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Full Length Research Paper

Alteration in chemical composition of red blood cells in iron deficiency anemia

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The alteration in the chemical composition of plasma in health and diseases is well studied, but the changes in *interior milieu* of erythrocytes are not properly explored. We have studied 15 parameters (carbohydrates, lipids, proteins, electrolytes / cations, anions, metabolites, iron compounds etc.). All the studied parameters were significantly decreased in iron deficient anemic erythrocytes, except inorganic phosphorus, which was actually increased significantly. Red cell's intracellular concentrations of iron and ferritin were found to be better indicator of iron deficiency anemia than plasma iron and ferritin.

Key words: Anemia, iron deficiency, red cells, chemical composition.

INTRODUCTION

The blood plasma is a complex mixture of several organic and inorganic compounds. Its chemical composition is affected by alteration in metabolism of other organs. This aspect has been very well studied in health and disease. On the contrary, there is not much information about changes in intracellular chemical composition of red blood cells.

Anemia is a very common condition and iron deficiency is the most common cause of it (Baker and DeMaeyer, 1979; Sharma et al., 1993). Innumerable workers have studied the variations in chemical composition of plasma/serum/whole blood in health and diseases, but there is paucity of information about changes in intracellular chemical composition of red blood cells in iron deficiency anemia (IDA). The aim of the present study was precisely to study alterations, if any, in the chemical composition of red blood cells in iron deficiency anemia.

MATERIALS AND METHODS

This study was carried out (after obtaining informed consent of the people) on 70 severely anemic subjects (32 males and 38 females; age 25 – 55 years; Hb 6.2 ± 1.5 g%) and 40 non-anemic purportedly healthy persons (30 males and 10 females; age 22 – 50 years; Hb 13.0 ± 1.1g %). 10 ml of blood was collected in the morning under fasting condition in a heparinized vial. The presence of severe anemia in anemic group subjects was confirmed by blood hemoglobin and other hematological parameters (Lewis et al., 2002) and that iron deficiency as the cause of anemia was established by iron parameters (plasma iron 50.5 ± 12.7 µg % vs. 118.6 ± 23.0 µg %; plasma ferritin 22.6 ± 13.3 ng/ml vs. 95.0 ± 45.6 ng/ml and low MCV with elevated RDW - CV (20.3 ± 3.3 vs. 13.9 ± 1.5) (Bessman et al., 1983).

An aliquot of whole blood was centrifuged, plasma discarded, cells twice washed with normal saline, then hemolysed by addition of 5 volumes of distilled water. The hemolysate was centrifuged and clear supernatant was used for analysis of various constituents.

Most of the chemical parameters were done on an auto analyzer, some on a semi auto analyzer using kits or reagents. A few parameters needed atomic absorption spectrophotometer and potassium was measured in a flame photometer

Total carbohydrates were estimated by anthrone reaction (Plummer, 1979), glucose by GOD – POD method. Total cholesterol was estimated by kit employing enzymes cholesterol esterase, cholesterol oxidase and peroxidase. Total proteins were measured by Biuret method, creatinine by Jaffe's reaction. Triglyceride estimation was based on its chemical hydrolysis followed by enzymatic determination of liberated glycerol. Uric acid was measured spectrophotometrically using uricase. Reduced glutathione was estimated using DTNB as chromogen and inorganic phosphorus essentially by Fiske and Subba Rao. The cations were estimated by different techniques – calcium by OCPC method, potassium by flame photometer and copper and zinc by atomic absorption spectrophotometer. Red cell iron was determined

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Table 1. Comparison of chemical composition of red blood cells of non-anemic and anemic subjects.

Parameters	Group	Mean ± SD
Total carbohydrates (mg/10^{10} RBC's)	Non anemic	14.5 ± 2.5
	Anemic	8.8 ± 3.4
Glucose (mg/10^{10} RBC's)	Non anemic	4.2 ± 0.92
	Anemic	2.2 ± 0.10
Total proteins (mg/10^{10} RBC's)	Non anemic	457.4 ±129.5
	Anemic	331.1 ± 141.6
Creatinine (µg/10^{10} RBC's)	Non anemic	19.9 ± 5.3
	Anemic	10.5 ± 5.5
Uric acid (mg/10^{10} RBC's)	Non anemic	0.37± 0.06
	Anemic	0.21 ± 0.13
Phosphorus (mg/10^{10} RBC's)	Non anemic	4.4 ± 0.77
	Anemic	6.1 ± 1.31
Total cholesterol (mg/10^{10} RBC's)	Non anemic	4.7 ± 1.1
	Anemic	2.6 ± 1.0
Triglycerides (mg/10^{10} RBC's)	Non anemic	6.7 ± 1.6
	Anemic	5.2 ± 1.8
Potassium (mg/10^{10} RBC's)	Non anemic	0.42 ± 0.24
	Anemic	0.15 ± 0.13
Calcium (mg/10^{10} RBC's)	Non anemic	1.9 ± 0.21
	Anemic	1.1± 0.41
Cu^{++} (µg/10^{10} RBC's)	Non anemic	9.5 ± 1.8
	Anemic	3.8 ± 2.0
Zn^{++} (µg/10^{10} RBC's)	Non anemic	11.2 ± 6.1*
	Anemic	9.0 ± 3.4
Reduced glutathione (GSH) (mg/10^{10} RBC's)	Non anemic	3.3 ± 0.56
	Anemic	1.6 ± 0.82
Iron (µg/10^{10} RBC's)	Non anemic	65.7 ± 22.0
	Anemic	18.6 ± 12.3
Ferritin (ng/10^{10} RBC's)	Non anemic	2060.5 ± 2044.5
	Anemic	223.3 ± 123.1

