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Original Research Article

Impact of anemia on HbA_1C level in type 2 diabetes mellitus patients

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ABSTRACT

HbA1c is one of the important laboratory markers of blood glucose levels monitoring over last 2-3 months. Studies have shown that many factors affect HbA1c levels, hence it is not the only investigation to diagnose diabetes mellitus. As both iron deficiency anaemia and diabetes mellitus are highly prevalent disorders, effect of iron deficiency anaemia on HbA1C need to be evaluated. Many factors such as haemolytic anaemia, vitamin deficiencies, pregnancy. Kidney disease, haemoglobin variants can affect HbA1C levels. This study had two groups of participants. Group 1 included diabetic patients with iron deficiency anaemia and group 2 included diabetic patients without iron deficiency anaemia. Significant correlation between HbA1C and iron deficiency anaemia has been found. PCV, MCH, MCHC, MCV are low in diabetics with Iron deficiency anaemia and high in diabetics without iron deficiency anaemia. Need for anaemia screening before treatment planning of diabetes based on HBA1C levels must be considered and for optimal diabetes control, treating anemia is necessary.

1. Introduction

Diabetes, a non communicable metabolic disorder in which either insufficient insulin or insulin resistance are responsible for persistent hyperglycemia. The population affected by diabetes is globally increasing at an alarming rate. Increase prevalence of metabolic syndrome, sedentary lifestyle causes homeostatic imbalance. Insulin resistance causing decreased glucose tolerance especially in muscle cells and adipocytes results in derangement of glucose metabolismand its consequences.

Plasma glucose estimation and urine glucose detection is now replaced by more reliable investigations like glycosylated hemoglobin. Compared to Oral glucose tolerance test, HbA1C test is fast and easy and gives idea about previous glycemic status of the patient also. However, before suspecting an improperly calibrated glucometer or poor patient record keeping, it is useful to consider the situations in which HbA1c may be spuriously elevated or depressed.

Although the American Diabetic Association recommends using hemoglobin A1c (HbA1c) to define diabetes, the relation between HbA1c and anaemia has not been completely evaluated.[1]

Conditions that affect erythrocyte turnover influence HbA1c concentrations and the International Expert Committee has warned clinicians to be aware of any conditions that could affect the turnover of red blood cells (RBC).[2]

HbA_1C has been established as a reliable marker not only for screening of diabetes but also for accurate prediction regarding risks and its complications...HbA1C is formed by ketoamine intermediate formation by glucose and beta chain Nterminal valine of the haemoglobin molecule. Non

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enzymatic addition of a sugar to a protein is termed glycation which occurs as a post translational modification,[3] glucose in the blood binds to hemoglobin by Amadori rearrangement. Hence, is an irreversible phenomenon.

Criteria for diagnosis of diabetes mellitus include, fasting plasma glucose > 126 mg%, post prandial plasma glucose > 200 mg% and HbA_1C > 6.5%.

It is hypothesised that in cases of hyperglycemia, with iron deficiency, total haemoglobin goes on decreasing, but glycation occurs at the same rate and HbA_1C remains same. With persistent hyperglycemia, overall rate of protein glycation is elevated, this reflects the glycemic status of the patient over previous 2 to 3 months. Hba1c is expressed as the percentage of HbA_1C to total haemoglobin. Also oxidative stress biomarker malondialdehyde increases glycation rate of haemoglobin. Diabetes can cause anemia by reduced iron absorption, gastrointestinal bleeding and also associated complications. 40% of diabetics develop complications of nephropathy. Inflammatory cytokines, reduced renal function in diabetic nephropathy are responsible for causing anaemia.[4] Advanced glycation end products (Age) formed due to persistent hyperglycemia increasing oxidative stress causes damage to DNA of the cells and result in apoptosis.[5]

