Type 2 Diabetes Mellitus is causing red blood cell hemolysis

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Abstract:

Background: Diabetes mellitus is a metabolic disease and its complications are interlinked and usually have a common soil. The clinical effect of diabetes on erythropoiesis is always interesting as the production of red cells is always at high rate and continuous, yet the red cell mean life span of 120 days is of an appropriate length, making it an excellent candidate for tests to detect the effect of diabetes and its complications and monitor for the response to treatment.

Aim of the study: To determine the level of reticulocyte percent in patients with uncomplicated type 2 Diabetes Mellitus and to evaluate the effect of folic acid treatment on this percent.

Patients, controls and methods: 140 patients with type 2 Diabetes Mellitus were included in this study. The exclusion criteria from this study were anemia, pregnancy, personal and family history of hemolytic anemia, overt microvascular complications of DM (retinopathy, neuropathy and nephropathy), acute infection and/or inflammation and history of chronic disease other than diabetes. The patient medical record was reviewed and peripheral blood specimen withdrawn for determination of hemoglobin concentration, PCV %, reticulocyte % and HbA_1c. A subgroup of (82) patients (45 males and 37 females) were chosen who consented on not to change their treatment for the next coming one month except for the addition of daily one 5mg tablet of folic acid. PCV %, hemoglobin concentration, reticulocytes % were tested for using manual techniques. HbA_1c % was measured using automated HPLC machine.

Results: This study revealed increased red cell destruction in type 2 diabetics in comparison to healthy control subjects of the same sex and age. Also the reticulocyte increment was more in those with higher HbA_1c %, although it was not in linear relationship with it. These findings are suggesting that the initiating event for red cell hemolysis is the increased blood glucose level.

Conclusion: Type 2 diabetes patients are subject to oxidative stress as a result of hyperglycemia. This study suggests that the addition of folic acid treatment to the regime of type 2 diabetic patients can be useful.

Introduction:

Diabetes mellitus (DM) is a syndrome of altered carbohydrate, fat, and protein metabolism resulting from an absolute or relative deficiency of insulin resulting in a group of common metabolic disorders that share the phenotype of hyperglycemia, the two broad categories of DM are type 1 and type 2 [1].

The burden of this disease is best demonstrated by the accompanying risk for microvascular and macrovascular complications and death [2], and the fact that the prevalence of hyperglycemia in Iraq is 10.4% [3]; and it is noticeably increasing [4].

The diagnosis of Diabetes is based on the intention to identify the fasting blood glucose concentration that best predicted the risk of developing specific diabetic microvascular complications (nephropathy, retinopathy and neuropathy) [5].

HbA_1c is a term used to describe stable minor hemoglobin components formed slowly non-enzymatically from post-translational modification of HbA by glucose. The rate of formation of HbA_1c is directly proportional to the ambient glucose concentration as erythrocytes are freely permeable to glucose; practically there is a strong relationship between levels of HbA_1c and the average blood glucose levels over the previous 3 months [6]. HbA_1c is the gold standard test of blood glucose control [7].

Hemolysis is defined as a reduction of the red cells life span, this usually results in anemia. While in compensated hemolysis a compensatory increase in erythropoiesis may be adequate to prevent the development of anemia. An elevated reticulocyte count is a reflection of the compensatory increase in erythropoiesis by the bone marrow, and it is, therefore, an indirect indication of shortened red cells life span [8].

Diabetes mellitus is a metabolic disease and its complications are interlinked and usually have a common soil. The precise effect of diabetes on red blood cells is incompletely studied.

The clinical effect of diabetes on erythropoiesis is always interesting as the production of red cells is always at high rate and continuous, yet the red cell mean life span of 120 days is of an appropriate length, making it an excellent candidate for tests to detect the effect of diabetes and its complications and monitor for the improvement and response to treatment.

Aim of the study:

To determine the level of reticulocyte percent in patients with uncomplicated type 2 Diabetes Mellitus and to evaluate the effect of folic acid treatment on this percent.

