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Research Article

IMPACT OF HYPOTHYROIDISM ON GLYCATED HAEMOGLOBIN IN NON- DIABETIC PATIENTS

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A B S T R A C T

Background: Hypothyroidism is a common but underdiagnosed disorder because of its nonspecific clinical presentation. The prevalence of hypothyroidism in developed countries is about 4%–5%, whereas, in India, it is reported to be around 10.95%. Glycatedhaemoglobin is an important tool for monitoring diabetes. There are several factors other than glycemic status which can influence HbA1c level. Altered RBC turnover is one of them. Hypothyroidism is known to cause decreased RBC turnover. A decrease in RBC turnover leads to increase in HbA1c levels. Because of this we excogitate that HbA1c level does not accurately reflect glycemia in hypothyroidism. Hypothyroidism is widely prevalent worldwide. For this reason the present study was aimed to know the effect of hypothyroidism on HbA1c level in non-diabetic patients.

Methods: This cross sectional, observational study was conducted at the Department of Medicine, Narayan Medical College and Hospital, Sasaram over a period of two years from 1st September, 2018 to 31st August, 2020. Total 100 patients with hypothyroidism and 100 control subjects were considered for final analysis.

Results: Our study revealed that serum TSH, fT4, HbA1C, and FBG were found significantly different between the hypothyroid and control groups (p values being <0.0001, <0.0001, <0.0001 and 0.0003 respectively). The difference of mean HbA1c with both groups was statistically significant (p<0.0001). HbA1C was seen higher in hypothyroid patients. Mean FPG in cases was significantly higher as compared to the control group (p=0.0003). In our study, the mean baseline HbA1c level in hypothyroid patients was found to be significantly higher relative to the matched control population (p<0.0001).

Conclusion: Mean glycatedhaemoglobin level was found to be significantly higher in hypothyroid patients than control subjects. Thus we conclude that HbA1c may not be a reliable marker of glycemic status in presence of untreated hypothyroid subjects.

INTRODUCTION

Hypothyroidism is a common but under diagnosed disorder because of its nonspecific clinical presentation.[1,2] Primary hypothyroidism is an abnormality in the thyroid gland itself – it can be subclinical or overt. Secondary or central hypothyroidism is caused by the disorders affecting pituitary gland or hypothalamus. Untreated hypothyroidism may lead to serious cardiovascular and neurological complications.[3, 4]The prevalence of hypothyroidism in developed countries is about4%–5%, whereas in India, it is reported to be around 10.95%.[5,6] As per the epidemiology study conducted by Unnikrishnan *et al*. in eight cities of India, the prevalence of subclinical hypothyroidism, a mild thyroid failure, was found to be 8.02%.[7] The prevalence of subclinical hypothyroidism ranges between 4% and 15% worldwide and is reported to be 11.4% for women and 6.2% for men in India.[7,8]

Diabetes mellitus is a disease known to the mankind since ancient times. It is the most common non-communicable disease globally. The estimated number of adults with diabetes in 2019 was more than 463 million [9], 80% of these living in developing countries, the largest number in the Indian subcontinent and in China. Worldwide 4.8 million people died due to diabetes in the year 2019. The number of people with diabetes are increasing in every country. Half of the people with diabetes are undiagnosed. Currently, India has approximately 77 million diabetics in the age group of 20-79 years.[10]

Glycated haemoglobin (HbA1c) is an important tool for monitoring diabetes. Major outcome studies have

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demonstrated the correlation of HbA1c with diabetes complication.[11,12,13,14,15] Major guidelines endorse the use of HbA1c for monitoring of diabetes.[16,17] The American Diabetes Association (ADA) also proposed HbA1c ≥ 6.5% for the diagnosis of diabetes and 5.7– 6.4% for the highest risk to progress to diabetes.[18]There are several factors other than glycemic status which can influence HbA1c level. Altered RBC turnover is one of them. Hypothyroidism is known to cause decreased RBC turnover. Decrease in RBC turnover leads to increase in HbA1c levels. Because of this we excogitate that HbA1c level does not accurately reflect glycemia in hypothyroidism. Hypothyroidism is widely prevalent worldwide. For this reason it is very important to know the influence of hypothyroidism on HbA1c. Patients with hypothyroidism show a greater propensity for comorbidities and complications as compared to the general population. Among the Indian population, patients with asthma, obesity, diabetes, dyslipidemia, and hypertension had the higher prevalence of hypothyroidism.[2] Patients with Sub clinical hypothyroidism have a high rate of progression to hypothyroidism, 4.2% if there is presence of thyroperoxidase antibodies, and 2.7% per year if there is absence of thyroperoxidase antibodies. The rate of progression is also higher in the case of females and the elderly.[19, 20]