Complications like diabetic nephropathy can even more impact the HBA1C levels, because a uremic environment shortens the red blood cell (RBC) lifespan, and carbamylated Hb formed in the presence of high urea interferes with glycosylation of Hb.[6]

Diabetic autonomic neuropathy interfering with the erythropoietin production and reduce iron absorption again complicate and increase the prevalence of anaemia in diabetes with CKD than the non diabetic controls.[7]

RBC is a biconcave disc shaped cell with dense and dark outer ring and pale centre. The changes in RBC morphology is a feature of many diseases and hence Complete Blood Count can be used to assess the disease extent and efficacy of treatment.[8]

Anemia is defined as a reduction in the oxygen-carrying capacity of blood and measured by the cut-off value of Hb level <12.0 mg/dl in adult non-pregnant women and <13.0 mg/dl in adult men.[9]

Iron deficiency anaemia is commonest anaemia in India. Iron deficiency can be due to inadequate iron stores, impaired utilisation or increased loss. Iron is inside haemoglobin molecule. Chronic iron deficiency anaemia results in shortened lifespan of RBCs, decrease in haemoglobin. All these changes have profound effect on the HbA_1C levels. Many researchers brooks, et al, Hansen et al, Coban et al, found significant decrease in HbA1C in diabetes with iron deficiency anaemia after treating with iron therapy.[10–12]

RBC is having major antioxidant system, anemia leads to oxidative stress, functionally defective HDL particles. Majority of microvascular complications of diabetes have direct link in anaemia.[9]

A complete blood count includes – PCV, RBC indices, MCV, MCH, MCHC, platelet count and WBC. Three major factors on which HbA_1C levels depends on are HbA_1C c in reticulocytes when released from the bone marrow, glycation rate and Mean age of RBC s in the circulation. RBC lifespan affects HbA_1C levels, independent of blood glucose levels.[11]

HbA_1C has many factors that modifies its levels. Uremia, hyperbilirubinemia, hypertriglyceridemia, chronic alcoholism, chronic ingestion of salicylates, vitamin C ingestion, and opiate addiction have all been reported to interfere with some assay methods, falsely increasing results.[13]

Nitin et al found direct unidirectional correlation between iron deficiency anaemia and HbA_1C levels. As sustainable data is required, the current study was under taken to correlate HbA_1C levels in diabetics with IDA and diabetics without IDA.[14]

Depending upon the methodology used to measure HbA1C, some diseases and pathological states like anaemia and Hemoglobinopathies can significantly affect HbA1C levels.[14]

HbA_1C clearly identifies the need for more evidence, especially in identifying the types and degrees of anemia likely to have significant impact on the reliability of HbA_1C.

Both iron deficiency anaemia and diabetes mellitus account for vast majority of health disorders, it is of utmost importance to exclude the factors that modify its actual levels.

Taking into account all these factors, it becomes very important as to what reduced haemoglobin causes to the HbA_1C levels in the diabetic population.

As a result, the present study was undertaken to find the correlation between HbA_1C levels and iron deficiency anaemia.

2. Materials and Methods

The study was done in Department of Medicine and Department of CCL in SKNMC&GH, Pune from period of June 2021 to December 2021. This cross sectional study was done after taking approval from the Institutional Ethical Committee. (IEC)

All participants were in the age group of 30 to 65 years.

Cases: 100 IDA patients with type II diabetes mellitus.

Control: 100 non anaemic patients with type II diabetes mellitus.

Both IPD and OPD patients were included in this study.

2.1. Exclusion criteria

Patients having history of renal disorders, liver disorders or any other inflammatory conditions or subjects on

any hematinic supplementation, hemoglobinopathies were excluded. Similarly, patients with complications of diabetes, type 1 diabetes mellitus, any acute illness, recent surgery, trauma, acute blood loss, transfusions were excluded from this study.

All the participants were given questionnaire regarding use of medications, food supplements, history of major surgery, smoking, physical activity.

