



Original Research Article

A Study of Glycated Hemoglobin (HbA1c) in Non Diabetic Hypothyroid Population

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ABSTRACT

Background: Protein glycation is a spontaneous reaction that is believed to play a key role in the pathogenesis of many clinical disorders. The glycation of proteins is enhanced by elevated glucose concentrations. The major form of protein glycation with a clinical consideration is glycated haemoglobin (HbA1c). The HbA1c fraction is abnormally elevated in chronic hyperglycaemic diabetic patients and it correlates positively with the glycaemic control. However, increased glycated haemoglobin levels have been documented in iron deficiency anaemic patients without any history of diabetes.

Aim: Studies have shown elevated HbA1C in non-diabetic hypothyroid patients. Hypothyroid patients often show anaemia as an associated feature which is an another condition showing falsely elevated A1C. Hence this study is aimed to investigate whether elevated A1C in hypothyroidism can be attributed to anaemia.

Materials and Methods: HbA1C levels of 140 non-diabetic hypothyroid patients (35 microcytic hypochromic anaemia, 35 normocytic normochromic anaemia and 70 non anemic patients) with 140 age, sex, plasma glucose levels and anaemia status matched controls were assessed. Anaemia status was determined by Haemoglobin, red cell indices and peripheral smear Glycemic status was determined by fasting Plasma glucose.

Results: HbA1C levels in hypothyroid patients with hypochromic microcytic anaemia and normocytic normochromic anaemia were $6.88 \pm 0.57\%$ & $6.22 \pm 0.41\%$ against $6.42 \pm 0.98\%$ & $6.0 \pm 0.39\%$ of euthyroid anaemia matched controls respectively. While hypothyroid non anemic patients showed A1C levels of $5.8 \pm 0.29\%$ against $5.45 \pm 0.55\%$ of euthyroid non anemic controls.

Discussion and Conclusion: Non-diabetic hypothyroid individuals with anaemia shows elevate A1C levels in prediabetes range. Hence care should be exercised while using HbA1C as a diagnostic tool for diabetes in such patients.

Keywords: Hypothyroidism, Diabetes, HbA1C, Red cell survival time, Anaemia

INTRODUCTION

Glycated haemoglobin is produced by a ketoamine reaction between glucose

and the N-terminal valine of both β -chains of the haemoglobin molecule. The major form of glycated haemoglobin is

haemoglobin A1c (HbA1c). The term HbA1C refers to glycated haemoglobin. It develops when haemoglobin, a protein within red blood cells that carries oxygen throughout your body, joins with glucose in the blood, becoming 'glycated'. By measuring glycated haemoglobin (HbA1c), clinicians are able to get an overall picture of what our average blood sugar levels have been over a period of weeks/months. For people with diabetes this is important as the higher the HbA1c, the greater the risk of developing diabetes-related complications. The HbA1c fraction is abnormally elevated in patients with chronic hyperglycaemic diabetes mellitus and it correlates positively with the metabolic control. [1] According to the American Diabetes Association (ADA) guidelines, the value of HbA1c should be kept below 7% in all the diabetics HbA1C is widely used for the assessment of glycemic status of the diabetic patients and the American Diabetes Association (ADA) recommended its use for diagnosing diabetes. Studies have shown variation in HbA1C levels in different conditions like Haemoglobinopathies, chronic kidney diseases, pregnancy even in the absence of diabetes mellitus. [2] Conditions that can affect erythrocyte turnover or survival may falsely elevate or lower the A1C levels. [3-6] Recent studies have shown its spurious elevation in hypothyroidism in the absence of diabetes. [7]

Decreased production of thyroid hormone is the key feature of hypothyroidism. [8] It is often complicated by conditions such as dilutional hyponatremia, anaemia and hyperlipidemia. [9] Anaemia in hypothyroidism can be normochromic normocytic, microcytic hypochromic and macrocytic. The most frequent type of anaemia encountered in hypothyroidism is normochromic normocytic. The etiology of anaemia in

hypothyroidism can be related to the nutritional iron deficiency or to the endocrine disorder itself where the lowered thyroid hormone levels repress the bone marrow often resulting in decreased erythrocyte production which may affect the life span of erythrocytes. Altered erythrocyte life span may be partially responsible for spurious elevation in HbA1C levels. [10-12] This study was aimed at determining whether spuriously elevated A1C levels in non-diabetic hypothyroid individuals can be attributed to anaemia.