All values are statistically highly significantly different at $P<0.001$, except marked by * which is at $P<0.05$.

by ferrozine method and ferritin by ELISA. All these methods are described in detail by Hemkar (2002).

The results were statistically evaluated by applying Student's ' t ' test (Mahajan, 2006).

RESULTS AND DISCUSSION

Innumerable substances are present in the cytoplasm of the red blood cells. We have measured some of them which include carbohydrates, proteins, lipids, anions, cations, metabolites – creatinine and uric acid and physiologically important GSH and ferritin. All the results are expressed in terms of fixed number of red cells (10^{10}) in order to nullify the effect of reduction in RBC count in IDA. These are shown in Table 1.

Red cell iron and ferritin: The red cell iron and ferritin were measured as they are of paramount importance in hemopoiesis. Both were drastically reduced in IDA. Red cell intracellular concentrations of iron and ferritin are better indicator of iron status as these are not influenced by infection, inflammation, tissue necrosis and tumors, just as plasma iron and ferritin. Also, red cell ferritin, being a residual of erythroblast ferritin, it reflects the balance between the iron supply to the erythroid marrow and need for the hemoglobin synthesis (Guillemin et al., 1993).

Red cell cholesterol and triglycerides: In an earlier study, we found a decrease in red cell membrane cholesterol and increase in total lipids (Sharma and Hemkar, 1999). In that study, cytoplasmic lipids were not measured. Now we have measured red cell intracellular cholesterol and triglycerides after removal of membranes. Both these were significantly decreased in iron deficiency. It may be mentioned that Kirilenko and Paramonova (1990) had found an increase in cholesterol but decrease in triglycerides. It is not clear whether these authors separated membranes from red cells and severity of iron deficiency was also not reported.

Total carbohydrates and glucose: Both the red cell intracellular glucose as well as total carbohydrates were significantly decreased in IDA. It is consistent with our observation of decreased glucose uptake by red cells of IDA (Hemkar, 2002). The decreased glucose in erythrocytes would lead to subdued metabolism.

Total proteins: Total proteins in the red cells of IDA subjects were significantly reduced in IDA. This finding was not unexpected in view of Hb being major protein in the red cells.

Red cell cations: We measured four cations - potassium, calcium, zinc and copper in IDA. All of them were significantly decreased. This observation may be explained on the basis of finding of Yip et al. (1983) that the leakiness of membrane for cations is increased during IDA. The contrary findings of Shimoda and Yawata (1985) about red cell calcium and Seino (1976) about zinc can be explained on the basis of differences in expressing the results. We have used a very scientific unit for comparison that is mg / 10^{10} RBC's. Previous workers have found increased erythrocyte zinc protoporphyrin in IDA (Schifmann et al., 1989). The incorporation of zinc into protoporphyrin to form zinc protoporphyrin would deplete free zinc in the cell.

Phosphorous: The only anion measured in our study was inorganic phosphate which was found to be significantly increased. Since erythrocytes utilize glucose mainly by anaerobic glycolysis, depressed glycolysis is expected to result in reduced 'substrate level phosphorylation'. This may explain increased intracellular inorganic phosphorus found in this study.

Creatinine and uric acid: These two metabolites were measured and were found to be significantly decreased. These changes may be a reflection of decreased muscle mass and decreased nucleoprotein turnover in anemic subjects.

Reduced glutathione: GSH is important for red cell integrity and metabolism. We have found a significantly decreased level in IDA. This observed decrease in GSH may be explained on the basis of lowered regeneration of GSH (Kurata and Suzuki, 1994) or due to reduced amount of ATP in iron deficiency (Acharya and Grimes, 1986) or to both.

This study reports 15 biochemical parameters in erythrocytes in severe IDA. It indicates that iron deficiency and consequent anemia results in profound alteration in the chemical composition of red cells. Most of the studied constituents were decreased probably because of increased leakiness of erythrocyte membrane. It also appears that there is general slowdown of metabolism within the red cells. Future studies might examine this possibility.

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