For diagnosis IDA, Diabetes mellitus using ADA and WHO criteria of 6.5%. Fasting blood glucose >126mg% and post prandial blood glucose > 200 mg%

Patients were anaemic when Hb < 13 gm in males and Hb < 12 gm in females.

RBC indices used for diagnosis of microcytic hypochromic anaemia were MCV < 80 fl, MCH < 26pg/L.

2.2. Collection of blood sample

After overnight fasting, the sample was collected under all aseptic precautions by needle and syringe technique.

After written informed consent, about 10 ml venous blood sample was collected in EDTA and fluoride bulb for estimation of red blood cell indices, HbA_1C and plasma glucose respectively. Fasting as well as postprandial blood glucose estimations are done.

2.3. Sample estimations

Red blood indices were estimated on Sysmex Hematology analyser, plasma glucose was analysed on VITROS 5600 dry chemistry analyser and HbA_1C were BIORAD D 10 HPLC system.

2.4. Statistical analysis

Data was analysed using SPSS software. Pearson's correlation, chi-square, and independent tests were calculated. The data was presented as mean + SD. A p value of < 0.05 was taken as statistically significant.

3. Results

A total of 200 Diabetic patients were included in this study. This study had two groups of participants. Group 1 included diabetic patients with iron deficiency anaemia and group 2 included diabetic patients without iron deficiency anaemia.

In this study we found significantly elevated HBA1C levels in diabetic patients with IDA (8.1) as compared to diabetic patients without IDA (6.1).

PCV in diabetic patients with IDA (32.2) is significantly low (p < 0.0001) as compared to diabetic patients without iron deficiency anaemia (34.7).

MCV in diabetic patients with IDA (79.3) is significantly low (p< 0.0001) as compared to diabetic patients without iron deficiency anaemia (80.9).

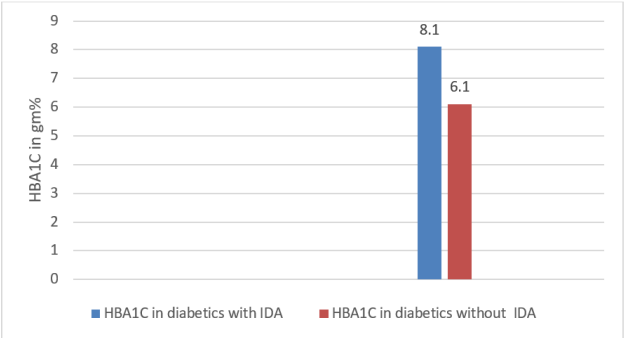

Fig. 1: Comparison between HbA_1C in diabetics with IDA and HBA1C in diabetics without IDA