Because of multiple manifestations it is very challenging and of utmost importance to do early accurate diagnosis and treatment of hypothyroidism in clinical practice; this challenge is even more crucial in subclinical hypothyroidism. Preferred test for diagnosing hypothyroidism is thyroid stimulating hormone (TSH), yet in some situations it can be misleading. The guidelines suggest that when the hypothyroidism state (such as subclinical, obvious or secondary hypothyroidism) is correctly classified, it is necessary to estimate T4 and TSH.[21]

Further, based on this classification, a tailored treatment approach for each patient can be adapted.[21] Both type 2 diabetes and hypothyroidism are highly prevalent disorders in the community. Appropriate treatment of hypothyroidism requires accurate diagnosis.

AIMS AND OBJECTIVES

To know the effect of hypothyroidism on Glycated haemoglobin (HbA1c) level in non-diabetic patients.

MATERIAL AND METHODS

This cross sectional, observational study was conducted at the Department of Medicine, Narayan Medical College and Hospital, Sasaram over a period of two years from 1st September, 2018 to 31st August, 2020.It was approved by the Institutional Ethics Committee. After screening around 1500 Patients attending Medicine OPD of Narayan medical college and hospital with hypothyroidism, and then after applying exclusion criteria, 100 patients with hypothyroidism and 100 control subjects were considered for final analysis. Age and gender of the patients in all the groups were well matched with controls (Their relatives and hospital staffs).Patients of above 18 years of age, recently diagnosed hypothyroidism, those who had no thyroid surgery or trauma to the neck, no history of previous exposure to radiation to the neck, those consenting to the study were included in the present study.

Whereas those excluded were patients with diabetes, haemoglobin <10gm/dl, renal failure (creatinine clearance < 60ml/min), hepatic dysfunction (increased bilirubin, reduced albumin [<3.5mg/dl],SGOT and SGPT 3 times upper limit of normal), acute or subacute thyroiditis, known haemoglobinopathies, patients who are on aspirin or vitamin C (>500mg/day), pregnancy and non-consenting patients Initial blood samples were drawn after an overnight fast of 8 hours, which were meant for testing of plasma glucose, TSH, Free T4, HbA1c, urea, creatinine, Liver function test. Then the subjects were given 75 gm of glucosein 300 ml of water and asked to drink it within 5 minutes. Blood glucose was measured after 2 hours.

Hypothyroidism was defined as free T4 below the normal lower limit and more than 15 micro IU / ml of TSH. Diabetes was diagnosed when fasting plasma glucose (FPG) was ≥126 mg/dl and/or post 75 gm glucose plasma glucose was ≥ 200 mg/dl.

Impaired fasting glucose was diagnosed when FPG was between 100 mg/dl to 125 mg/dl. Impaired glucose tolerance was defined as post 75 gmglu cose plasma glucose between 140 and 199 mg/dl.

HbA1c was measured by using ion-exchange high-performance liquid chromatography (HPLC) method using Bio-Rad D 10 HbA1c program which is certified by NGSP as having reported traceability to the reference system for Diabetes Management and Complications Trial (DCCT).

TSH was measured by Immulite 1000 third generation solid-phase, two-site, chemiluminescent immunometric assay. Normal reference range of TSH using this assay is 0.25 to 5.0 microIU / ml.

Free T4 is measured by competitive analog assay using same analyzer. Normal reference range was between 0.8 and1.8 ng/dl.