Patients, controls and methods:

140 patients, 70 males and 70 females, aged 30-62 years, with type 2 Diabetes Mellitus attending the national center for Diabetes Mellitus at Almustansiriya University in Baghdad-Iraq, during the period from August-November 2010 were included in this study.

The exclusion criteria from this study were anemia, pregnancy, personal and family history of hemolytic anemia, overt microvascular complications of DM (retinopathy, neuropathy and nephropathy), acute infection and/or inflammation and history of chronic disease other than diabetes.

The patient medical record was reviewed and peripheral blood specimen withdrawn for determination of hemoglobin concentration, PCV %, reticulocyte % and HbA_1c.

A subgroup of (82) patients (45 males and 37 females) were chosen who accepted to consent on not to change their treatment for the next coming one month except for the addition of daily one 5mg tablet of folic acid.

A total of 140 healthy controls, 70 males and 70 females, were included in this study; they were selected in a way to be age (range) and sex (ratio) matched for the patients group.

PCV % was measured using the microhematocrit technique. Hemoglobin concentration was measured using cyanmethemoglobin method. Reticulocytes % were counted using peripheral blood smears stained with new methylene blue. HbA_1c % was measured using automated HPLC machine.

Statistical analyses were performed by using Statistical Package for Social Sciences (SPSS) version 18.0 software, with p-value of less than 0.05 considered significant.

Results:

There was a highly statistical significant difference between hemoglobin concentration, PCV % and reticulocyte % between type 2 diabetic patients and healthy control subjects (Table 1).

Also there was a significant difference between the different groups of type 2 diabetic patients that were stratified according to HbA_1c % using ANOVA test. However, there was no linear correlation between each patient HbA_1c % and its correspondent reticulocyte % (Table 2).

The addition of folic acid treatment would considerably improve the reticulocyte percent (Table 3).

Table 1: Differences in mean Hb concentration,anbbbd PCV% and reticulocytes% between healthy subjects and type 2 diabetic patients:

Parameter		Healthy subjects	Type 2 diabetic patients	P-value
Hb (g/dl) *	Males	14.6 (13-16.9)	14.1(13-15.2)	< 0.001
	Females	12.9 (12-14.6)	12.4 (12-13.1)	< 0.001
PCV % **	Males	46 (40-50)	42 (40-46)	< 0.001
	Females	39 (36-45)	37 (36-40)	< 0.001
Reticulocytes %		1 (0.3-2)	3.3 (1.8-4.6)	< 0.001

* Normal Hemoglobin concentration for males 13-17 g/dl and females 12-15 g/dl [9].
** Normal PCV % for males 40-50 % and females36-46 % [9].

Table 2: Relation between HbA_1c % and reticulocytes %:

HbA_1c %	Reticulocytes % mean and range	P-value
< 6	2.2 (1.8-2.5)	0.02
6.1-7	3.2 (2-3.8)	
> 7	3.8 (2.9-4.6)	

Table 3: Effect of folic acid treatment on reticulocyte %:

Reticulocytes % mean and range		P-value
Before folic acid treatment	After folic acid treatment	
3.5 (2.2-4.3)	2.4 (1.6-3.7)	< 0.01

Discussion:

This study revealed increased red cell destruction in type 2 diabetics in comparison to healthy control subjects of the same sex and age. Also the reticulocyte increment was more in those with higher HbA_1c %, although it was not in linear relationship with it. These findings are in consistence with Hudson et al [10], suggesting also that the initiating event for red cell hemolysis is the increased blood glucose level.

Type 2 diabetes patients are subject to oxidative stress as a result of hyperglycemia. Folate supplementation improves markers of oxidative stress [11]. This study suggest that addition of folic acid treatment to the regime of type 2 diabetic patients can be useful, but the prospect of applying these data to the overall diabetes management and follow up for improvement in diabetes control with reticulocyte% (or preferably absolute reticulocyte count if available) necessitate studying this subject even more.

There should be more detailed investigations to answers the questions about the exact mechanism of red cell destruction in type 2 diabetes mellitus, as whether it is due to the oxidative stress imposed by the hyperglycemia.

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