MATERIALS AND METHODS

The data of the patients attending Geetanjali medical college and hospital from January 2014 to November 2014 was collected. We collected the data of 613 subjects aged 20 years and above who had HbA1C, peripheral smear, Haemoglobin, mean corpuscular Haemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular Haemoglobin concentration (MCHC), serum TSH and plasma glucose levels estimated. A total of 195 were found to be non-diabetic who were diagnosed as having Hypothyroidism based on their TSH levels. It was made sure that the patients were non-diabetic (Fasting Plasma Glucose <100 mg/dl) and HbA1C estimation was carried out as a part of our laboratory package in an attempt to investigate the endocrinological disorders in detail. Of these 70 non anemic subjects and 125 anemic subjects were selected. Out of the anemic cases 35 subjects with hypochromic microcytic anaemia and 35 subjects with normocytic normochromic anaemia were selected and 55 were excluded based on exclusion criteria. Similarly 35 microcytic hypochromic anemic, 35 normocytic normochromic anemic and 70 non anemic euthyroid controls matched for sex and plasma glucose levels were included in our study. Microcytic hypochromic anaemia was

defined as microcytic hypochromic picture on peripheral smear, low Hb levels (<12g% in males, <11g% in female), predominantly microcytic indices (MCV<76 fL) and hypochromic indices (MCH<27 pg/cell). Normocytic normochromic anaemia was defined as low Hb levels, normocytic normochromic red cell indices and peripheral smear picture.

Exclusion criteria: Pregnant patients, patients having haemolytic anaemia, other Haemoglobinopathies, anaemia due to other chronic illnesses and abnormal renal function test (Serum Urea, Creatinine and eGFR) were excluded from our study.

Measurement of HbA1C was done by turbidometric method in Cobas C311 fully automated analyser. Haemoglobin and red cell indices were estimated using Sysmax kx 21i automated counter. Serum TSH (Elecsys TSH immunoassay kit), were estimated by Electrochemiluminescence method using Roche Hitachi cobas e411 analyser (Roche Diagnostics GmbH, Mannheim). Plasma glucose estimation was done by glucose oxidase peroxidase method using Roche Cobas C 311 analyser.

Data was analysed by using online student t-test calculator. P-value less than 0.01 was considered as a significant.

RESULTS

Subject characteristics are as shown in [Table/Fig-1]. Females showed more predisposition towards being hypothyroid and anemic than males.

HbA1C was around 6.51 ± 0.49 in anemic cases as compared to 5.8 ± 0.29 of non anemic cases. HbA1C levels in different types of anaemia are as shown in [Table/Fig-2]. According to the table HbA1C levels in normocytic normochromic anaemia were lower (6.22 ± 0.41) as compared to the microcytic hypochromic anaemia (6.8 ± 0.57) and differences between their respective control groups were statistically significant.

Difference in level of HbA1c between anemic and non anemic group of both hypothyroid and euthyroid patients were calculated by using P-value. There was significant difference found between anemic and non anemic group of both hypothyroid and euthyroid patients.

Table/Fig 1: Subjects characteristic

Type	Microcytic hypochromic	Normocytic normochromic	Non anemic
Hemoglobin(gm /dl)	9.26 ± 2.1	9.8±0.92	13.1±1.21
Feritin(ng/ml)	8.32±5.1	178.3±35.12	215.9±19.2
MCV(fl)	55.56±12	65±8.9	78.9±5.1
MCH(pg/cell)	16.2±5.7	32.9±6.1	54.5±2.4
TSH (µIU/ml)	33.3±11.2	29.9±10.1	36.45±15.1
Plasma Glucose(mg/dl)	92±8.1	88±6.5	87.5±7.1
Female:male ratio	30:5	28:7	55:15

Table/Fig 2: Distribution of HbA1C (%) in hypothyroid and euthyroid cases

Group	Hypothyroid [HbA1C(%)]			Euthyroid [HbA1C(%)]		
	Total(70)	Male(35)	Female(35)	Total(70)	Male(35)	Female(35)
Microcytic Hypochromic	6.8±0.57	6.61±0.33	6.92±0.81	6.42±0.98	6.46±0.77	6.39±1.2
Normocytic Normochromic	6.22±0.41	5.87±0.31	6.57±0.51	6.0±0.39	6.12±0.23	5.9±0.56
Non anemic	5.8±0.29	5.82±0.23	5.71±0.35	5.45±0.55	5.39±0.68	5.51±0.43

Table/Fig 3: Comparison of HbA1C (%) in hypothyroid anemic VS Non anemic

Group	comparison	Subject(N)	Result [HbA1C(%)]	P-value
Microcytic Hypochromic VS Non anemic	Microcytic Hypochromic	70	6.8	<0.01
	Non anemic	70	5.8	
Normocytic Hypochromic VS Non anemic	Normocytic Hypochromic	70	6.22	<0.01
	Non anemic	70	5.8	