For statistical analysis data were entered into a Microsoft excel spread sheet and then analyzed by SPSS (version 27.0; SPSS Inc., Chicago, IL, USA) and GraphPad Prism version 5. Data had been summarized as mean and standard deviation for numerical variables and count and percentages for categorical variables. Two-sample t-tests for a difference in mean involved independent samples or unpaired samples. Paired t-tests were a form of blocking and had greater power than unpaired tests. The chi squaretest ($\chi 2$ test) was any statistical hypothesis test in which, if the null hypothesis is valid, the sampling distribution of the test statistics is a chisquared distribution. The 'chi-square test' is also used as a short for the Pearson chi-square test without other credentials. Unpaired proportions were compared by Chi-square test or Fischer's exact test, as appropriate. Once a t value is determined, a p-value can be found using a table of values from Student's t-distribution. If the calculated p-value is below the threshold chosen for statistical significance (usually the 0.10, the 0.05, or 0.01 level), then the null hypothesis is rejected in favour of the alternative hypothesis. p-value ≤ 0.05 was considered for statistically significant.

RESULTS AND ANALYSIS

The present study was aimed to know the effect of hypothyroidism on HbA1c level in non-diabetic patients. Our study revealed that serum TSH, fT4, HbA1C and FBG were found significantly different between the hypothyroid and control groups (p values being <0.0001,<0.0001,<0.0001 and

0.0003 respectively). There was no statistically significant difference in age distribution between the groups. [Numerical variables between groups compared by t-test; (p=0.0625)]. Thus age was matched in two groups. In our study, 143 (71.5%) patients were Female and 57 (28.5%) patient were male. Difference of mean BMI with both Group was statistically significant (p<0.0001). Mean BMI was seen higher in hypothyroid patients as compared to control subjects. Mean TSH was higher in hypothyroid cases as compared to control subjects. Mean FT4 was significantly lower in hypothyroid cases as compared to control subjects. (p<0.0001).Difference of mean HbA1c with both Group was statistically significant (p<0.0001). HbA1C was seen higher in hypothyroid patients. Mean FPG in cases was significantly higher as compare to control group (p=0.0003).The difference of mean 2Hr PPPG with both Group was not statistically significant (p=0.0813). In our study, the mean baseline HbA1c level in hypothyroid patients was found to be significantly higher relative to the matched control population (p<0.0001).

Table 1 Comparison of baseline characters between hypothyroid and control groups

	CASE (HYPOTHYROID) n=100	CONTROLS n=100	P VALUE
AGE	35.36± 9.3652	37.97±10.3137	0.0625
GENDER (M/F)	29/71	28/72	
TSH (µIU/ml)	30.6480± 14.4139	2.5719± 1.0051	<0.0001
FT4 (ng/dl)	0.5131± 0.1851	1.2014± 0.1934	<0.0001
HbA1C	5.8490± 0.4286	5.1470± 0.2311	<0.0001
HEIGHT (meter)	1.5682± 0.1033	1.5617± 0.0600	0.5870
WEIGHT (kg)	62.2682± 11.7011	57.6700± 6.6112	0.0008
BMI (Kg/m²)	25.2114±3.6260	23.5942± 1.8365	<0.0001
FPG (mg/dl)	89.7900± 5.8694	8888886.8000±5.6854	000000.0003
2HrPPG (mg/dl)	112.6100± 13.5995	109.480± 11.5806	0.0813

Table 1 showing Comparison of baseline characters between hypothyroid and control groups. All values (except gender) have been elaborated as mean ±standard deviation; P values were calculated by unpaired t-test; P<0.05 was considered statistically significant. Serum TSH, fT4, HbA1C and FBG were found significantly different between the hypothyroid and control groups (p values being <0.0001, <0.0001,<0.0001 and 0.0003 respectively)

Table 2 Association between HbA1c distribution among cases and control

	GROUP		
HbA1c GR	Case (Hypothyroid)	Control	TOTAL
5.0-5.5	3 (11.5)	23 (88.5)	26
5.6-6.0	20 (21.3)	74 (78.7)	94
6.1-6.5	52 (96.3)	2 (3.7)	54
6.6-7.0	15 (93.8)	1 (6.3)	16
7.1-7.5	10 (100)	0	10

Chi-square value: 114.9522; p-value: <0.0001

Above table depicts HbA1c distribution among cases and control was found to be statistically significant (p<0.0001). HbA1C was seen higher in hypothyroid patients as compared to control subjects.

