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Original Article

Biochemistry Section

Effect of Iron Deficiency on Glycation of Haemoglobin in Nondiabetics

BALASUBRAMANIAN SHANTHI, CARNAGARIN REVATHY, ARCOT JAGDEESHWARAN MANJULA DEVI, SUBHASHREE

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.

Aims and Objective: The aim of this study was to determine the effect of IDA on the HbA1c levels in nondiabetic patients, so as to consider IDA as an important factor which influenced the HbA1c levels, while monitoring the glycaemic status of diabetics.

Methodology: Fifty non-diabetic, anaemic patients and 50 age-matched healthy subjects were enrolled in this study. The patients who had glucose tolerance abnormalities (impaired glucose tolerance or diabetes mellitus), haemoglobinopathies, haemolytic anaemia, infestation, chronic alcohol ingestion and chronic renal failure were excluded from the study. Haematologic investigations were done and the fasting and postprandial glucose and HbA1c levels were measured in all the subjects.

Results: The mean HbA1c (7.6 ± 0.5%) level in the patients with IDA was higher than that in the control group (5.5% ± 0.8) ($p < 0.001$). There were no differences in the levels of fasting and postprandial glucose between the IDA and the control groups ($p > 0.05$). The haemoglobin, serum ferritin, fasting and postprandial glucose, and the HbA1c levels were normal in the control group ($p > 0.05$).

Conclusion: HbA1c is not affected by the blood sugar levels alone, and there are various confounding factors when HbA1c is measured, especially that of iron deficiency, which is the commonest of the deficiency diseases worldwide. It is hence prudent to rule out IDA before making a therapeutic decision, based on the HbA1c levels.

Key Words: Iron deficiency, HbA1c, Protein glycation

INTRODUCTION

Glycated haemoglobin is produced by a ketoamine reaction between glucose and the N-terminal valine of both ß-chains of the haemoglobin molecule. The major form of glycated haemoglobin is haemoglobin A1c (HbA1c) [1,2]. The measurement of glycated haemoglobin is the standard method for assessing the long-term glycaemic control. When plasma glucose is consistently elevated, the nonenzymatic glycation of haemoglobin increases; this alteration reflects the glycaemic history over the previous 2–3 months, since erythrocytes have an average lifespan of 120 days [3,4]. The HbA1c fraction is abnormally elevated in patients with chronic hyperglycaemic diabetes mellitus and it correlates positively with the metabolic control [5]. According to the American Diabetes Association (ADA) guidelines, the value of HbA1c should be kept below 7% in all the diabetics [6]. The values which are greater than 7% indicate an increased chance of progression to the diabetic complications, especially the microvascular ones.

HbA1c is majorly affected by the blood glucose levels alone. However, certain studies have proven that the HbA1c levels are altered by various other coexisting factors, along with diabetes, especially that of iron deficiency anaemia, which is a major public health problem in developing countries like India.

According to the World Health Organization (WHO), iron deficiency is the commonest of the deficiency diseases worldwide [7]. One of the well-studied pathological ill-effects of IDA in the biological system is the glycation of proteins [8]. The nonenzymatic glycation of proteins has pronounced effects on the structure and the function of proteins. The pathological consequences of these alterations depend on the nature of the proteins which are involved, as well as on their functions and concentrations in specific tissue localizations [9].

The two known factors which can modulate the glycation of proteins are the prevailing concentration of glucose and the half life of the protein [10]. But evidences in the literature have documented increased glycated protein levels in some non-diabetic pathological states, like iron deficiency anaemia. Some authors have also found that on supplementation with iron therapy, there was a significant decrease in the levels of glycated haemoglobin [11]. Evidence has accumulated, which supports the hypothesis that the glycation reaction, apart from the traditional chronic hyperglycaemia ,can be modulated by the iron status of the patient. If the degree of glycation of other proteins in anaemic patients was similar to that of the glycated haemoglobin, it would have important clinical implications. Thus, the objective of the present study was to determine whether the HbA1c levels were increased among the anaemic patients without diabetes. If so, the iron deficiency had to be corrected before any diagnostic or therapeutic decision was made based on the HbA1c level.

