Original Article

DOI: 10.21276/APALM.2355

Role and Significance of Hematological Parameters in Diabetes Mellitus

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ABSTRACT

Background: Diabetes mellitus is a global metabolic disorder characterized by persistent hyperglycemia and increased risk of microvascular and macrovascular complications. Consistent elevation of HbA1c can be associated with functional and structural changes in hemoglobin molecule, cytoplasmic viscosity and osmotic disturbances within red cell. These changes may be reflected in the red cell analytical parameters.

Methods: 120 subjects were studied. 41 were non diabetic healthy subjects (Group1). 79 were known diabetic patients, divided into two groups: HbA1c <7(39 subjects) Group 2 and HbA1c >7(40 subjects) Group 3.

Sample for glucose estimation and CBC were collected in vacutainers containing sodium Fluoride and K 2EDTA respectively. Glucose, HbA1c and CBC estimation was carried out by auto analyzers.

Result: Mean RBC count of diabetics with HbA1c<7 was less when compared to non-diabetic individuals. Significant decrease was noted in the mean RBC count, Hb, HCT and MCV of diabetics with HbA1c>7 when compared to non diabetic individuals. There was significant decrease in mean Hb, HCT, MCV, MCH of diabetics with HbA1c >7 when compared to diabetics with HbA1c <7.

Conclusion: The findings in the present study suggest that diabetic patients with poor control are more prone to develop anemia. This indicates a need for routine CBP examination for early detection and management of anemia in diabetic patients at the primary care setting to reduce associated morbidity due to lowered immunity associated with anemia and complications such as diabetic ketoacidosis.

Keywords: *Diabetes Mellitus, HbA1c, CBC, Red Cell Parameters*

Introduction

Diabetes mellitus is a global metabolic disorder. Incidence of diabetes mellitus continues to rise with almost equal propensity in elderly and also in young age group due to adverse life style changes like excess calorie intake and sedentary life style[1]. Diabetes mellitus is a chronic metabolic syndrome characterized by persistent hyperglycemia and increased risk of microvascular and macrovascular complications and these complications are responsible for majority of morbidity and mortality associated with the disorder[2]. A majority of patients with diabetes mellitus have signs of metabolic syndrome/ syndrome X, which is a cluster of phenotypes associated with increased risk of cardiovascular disease. Insulin resistance play a central role in this syndrome[3].

When plasma glucose is episodically elevated over time, small amount of hemoglobin A are nonenzymatically glycated to form glycated hemoglobin (HbA1c). HbA1c has glucose attached to terminal valine in each β chain. The concentration of HbA1c depends on the concentration of glucose in the plasma and the duration of hyperglycemia and is an index of diabetic control for a period over past 12 weeks. High levels of glycated hemoglobin have shownto impair endothelium mediated vasoactive responses, which can lead to hypertension and vascular diseases in diabetic patients[4]. Watala et al. attributed an increase in erythrocyte internal viscocity to glycation derived structural alterations in hemoglobin molecules[5,6].

The slow glycosylation and consistent elevation of HbA1c can be associated with functional and structural changes in hemoglobin molecule and cytoplasmic viscosity within each red cell along with osmotic disturbances within the cell. These changes may be reflected in any one or all the red cell analytical parameters such as Hb, RBC count HCT, MCV, MCH and MCHC. Since microvascular complications are attributed to increase in HbA1c, changes in red cell deformability and other hemorrheological alterations, red cell indices may be used to monitor the disease progression in diabetic patients and there has been no in depth study done in this direction.

The quantitative and qualitative analysis of red cell parameters are measured by RBC count, Hematocrit(HCT), Mean Corpuscular Volume(MCV),Mean Corpuscular Hemoglobin(MCH) and Mean Corpuscular Hemoglobin Concentration(MCHC) which gives the indication of red cell deformability and hemorrheological state. The Red

Blood cell distribution width(RDW) is a measurement of the size variation among the circulating red cells and is calculated as a part of routine complete blood count.RDW along with MCV is useful in the differential diagnosis of the causes of anemia[7].RDW is now being considered as an inflammatory marker and an elevated RDW value is shown to be significantly associated with diabetic nephropathy in type 2 diabetes patients independent of traditional risk factors including diabetes duration and glycemic control[8].

This study was planned to show the association of HbA1c with variations in red cell parameters in diabetics with HbA1c<7 and diabetics with HbA1c>7 when compared to non diabetic healthy individuals and also as a predictive maker for any impending morbidities.