Table 3 Association between TSH and HbA1c among hypothyroid group

	HBA1C GR					
TSH GR	5.0-5.5	5.6-6.0	6.1-6.5	6.6-7.0	7.1-7.5	TOTAL
5-14	2 (66.7)	0	1 (33.3)	0	0	3
15-25	1 (2.0)	19 (38.0)	23 (46.0)	2 (4.0)	5 (10.0)	50
26-35	0	0	15 (78.9)	2 (10.5)	2 (10.5)	19
36-45	0	1 (6.3)	7 (43.8)	6 (37.5)	2 (12.5)	16
46-55	0	0	1 (16.7)	4 (66.7)	1 (16.7)	6
56-65	0	0	3 (100)	0	0	3
66-75	0	0	2 (66.7)	1 (33.3)	0	3

Chi-square value: 88.0361; p-value: <0.0001

Above table depicts association between distribution of TSH ranges (ranging from 5-14, 15-25, 26-35, 36-45, 46-55, 56-65, 66-75) and HbA1c (ranging from 5.0-5.5, 5.6-6.0, 6.1-6.5, 6.6-7.0, 7.1-7.5) among hypothyroid group and was found to be statistically significant (p<0.0001).

Table 4 Association between FT4 group and HbA1c group

	HBA1C GR					
FT4 GR	5.0-5.5	5.6-6.0	6.1-6.5	6.6-7.0	7.1-7.5	TOTAL
0.00-0.10	0	2	3	1	0	6
0.21-0.30	0	2	4	2	0	8
0.31-0.40	0	7	19	3	2	31
0.40-0.50	0	1	1	2	2	6
0.51-0.60	0	0	6	2	2	10
0.61-0.70	0	0	9	3	2	14
0.71-0.80	3	8	10	2	2	25

Chi-square value: 29.8150**; p-value:** 0.1910

Above table depicts association of FT4 (ranging from 0.00-0.10, 0.31-0.40,0.40-0.50, 0.51-0.60, 0.61-0.70, 0.71-0.80) and HbA1c (ranging from 5.0-5.5,5.6-6.0, 6.1-6.5, 6.6-7.0, 7.1-7.5) which was not statistically significant(p=0.1910).

DISCUSSION

Out of total 200 study subjects in our study, 143 (71.5%) were males and 57 (28.5%) were females. In a study conducted in Sri Venkateswara Institute of Medical Sciences, Tirupati, Andhra Pradesh, India by Anantarapu S *et al*.[22] there were 35 female and 3 male out of total 38 study subjects. In the study conducted by Islam S *et al*.[23] in Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM), out of 50 controls 40 were male and 10 were female. Out of 472 study subjects 198 (41.9%) were male and 274 (58.1%) were female by OgbonnaSU *et al*.[24] in Department of Internal Medicine, Federal Medical Center, Umuahia, Abia State, Nigeria.

In the present study mean age was 35.36 years. Mean age was 45.50 by Islam S *et al*.[23] which is higher than that in our study. Anantarapu S *et al*.[22] reported mean age as 37.8 years which is higher than that in present study. Mean age was 47 by Pasupathi P *et al*.[25] which was higher than that in present study.

In our study, the mean BMI (mean± s.d.) of patients (cases) was 25.2114± 3.6260 while for controls, the mean BMI (mean± s.d.) of patients was 23.5942± 1.8365. Mean BMI was seen higher in hypothyroid patients as compared to control subjects. This difference was unlikely to influence the results because BMI per se is not known to affect HbA1c level. Islam S *et al* [23]reported mean BMI (mean± s.d.) 21.90±2.73 in case of control and mean BMI (Mean± S.D.) was 21.57±5.08 in

cases. Anantarapu S *et al* [22] reported BMI (mean± s.d.) 25.0 ± 4.2 kg/m^2 in study subjects. BMI (mean± s.d.) in nondiabetic subjects was 32 ± 5.9 as reported by Pasupathi P *et al*.[25]

In our study subjects, the mean TSH (mean± s.d.) of patients (cases) was 30.6480± 14.4139 while for Control, the mean TSH (mean± s.d.) of patients was 2.5719± 1.0051. The association between distribution of TSH ranges and hypothyroid group and was found to be statistically significant whereas study done by Christy AL *et al*.[26] found that there was no significant correlation found between HbA1C and TSH. In the study by Komarica EB *et al*.[27] conducted on 50 subjects from Clinic for Endocrinology, Diabetes and Metabolic Diseases, Clinical Centre of Sarajevo University, Bosnia and Herzegovina, TSH and HbA1c were significantly correlated in hypothyroid patients (r =0.46 p <0.05) which is similar to our study. In the study by Ali AY *et al*.[28] conducted in Iraq on 95 Hypothyroid patients and 40 controls, revealed a significant relation with HbA1Cin both controlled and uncontrolled hypothyroid groups against the control group($P < 0.05$), but there is no significant difference between the controlled and uncontrolled hypothyroid groups ($P = 0.08$). Among the uncontrolled hypothyroid and diabetic, hypothyroid groups The TSH significantly positive relation with HbA1c was found. (r = 0.401 and 0.58, respectively).