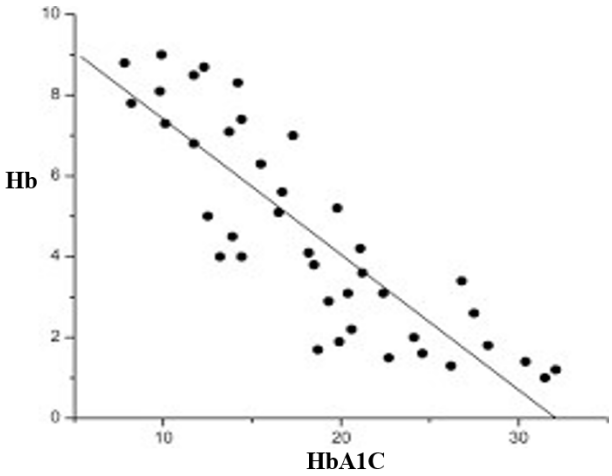

Fig. 2: Scatter diagram of hemoglobin and HbA_1C

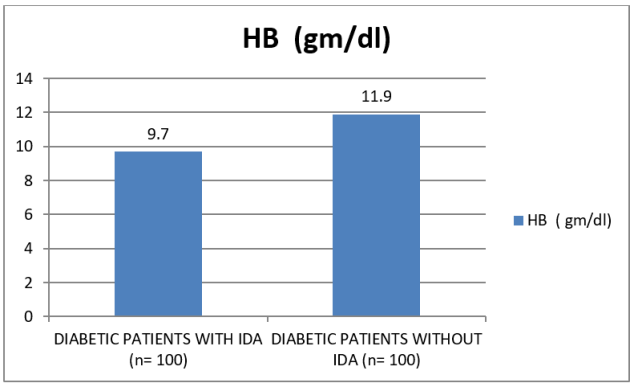

Fig. 3: Comparison of Hb between diabetic patients with IDA and diabetic patients without IDA

Table 1: Comparison of laboratory parameters in diabetics with iron deficiency anemia and diabetics without iron deficiency anemia

Parameters	Diabetic Patients with IDA (n= 100)	Diabetic Patients Without IDA (n= 100)	P value
HB (gm/dl)	9.7±2.4	11.9±2.0	< 0.0001
PCV (hematocrit)	32.2±5.6	34.7±5.0	<0.0001
MCV (fl)	79.3±6.8	80.9±7.4	<0.0001
MCH (pg)	31.3±12.6	37.3±24.7	<0.0001
MCHC (gm/dl)	31.6±2.6	32.7±1.8	<0.0001
RDW CV %	14.9±2.3	19.5±7.3	<0.0001
HbA_1C	8. 1±1.9	6.1±0.3	<0.0001

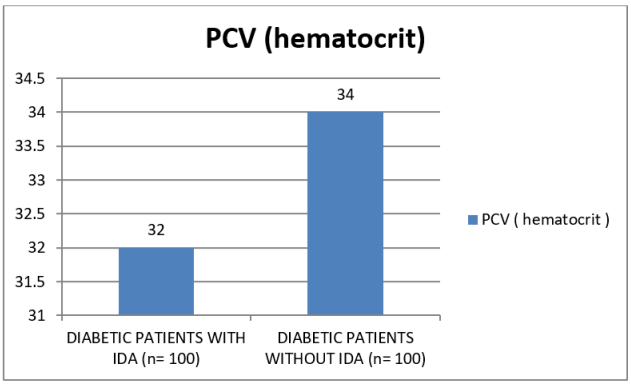

Fig. 4: Comparison of PCV between diabetic patients with IDA and diabetic patients without IDA

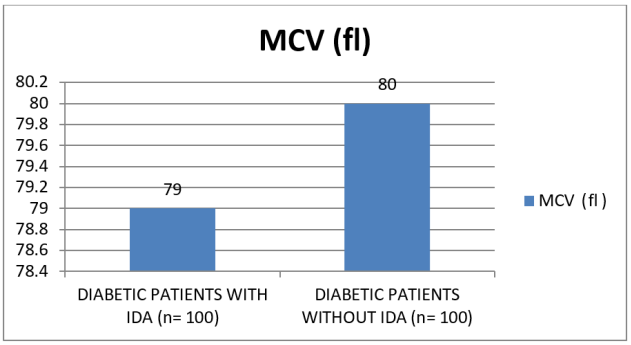

Fig. 5: Comparison of MCV between diabetic patients with IDA and diabetic patients without IDA

Indicates average volume of RBCs [15]

MCH in diabetic patients with IDA (31.3) is significantly low (p< 0.0001) as compared to diabetic patients without iron deficiency anaemia (37.3).

MCHC in diabetic patients with IDA (31.6) is significantly low (p< 0.0001) as compared to diabetic patients without iron deficiency anaemia (32.7).

RDW in diabetic patients with IDA (14.9) is significantly low (p< 0.0001) as compared to diabetic patients without iron deficiency anaemia (19.5).