Materials and Methods

This comparative prospective study was conducted in the Department of Pathology, Deccan College of Medical Sciences, Hyderabad. A total of 120 subjects were included in the study out of which 41 were non diabetic healthy subjects which were taken as group1 and 79 were known diabetic patients which were divided into two groups with HbA1c <7(39 subjects) as group 2 and HbA1c >7(40 subjects) as group 3.

Sample for glucose estimation and CBP were collected in vacutainers containing sodium Fluoride and di potassium salt of EDTA respectively. All tests were conducted within one hour of sample collection after taking consent from the subjects. 2 ml of blood was collected in each vacutainer under aseptic precautions.

Glucose estimation was carried out by auto analyzer using enzymatic Hexokinase oxidation method for plasma glucose levels. HbA1c estimation was done using auto analyzer (COBAS C III) which is based on turbidometric inhibition immuno assay. Estimation of red cell parameters including the RBC count, Hb, HCT, MCV, MCH, MCHC was carried out using blood cell count auto analyzer (Horiba Pentra ES60) which is based on impedence (real cell volume measurement) and optical (analysis of internal cell structure by light absorbance) Double Hydrodynamic Sequential system.

The biological reference range in both males and females for RBC count is 3.8-6.5 millions/cu.mm, Hb is 11.5-17mg/dl, Hematocrit is 37-54%, MCV is 80-100µm3 , MCH IS 27-32 pg, MCHC is 32-36g/dl and RDW is 11-16 %.

Exclusion criteria includes subjects having other inflammatory disorders (SLE, RA), hypo/hyperthyroidism, chronic kidney diseases(all causes except diabetic kidney disease), congenital heart diseases, alcoholism, hemolytic anemias like sickle cell trait and S beta thalassemia and malignancy.

Statistical Analysis: All the data from the patients and controls were analyzed and compared using Students t-test in the Microsoft Excel. Results were considered significant at $p<0.05$

Result

In this study, a total of 120 subjects were included out of which 41 were non-diabetic healthy subjects (group 1) with their age ranging from 19-79 years and 79 were type-2 diabetic subjects with their age ranging from 30-99 years . The mean age of non diabetics was 30.4 years and mean age of diabetics was 54.1 years. The diabetic subjects were divided into 2 groups, one with HbA1c<7(group 2) and other with HbA1c>7(group 3). The majority of the subjects among all the groups were females. In this study it was observed that the mean RBC count of diabetics with HbA1c<7 was less when compared to non-diabetic healthy individuals. There was a significant decrease in mean RBC count, Hb, HCT of diabetics with HbA1c>7 when compared to non diabetic individuals. There was significant decrease in mean MCV and MCH of diabetics with HbA1c >7 when compared to diabetics with HbA1c <7.

Overall, in diabetics the mean RBC, HB, PCV, MCV and MCH values were lower when compared to non-diabetic individuals.

Table 1: Patient Demographics.

	Group 1		Group 2		Group 3		Total
Age	Male	Female	Male	Female	Male	Female	
10-19	2	1	0	0	0	0	3
20-29	3	14	0	0	0	0	17
30-39	7	8	1	1	1	0	18
40-49	0	1	2	8	3	9	23
50-59	2	1	2	6	2	5	18

	Group 1		Group 2		Group 3		Total
Age	Male	Female	Male	Female	Male	Female	
60-69	0	1	3	4	5	6	19
70-79	1	0	2	6	2	2	13
80-89	0	0	2	1	1	2	6
90-99	0	0	0	1	1	1	3
Total	**15**	**26**	**11**	**28**	**15**	**25**	**120**

Tables 2: Comparison of RBC indices in Group 1 (Non Diabetics) with Group 2 (Diabetics with HbA1c<7).

Parameters	Group 1	Group 2	p value
RBC	4.5	4.2	0.001
Hb	12.4	11.7	0.14
HCT	37.6	35.5	0.06
MCV	82.3	84.3	0.23
MCH	27.1	27.3	0.3
MCHC	32.8	32.3	0.4
RDW	13.3	13.6	0.5

A Significant decrease was noted in RBC count of diabetics with HbA1c <7(group 2) However, the values are within normal reference range.

Table 3: Comparison of RBC indices in Group 1(Non Diabetics) with Group 3(Diabetics with HbA1c>7).

Parameters	Group 1	Group 3	p value
RBC	4.5	4.1	0.02
Hb	12.4	10.9	0.01
HCT	37.6	32.9	0.006
MCV	82.3	78.6	0.15
MCH	27.1	25.9	0.13
MCHC	32.8	31.9	0.8
RDW	13.3	12.7	0.9

A significant decrease was noted in RBC count, Hb and Hct in Diabetics with HbA1c >7 (group 3) when compared with group 1. Group 3 values were below normal reference range.