PATIENTS AND METHODS

Blood samples (3ml) were obtained from 50 anaemic patients of the mean age, 43.52±7.79 years, among which 19 were males and 31 were females and 50 age-matched healthy subjects. The anaemic patients were recruited from the Medicine Outpatients Department of our institute, Sree Balaji Medical College, Chrompet, Chennai, India. The anaemic patients were selected, based on their haemoglobin levels (Hb < 11 g/dl), ferritin levels (<9 ng/ml for women, <15 ng/ml for men) and on their peripheral blood smears (mostly microcytic hypochromic), which suggested iron deficiency anaemia and on their haematologic investigations and serum fasting and postprandial glucose levels. The patients who had glucose tolerance abnormalities (impaired glucose tolerance or diabetes mellitus), haemoglobinopathies, haemolytic anaemia, chronic alcohol ingestion, and chronic renal failure were excluded from the study.

LABORATORY INVESTIGATIONS

The blood specimens were drawn after an overnight fast. A Sysmax automated haematology analyser was used for the whole blood counts [haemoglobin (Hb), haematocrit (Hct), mean corpuscular volume (MCV), and mean corpuscular haemoglobin (MCH)]; the serum ferritin levels were measured by using a Diatek kit in a Labowind semiautomated analyser, and the peripheral blood smears were examined in all the patients. The HbA1c levels were determined by turbidimetric immunoinhibition.

This study was approved by the ethics Committee of Sree Balaji Medical College and Hospital, Chrompet ,Chennai, India. An informed consent was obtained from all the subjects.

STATISTICAL ANALYSIS

All the results are presented as mean ± S.D. The statistical significance of the difference between the groups was evaluated by the Student's t-test. The correlation was assessed by the partial correlation analysis. A p value of 0.05 was selected as the point of minimal statistical significance.

RESULTS

All the parameters which were tested in both the groups have been reported in [Table/Fig-1]. The fasting and the postprandial blood glucose levels confirmed the nondiabetic status. The serum ferritin levels (index of the iron deficiency status) were low among the iron deficient subjects (3.68±1.8 ng/ml) and the peripheral blood smears showed a hypochromic microcytic picture. The HbA1c levels were significantly increased among the IDA patients as compared to those in the controls. The mean HbA1c (7.6 ± 0.5%) level in the patients with IDA was higher than that in the control group (5.5% ± 0.8) ($p < 0.001$). There were no differences in the levels of fasting and postprandial glucose between the IDA and the control groups ($p > 0.05$). The haemoglobin, serum ferritin, fasting and postprandial glucose, and the HbA1c levels were normal in the control group ($p > 0.05$).

Parameters	IDA group (n=50)	Control group (n=50)
Hb, g/dl	10.6±1.4*	13.4±0.96
Hct, %	33.2±3.0*	41.4±2.7
MCV, fl	72.3±4.2*	84.2±4.6
MCH, pg	22.6±2.2*	32.9±1.7
Ferritin, ng/ml	3.68±1.8*	22.3±6.1
Fasting glucose, mg/dl	92.8±9.4	90.7±10.6
Postprandial glucose, mg/dl	104.6±6.1	101.9±5.8
HbA1c, %	7.6 ± 0.5%*	5.5% ± 0.8

[Table/Fig-1]: Laboratory parameters of study groups

DISCUSSION

Our results suggested that IDA was associated with higher concentrations of HbA1c. Similarly, Brooks et al., [12] showed higher HbA1c concentrations in iron-deficient nondiabetic adults, which decreased to normal after iron replacement. Hansen et al., [13] showed normal HbA1c concentrations in iron deficiency, which dropped to subnormal levels after iron supplementation. Rai et al., [14] investigated the different methods and no difference was detected among the colorimetric methods, ion-exchange chromatography and affinity chromatography. The commonly performed immunoturbidometric method was performed to determine the HbA1c levels in this 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 [15]. 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 immunoturbidometry 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 [16,17].

CONCLUSION

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.

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AUTHOR(S):
1. Dr. Balasubramanian Shanthi
2. Dr. Carnagarin Revathy
3. Dr. Arcot Jagdeeshwaran Manjula Devi
4. Dr. Subhashree

PARTICULARS OF CONTRIBUTORS:
1. Associate Professor, Department of Biochemistry,
2. Postgraduate, Department of Biochemistry,
3. Professor & Hod, Department of Biochemistry,
4. Associate Professor, Department of Pathology,
Sree Balaji Medical College and Hospital,
Chennai, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Carnagarin Revathy,
Postgraduate, Department of Biochemistry,
Sree Balaji Medical College And Hospital, Clc Works Road ,Chrompet, Chennai-600044, India.
Phone: 9952264962
E-mail: revathycarnagarin@yahoo.com

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