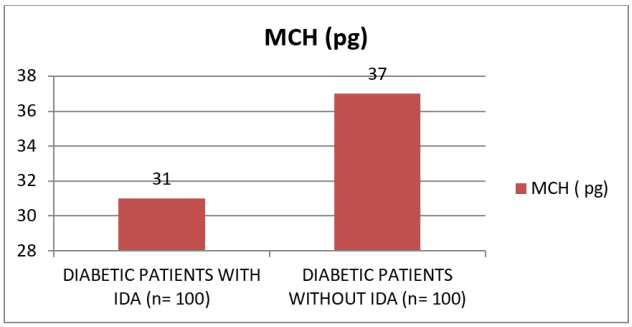

Fig. 6: Comparison of MCH between diabetic patients with IDA and diabetic patients without IDA

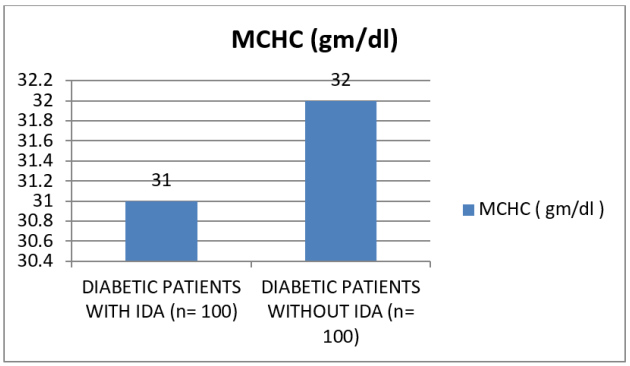

Fig. 7: Comparison of MCHC between diabetic patients with IDA and diabetic patients without IDA

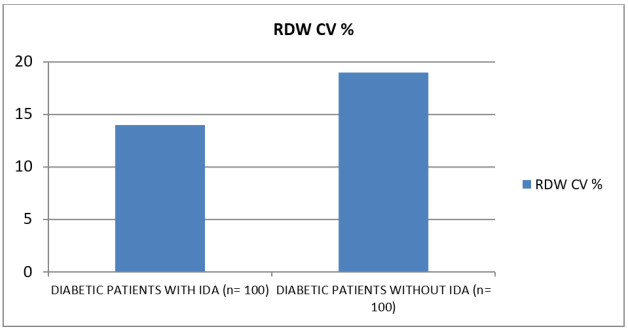

Fig. 8: Comparison of RDW between diabetic patients with IDA and diabetic patients without IDA

4. Discussion

Diabetes is world's most significant health disorder. Quality of life and development of complications are major setbacks for treatment goal Being a metabolic disorder, diabetes influences RBC synthesis also, with changes in its morphology. Hence, many studies have been carried out to delineate the effect of diabetes on red blood cell indices and to what extent anaemia can affect HbA1C levels.

The mean haemoglobin among 1^{st} group were significantly lower than group 2 patients. Similar studies were done in Bangladesh, Egypt.[11,12,16,17] since major portion of Hb molecule is getting glycated in persistent hyperglycemia.

In NIDDM with Angiotensin II antagonist (RENAAL) trial, suggested reduced Hb levels, show diabetic patients at increased risk of progressive renal disease.[16]

PCV, MCV values are also significantly low in 1^{st} group than 2^{nd} group. Oxidative stress and inflammatory response due to persistent hyperglycaemic status may be the cause of above finding.[6]

MCH and MCHC are low significantly less in group 1 than in group 2. Generation of free radicals alter shape of RBCs in diabetes patients. Reduced haemoglobin results in microcytic hypochronic picture.[5]

RDW is a marker of anisocytosis. High RDW suggests red cell distortion. It is due to oxidative stress in diabetes which results due to persistent hyperglycemia.

Red blood cell indices as discussed above are also showing significant correlation with HBA1C levels of these patients, included in this study.