Table/Fig 4: Comparison of HbA1C (%) in Euthyroid anemic VS Non anemic

Group	comparison	Subject(N)	Result [HbA1C(%)]	P-value
Microcytic Hypochromic VS Non anemic	Microcytic Hypochromic	70	6.42	<0.01
	Non anemic	70	5.39	
Normocytic Hypochromic VS Non anemic	Normocytic Hypochromic	70	6.12	<0.01
	Non anemic	70	5.39	

DISCUSSION

Our results showed elevation of HbA1C in microcytic hypochromic and normocytic normochromic anaemia patients. Elevation was more in microcytic hypochromic anaemia. Etiology of microcytic anaemia may be iron deficiency, early stages of anaemia due to endocrine diseases, thalassemia and anaemia of chronic diseases. Because we had excluded anaemia due to other Haemoglobinopathies and chronic diseases, based on the ferritin levels we could ascertain that those with iron deficiency anaemia had higher levels of HbA1C when compared to anaemia of endocrine diseases. There was no significant correlation found between HbA1C and TSH & Plasma glucose levels and TSH.

Hypothyroidism and diabetes are the most common endocrine disorders found in Indian population. Both the diseases co-exist. The prevalence of thyroid disease in patients with diabetes mellitus is approximately 10-15%. [13,14] 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 especially when American Diabetes Association has endorsed it as diagnostic criteria for diabetes mellitus. When studies were done to evaluate the cause of these elevated HbA1C, Kim et al., [7] found in their study that they were attributed to anaemia associated with it. A number of studies have shown that iron deficiency anaemia is mostly associated with elevated HbA1C levels. [2] Other conditions where iron deficiency anaemia

play a pivotal role in elevating HbA1C levels are chronic kidney diseases and pregnancy. [15,16] Hypothyroidism is mainly complicated by normocytic normochromic anaemia which may be early iron deficiency anaemia due to nutritional deficiency or it may be secondary to hypothyroidism itself. [17]

Since we could not obtain the post therapy data of the patients, our study could not explain the effect of thyroid hormone therapy on HbA1C levels.

There was no significant correlation found between HbA1c and erythrocyte indices in case of normocytic normochromic anaemia. Previous studies have found association between red cell survival and elevated A1c levels. [18,19] Hence, red cell morphology alone may not completely explain the elevated A1C levels; rather red cell survival time gives a better explanation of it. We did not measure the erythrocyte lifespan, which was one of the limitations of our study.

In anaemic patients, the concentration of glycated haemoglobin has been reported to be increased despite the shortened life span of the erythrocytes. Several mechanisms have been advocated for this increase in the levels of glycated haemoglobin in anaemic patients. It has been proposed that in iron deficiency, the quaternary structure of the haemoglobin molecule may be altered, and that the glycation of the β -globin chains occurs more readily. According to some investigators, the increase in the glycated haemoglobin levels in non-diabetic anaemic patients has been mainly attributed to the decrease in the

haemoglobin levels in these patients. But studies which have investigated the glycation levels of other proteins have not been carried out.

This study has got a significant relevance because iron deficiency anaemia is very highly prevalent in a tropical country like India. IDA, being a common variable, influences the HbA1c levels when they are estimated by the most commonly employed methods like immunoturbidimetry and so, the IDA must be corrected before making any diagnostic or therapeutic decision based on the HbA1c levels. HbA1c is commonly used to assess the long-term blood glucose control in the patients with diabetes mellitus, because the HbA1c value has been shown to predict the risk for the development of many of the chronic complications in diabetes.

There was no significant correlation found between plasma glucose levels and TSH. Patients suffering from diabetes showed association in previous studies. Our study subjects were non-diabetic; hence presence of diabetes could be criteria for plasma glucose levels to be associated with TSH.

All the cases are non-diabetic and most of the factors which can interfere with glycation of Haemoglobin like chronic kidney diseases, Haemoglobinopathies, pregnancy and Haemolytic anaemia were excluded, which strengthen our study.

The limitations however include inability to measure the RBC life span & also the lack of knowledge of treatment. The findings of the study also need to be validated in larger cohort.

CONCLUSION

Elevated HbA1C in hypothyroidism can be attributed to anaemia. Hence it is recommended to consider it before diagnosing diabetes solely on the basis of HbA1C. Our results showed that iron

deficiency was associated with higher proportions of HbA1c, which could cause problems in the diagnosis of uncontrolled diabetes mellitus in iron-deficient patients. The iron status must be considered during the interpretation of the HbA1c concentrations in Diabetes mellitus. The iron replacement therapy is thus especially important in diabetic patients with iron deficiency, as it would also increase the reliability of the HbA1c determinations. Still a RBC life span study is needed to prove the exact pathology behind this elevation.

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