (impaired glucose tolerance) between subjects having TSH <4.2 uIU/ml & TSH ≥ 4.2 uIU/ml.

Discussion:

A persistent high blood sugar level leads to non enzymatic addition of the excess sugar to some amino acid residues of protein which is termed as glycation of proteins. Human adult hemoglobin usually consists of HbA (97% of the total), HbA2 (2.5%) and Hb F (0.5%). Chromatographic analysis of HbA identifies several minor hemoglobin, namely HbA1a, HbA1b, and HbA1c, which are collectively known as HbA1 (fast hemoglobin) because they migrate rapidly) or glycated hemoglobin. HbA1c which contributes to almost 80% of the total HbA1, forms the major fraction of glycated hemoglobins **[1].**

Lowering A1C to below or around 7% has been shown to reduce microvascular and neuropathic complications of type 1 and type 2 diabetes. It may rise within 1 week after rise in blood glucose due to stopping therapy but may not fall for 2–4 weeks after blood glucose decrease when therapy is resumed.

HbA1c level decreased in Shortened RBC life span (e.g.,

hemolytic anemias, blood loss), following blood transfusions, in pregnancy and with ingestion of large amounts (>1 g/day) of vitamin C or vitamin E. Depending upon the types, Hemoglobinopathies affect the HbA1c value in various way, either decrease or increase it. HbA1c value increased in chronic renal failure with or without hemodialysis, iron deficiency anemia, splenectomy, increased serum triglycerides, Alcohol ingestion, Lead and opiate toxicity. **[2**]

Thyroid hormone (TH) plays a critical role in glucose metabolism. Its effects on the glucose metabolism is exhibited in several organs like the liver, gastrointestinal tract, pancreas, adipose tissue, skeletal muscles, and the central nervous system. Its stimulatory effects on gastrointestinal motility causes an enhanced absorption of glucose through intestine.**[9].** Its stimulatory effects on the glucose transporter GLUT 2 in the liver leads to an increased hepatic glucose uptake. Its effects on the liver also leads to increased hepatic gluconeogenesis and glycogenolysis. The increased hepatic gluconeogenesis is mostly due to TH induced lipolysis, and a consequent rise in the levels of free fatty acid that finally stimulates hepatic gluconeogenesis. Hyperthyroidism increases glucose transporter type 4 (GLUT4) gene expression and glucose uptake in skeletal muscles. **[10]**

From the findings of many previous studies it is evident that blood sugar level is affected in hypothyroidism and levels may increase. Indeed, it has been noted that patients with diabetes who also have hypothyroidism may have higher levels of Hemoglobin A1C (HBA1C). Quite a few articles in abroad and also in India hypothesize that hypothyroidism may falsely increase the level of the HBA1C and with successful thyroid hormone therapy the HBA1C results decreases without alteration in the anti-diabetic medication**. [8]**

In this context the present study emphasizes the effect of subclinical hypothyroidism on Blood HbA1c level in healthy urban adult population coming for annual health checkup in multi-disciplinary corporate hospitals of West Bengal as subclinical hypothyroidism often goes unnoticed but may have a noticeable effect on body's metabolic status.

The study was conducted among 177 healthy adult population attending NABL accredited corporate laboratories in West Bengal for one year duration. The study population has been divided into two groups (individuals having TSH < 4.2 uIU/ml and individuals having TSH (4.2 -10 uIU/ml) i.e. subclinical hypothyroidism. It was found that there is no significant association between hypothyroidism and sex distribution (**p value-0.109)** which is contrary to the fact that hypothyroidism is more common in female population found in previous other studies. **[11, 12]** The possible explanation to this finding could be that the present study population is representing only the affluent segment of the society having proper nutritious diet rich in iodine; i.e. the leading cause of hypothyroidism in India even in the World. **[13]** (Vide table-1).

Using unpaired student's t- test it has been found that there is significant differences between the mean values of HBA1C, FBS, PPBS level in subjects having TSH <4.2 uIU/ml & TSH ≥ 4.2 uIU/ml. (p value < 0.01). Whereas by Correlation test no significant concordance found between Serum TSH level and FBS, PPBS levels but a positive correlation found between TSH and HBA1c. (Vide table-2, 3) This finding is unison with the study findings of R. Bhattacharyya et al. It points towards a positive correlation between patient's thyroid status and HBA1c level irrespective of the influence of their glycemic control. A reduced rate of RBC production with a resulting reduced turn over rate has been observed in thyroid hormone deficiency due to a reduced stimulatory effect of thyroid hormones on these cells. These explanations corroborate well with the observations reported by Bhattacharya et al (2017) who reported increased reticulocyte counts following thyroid hormone replacement therapy. These increase in reticulocyte count is independent of the difference in HbA1c values in hypothyroid patients before and after achievement of stable euthyroid state as this difference was found to be statistically insignificant when adjusted for reticulocyte count. These findings suggested that decreased RBC turnover is responsible for altered HbA1c levels in hypothyroid patients. However, this finding is not found in the hyperthyroid patients who showed similar median HbA1c value in comparison to controls at the baseline. **[8]**

On further statistical analysis we need to find out whether this correlation is found by chance or there's a strong association existing between subclinical Hypothyroidism and elevated HBA1c level in normoglycemic individuals. So we performed Chi square test of association found that there was strong association found between subclinical hypothyroidism (TSH ≥4.2uIU/ml) and increased HBA1C value (HBA1C > 5.7) in this normoglycemic study population. (p value- <0.01) (Vide table-4).

With the help of this data and analyzing them, it can be said that hypothyroidism even in its subclinical stage elevates HBA1c value in a normoglycemic patients which might confuse us to comment on the glycemic control of the patient and initiate treatment. Iron deficiency is the most common nutritional deficiency nation-wide. It not only results in hypochromic microcytic anemia but also affects many other systems of the body. Many studies have reported that depletion of iron stores may alter the glycation rate of hemoglobin and elevate Hba1C concentrations, independent of glucose level. Hypochromic microcytic anemia results in decreased RBC turnover rate resulting in increased HBA1C value. **[14, 15]** On the other hand Subclinical hypothyroidism is also associated with iron-deficiency anemia. **[16]** Iron-deficiency anemia can affect thyroid metabolism through several mechanisms, such as decreased oxygen transport of anemia (similar to hypoxia), influence of iron deficiency on iodine deficiency disorders through alteration of central nervous system control of thyroid metabolism, and modification of nuclear T3 binding and altered thyroid peroxidase activity. Iron supplementation also has some beneficial effects on goiter with iron deficiency. **[17, 18]**

HBA1C level, Iron status and thyroid status of an individual might be interlinked among each other and affect each other in a complex way and iron status should be evaluated in a normoglycemic healthy individual with elevated HBA1c level and subclinical hypothyroidism.

Conclusion:

A strong positive correlation found between elevated HBA1c value and subclinical hypothyroidism in healthy adult individuals though they are all found to be normoglycemic and both the FBS and PPBS levels bear no significant concordance with serum TSH value within the Biological Reference range. This finding points towards an independent association of subclinical hypothyroidism and baseline elevation of HBA1c value and it should be taken into consideration by the doctors when they interpret the glycemic status of a subclinical hypothyroid patient who is otherwise healthy.

In this study Iron status of the study population was not evaluated; it should be assessed in future study and corrected accordingly as Iron deficiency might be the missing link which connect subclinical hypothyroidism with elevated HBA1c.

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