In the cross-sectional study by Aljabri KS *et al*.[29] conducted in the Diabetes centre at King Fahad Armed Forces Hospital, Jeddah, Saudi Arabia from January 2018 to December 2018, TSH, FT4 and HbA1c were measured. Patients with primary hypothyroid had statistically significant higher HbA1c compared to subclinical hypothyroidism, hyperthyroidism and subclinical hyperthyroidism (P=0.004). In addition, there was a statistically significant difference between HbA1c below and above 7% in correlation to thyroid dysfunctions (P=0.02). In a clinical trial by Kim M K *et al*.[30], A1C levels were measured in 30 non-diabetic patients with overt hypothyroidism before and after thyroid hormone replacement. A1C levels were significantly higher in patients with overt hypothyroidism compared with control subjects. In addition, A1C levels decreased after thyroid hormone replacement in patients with overt hypothyroidism. Here however it may be noted that hypothyroidism and diabetes are the most common endocrine disorders found in Indian population. Both the diseases co-exist. The prevalence of thyroid disease together with diabetes mellitus is substantial. Studies done in hypothyroid patients showed elevated HbA1C not only in the presence of diabetes but also in non-diabetic subjects. Hence the role of HbA1C as a marker of diabetes was questioned in such conditions.[26]

In our study mean fasting plasma glucose values varied between hypothyroid and control group patients. The difference of mean FPG between two groups was statistically significant. According to the study conducted by Islam S *et al.* [23] in the Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders, the mean FPG for both groups were statistically significant which was similar to our study. Haemoglobin levels were not significantly different.

In this study done by us the median baseline HbA1c level in hypothyroid patients was found to be significantly higher relative to the matched control group.[Median +/- inter-quartile range 5.8+/- 0.08 [hypothyroid] vs 5.1 +/- 0.03[controls]; p< .001]despite having similar fasting and post glucose values. This finding was in accord with the study by Kim M K *et al*.[30] In this study they found the mean A1c level to be significantly higher in patients with hypothyroidism compared with control subjects[mean (+/-SD) 5.8490 ±(0.4286) vs 5.1470± (0.2311); p< .0001].

They suggested that this influence may have been due to low RBC turnover in hypothyroid patients. In that study, they also showed that mean A1c level was decreased from baseline by thyroid hormone replacement. In same study they found that there was concomitant decrease in reticulocyte count with levothyroxine replacement, which indirectly supports the presence of low RBC turnover in hypothyroid state.

In our research, the HbA1c disparity between control and hypothyroid patients was greater than that of Kim M K *et al*.[30] This may be due to their patients' shorter period of hypothyroidism (4 weeks after thyroidectomy) than ours (most of whom may have a longer natural history of illness).As we know that haemoglobin is a decisive of HbA1c value, we adjusted the results forhaemoglobin, but even that did not change the outcome. A crucial aspect of our study was that we measured the effect of hypothyroidism on HbA1c level in same ethnic group. And to the best of our knowledge, same kindred study is not available in world literature till date.

A drawback of our research was that we could not explicitly calculate RBC turnover with investigations such as the RBC assay tagged with Chromium 51.

In diabetic patients, glycated haemoglobin is an important method for controlling glycemic status. The ADA has recently proposed its use in the diagnosis of diabetes and prediabetes[5]. In the previously mentioned study done by Kim M K *et al.*, they found that 33% of the patient had$HbA1C \geq 5.7\%$ during hypothyroidism, but after thyroid hormone replacement only 13% had $HbA1C \geq 5.7\%$.We suggest to exercise of caution while elucidating HbA1c data in patients with hypothyroidism.

CONCLUSION

Mean glycated haemoglobin level was found to be significantly higher in hypothyroid patients than control subjects. Thus we conclude that HbA1 cmay not be a reliable marker of glycemic status in presence of untreated hypothyroid subjects.

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