Table 4: Comparison of RBC indices in Group 2(Diabetics with HbA1c<7) with Group 3 (Diabetics with HbA1c>7).

Parameters	Group 2	Group 3	p value
RBC	4.2	4.1	0.7
Hb	11.7	10.9	0.2
HCT	35.5	32.9	0.2
MCV	84.3	78.6	0.01
MCH	27.3	25.9	0.02
MCHC	32.3	31.9	0.4
RDW	13.6	12.7	0.5

A Significant decrease was noted in MCV and MCH in Diabetics with HbA1c >7 (group 3). Values were below normal reference ranges.

Discussion

Diabetes mellitus is a metabolic disorder associated with abnormal substrate metabolism arising from insulin deficiency or decreased responsiveness of tissues to insulin. Insulin is the key hormone in substrate homeostasis and insulin deficiency results in wide variety of metabolic defects affecting carbohydrate, protein and lipid metabolism[9]. Insulin deficiency and hyperglycemia affects the tissues as well, resulting in complications of diabetes. In the present study, a total of 120 cases were included of which 79 individuals were suffering with type 2 Diabetes Mellitus and 41 individuals were non diabetic. The diabetics were divided into two groups, one with HbA1c <7 (group 2) which included 39 subjects and the other with HbA1c >7 (group 3) which included 40 subjects. Majority of the subjects in all the groups were females.

Present study showed that diabetics with HbA1c <7 and HbA1c >7 were having comparatively lower mean RBC counts compared to non-diabetic subjects. In type 2 Diabetes Mellitus, life span of RBC may be decreased due to disturbances in the hematopoetic milieu, such as chronic hyperglycemia and hyperosmolarity[10,11] . These disturbances can lead to increased internal viscocity and increased membrane rigidity in these blood cells so that number of red blood cells decreases[12]. The results in the study were in agreement with study by Agrawal R on RBCS deformability and related indices in type 2 DM[13].

This study also showed that the diabetics with HbA1c >7 when compared to non diabetic subjects were having statistically significantly lower RBC count, Hb and HCT values when compared with the non diabetics, suggesting that diabetics are prone to develop mild anemia. Similar observation of lower Hb concentration in type 2 diabetics when compared to non diabetics were made by Al Salhen KS et al[14].Chronic diseases such as DM, are accompanied by mild-to-moderate anemia[15] .Hyperglycemia has a direct relationship with the development of an inflammatory condition showed by the increased expression of proinflammatory cytokines such as IL-6, TNF-α, and NFκB. Thus, diabetes as well as hyperglycemia due to its nature, is also an inflammatory disease. Studies show that the longer the duration of the disease and/or loss of glycemic control, the higher the inflammatory process [16,17].By increasing especially IL-6, antierythropoietic effect occurs, since this cytokine changes the sensitivity of progenitors to erythropoietin (erythroid growth factor) and also promotes apoptosis of immature erythrocytes causing a decrease further in the number of circulating erythrocytes and consequently causing a reduction of circulating hemoglobin [16,18].Al khoury et al. demonstrated that for each chronic kidney disease stage, hemoglobin is 1gm/dl lower in patients with diabetes than in non-diabetics[19].

Apart from the above mentioned mechanisms, anemia in diabetes may also be due to hematotoxic effects associated with toxic substances on bone marrow leading to bone marrow depression caused by damage to multiple classes of hematopoetic cells and variety of hematopoetic functions[20,21].Waggiallah H et al also observed significant decrease in hemoglobin concentration, RBC count, MCH and MCHC values in diabetics when compared to non diabetic healthy individuals [22] .

In this study, it was observed that diabetics with HbA1c>7(group 3) had significantly lower mean MCV and MCH($p<0.05$) values when compared to diabetics with HbA1c<7(group 2) suggesting that good diabetic control is essential to prevent development of anemia in diabetic individals. No such comparison was made in any of the previous studies.

Conclusion

The findings in the present study suggest that diabetic patients with poor control are more prone to develop anemia. This indicates a need for routine CBP examination for early detection and management of anemia in diabetic patients at the primary care setting to reduce associated morbidity due to lowered immunity associated with anemia and complications such as diabetic ketoacidosis.

Acknowledgements

We would like to acknowledge our technical and supporting staff .

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Financial or other Competing Interests: None.