Koga et al suggested that RBC indices vary with HBA1C independent of plasma glucose.[8]

Rusak et al have found mean corpuscular volume (MCV) and HbA1C are having positive correlation.[4] Our results are similar to results of analysis of Diabetes Control and Complications Trial.[18]

This study has found that iron deficiency anaemia has significant correlation with HBA1C levels.

Naqash A et al have found the need for physicians to evaluate the confounding factors that affect HBA1C levels.[3]

While Kalasker V et al found no variation in IDA and controls regarding HBA1C. Less number of studies are carried out regarding the variables associated with the utility of HBA1C in terms of accuracy.[14]

Sluiter et al have put possible explanations to above mentioned findings. When blood glucose enters erythrocytes, it glycates amino terminals of haemoglobin. Half life of RBC is typically 60 days, the level of HbA_1C reflects mean blood glucose concentration over preceding 8-12 weeks.[2]

Ford et al have also found similar results and significant changes in the HbA1c levels in diabetic patients with or without iron deficiency anaemia before and after treatment with iron therapy.[12]

Kim et al. Found similar results to the above with slight upword shift of HbA1C iron deficiency.[19]

English et al suggest that HbA1c is likely to be affected by iron deficiency and IDA with a spurious increase in HbA1c values; conversely, non-IDA may lead to a decreased HbA1c value.[7]

Mishra TK et al suggests absolute increase in HBA1C levels with treatment of iron deficiency anaemia.[11]

Hardikar et al. Studied iron-deficiency in young population for diagnosis of DM using HbA_1C showing increased levels of MCV, MCH, MCHC and RDW as compare to non-anemic controls.Pre- and post-treatment HbA1c for anemia showed changes as large as -1.2%.[14]

This study are having contrast findings to the study done by Shariff et al who have not found any correation between HbA_1C and iron status of the patient.

Non diabetics as well as diabetics having iron deficiency anaemia were included in many other studies as far as HbA_1C status was considered. But what effect it has in well controlled diabetic patients is yet to be investigated.

For accurate diagnosis of diabetes mellitus clinicians should always consider plasma glucose post prandial as well as fasting levels and urine sugar also. Oral glucose tolerance test (OGTT) would be useful for correct diagnosis of diabetes.

Insulin deficiency affects the cellular level of all organs of the body resulting in complications of diabetes. RBC having peculiar characteristics definitely shows changes of hyperglycemia. Good glycemic control will be helpful to control further progression to complications.

Proper consideration of the erythrocyte indices, haemoglobin content of the patient must be taken into consideration before finally starting and monitoring the treatment of hyperglycaemic status of the individual.

5. Conclusion

In conclusion, this study found significant increase in HbA_1C in diabetic patients with IDA as compared to diabetic patients without IDA.

All these findings correlate with previous studies and further studies are required to be taken to define specific risks in diabetes and its complications. Iron status correction and iron replacement therapy becomes even more important when the patient is diabetic.

Until large scale trials are undertaken the existing theories about underlying mechanism remains elusive. Other non glycemic factors affecting HbA_1C levels also needs to be studied.

Those patient s having IFG or prediabetics need to be tested by alternative methods for HbA1C testing. Such patients need to be investigated for iron deficiency anaemia as well.

HbA1C levels not correlating clinically need to be evaluated thoroughly.

Further, for correct diagnosis and future management of diabetes mellitus and its chronic complications, individualized approach is a must and proved. The findings of significant effect of anaemia and other factors on HbA1C levels, it is important to correctly diagnose the hyperglycemia.

6. Limitations of the Study

The effect of other variables on HbA_1C like vitamin deficiencies, kidney disorders, haemolytic anemia, genetic disorders and hemoglobinopathies cannot be studied.

7. Abbreviations

RDW: Red cell distribution width; MCV: Mean corpuscular volume; MCH: Mean corpuscular haemoglobin; MCHC: Mean corpuscular haemoglobin concentration; IDA: Iron deficiency anaemia; PCV: Packed cell volume.

8. Source of Funding

Not applicable.

9. Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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