Indian J Physiol Pharmacol 2000; 44 (4) : 491–494

SOME BIOCHEMICAL CHANGES IN HEME SYNTHESIS IN IRON DEFICIENCY

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(Received on January 18, 2000)

Abstract : Some enzymes and intermediates of heme synthesis were determined in blood and urine of 26 women with severe iron deficiency anemia (IDA). Erythrocyte free protoporphyrin was almost doubled and delta-aminolevulinate dehydrase significantly raised. But urinary excretion of delta-aminolevulinic acid and reticulocyte ferrochelatase were significantly reduced in iron deficiency anemia. Hence these could serve as useful indices of iron deficiency and consequent anemia.

Key words: iron deficiency anemia delta-aminolevulinate dehydrase protoporphyrin ferrochelatase delta-aminolevulinic acid

INTRODUCTION

Anemia is the chief manifestation of iron deficiency. Several parameters are available for diagnosis of iron deficiency anemia (IDA). These include classical parameters, like, erythrocyte morphology, red cell indices, bone marrow iron, serum iron, iron binding capacity, transferrin saturation, and more recent tests-erythrocyte protoporphyrin, serum ferritin, transferrin receptors (1) and zinc protoporphyrin (2).

Iron is known to affect synthesis of heme and free protoporphyrin accumulates in erythrocytes in iron deficiency anemia (IDA) (3). This increase is because of failure of iron incorporation at the level of ferrochelatase. However, this enzyme was never measured in blood in IDA, despite reports of its decrease in human leucocytes (3) and pig heart muscle (4). Therefore, we decided to estimate it.

Another enzyme of heme biosynthesis--delta aminolevulinate dehydrase (ALAD) has been studied in IDA in the past but the reports were conflicting; ranging from normal (5) to higher (6-8) or reduced (3). Similar contradiction was also seen in the reported excretion of delta-aminolevulinic acid (ALA) in urine by iron deficient anemic persons in this study. It was expected that these parameters may prove useful in diagnosis of iron deficiency.

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METHODS

For this study, subjects of iron deficiency were selected from out patient Departments of Mahila Chikitsalaya and Zanana Hospital of Jaipur. The criteria of selection was freedom from infection and febrile disease and their hemoglobin level. The women having hemoglobin below 12 g% were considered anemic and an attempt was made to select women with hemoglobin as low as possible. The subjects of non-anemic control group were selected from among medical students, neighbours and college and hospital staff. Only those purportedly healthy females were selected whose hemoglobin was above 12 g%.

Ten ml of blood was drawn in the morning. Heparinised blood was used for determining hemoglobin, hematocrit, red cell and reticulocyte counts and for preparing peripheral blood film as usual. Serum was used for determining serum iron (SI) and total iron binding capacity (TIBC) (10) on an auto analyzer (Merck, Selectra). Heparinised whole blood was also used for spectrophotometric determination of enzymes 5-aminolevulinate dehydrase (EC 4.2.1.24) (11) and ferrochelatase (EC 4.99.1.1) (12) and for erythrocyte free protoporphyrin (EFP) (10).

The subjects were asked to collect a 24-hour urine sample which was immediately analyzed for 5-aminolevulinic acid (10).

RESULTS AND DISCUSSION

Table I shows hematologic values of non-anemic and anemic women which indicates the existence of severe anemia in anemic group women. That the cause of this anemia was iron deficiency, was indicated by serum iron and percent saturation values (Tables I) and the presence of hypochromic microcytic erythrocytes in peripheral blood films. The values of some enzymes and intermediates of heme biosynthesis are presented in Table II. All were significantly different in IDA group as compared to non-anemic control group.

TABLE I: Hematologic and iron values.

Parameter	*Control group(25)*	*IDA group (26)*
Hb (g%)	12.7±0.32	6.48±0.99*
TRBC (10^6/mm^3)	4.10±0.37	2.72±0.43*
PCV (%)	34.3±2.3	20.5±2.9*
MCV (fl)	84.3±6.6	73.9±7.5*
MCH (pg)	30.1±2.4	23.9±1.8*
MCHC (%)	35.5±1.7	31.6±2.1*
SI (µg/dl)	107.5±9.2	41.6±5.0*
TIBC (µg/dl)	365.1±10.7	396.7±28.9*
PS (%)	29.3±2.7	10.5±1.5*

All values are mean ± S.D.
*Significantly different (P<0.001)

Table II shows that EFP was raised in IDA group. The average value was more than double than the control group. Therefore, the present study confirms that the accumulation of free protoporphyrin in erythrocytes is a useful indication of iron deficiency.

TABLE II: Some parameters of iron deficiency.

Parameter	*Control group(25)*	*IDA group (26)*
ALAD (U/ml blood)	17.2±3.1	44.2±9.7*
ALAD (U/10^6 reticulocytes)	0.26±0.20	0.84±0.08*
Ferrochelatase (U/ml blood)	51.4±12.6	46.3±9.1**
Ferrochelatase (U/10^6 reticulocytes)	2.10±0.59	0.93±0.29*
EFP (µg/dl RBC)	44.7±23.5	93.6±35.3*
Urinary ALA (mg/24hrs)	5.9±1.2	3.1±1.2*

All values are mean ± S.D.
*Highly significant; P<0.001
**Insignificant; P>0.05

The ferrochelatase activity in blood has not been reported so far. We have for the first time reported significantly decreased level of ferrochelatase in iron deficiency anemia when expressed as units/10^6 reticulocytes (Table–II) The decrease in enzyme is compensated by increase in number of reticulocytes, hence the activity when expressed as units/ml blood did not attain significant limits.

The behaviour of ALAD in iron deficiency was just opposite to that of ferrochelatase. ALAD was increased significantly in iron deficiency, whether expressed as units/ml blood or units/10^6 reticulocytes (Table–II). Earlier reports on ALAD in iron deficiency in humans were contradictory (3, 5–8). This variation may be due to differences in the techniques employed, severity of iron deficiency/ anemia and reticulocytosis, etc. The increase in ALAD is supported by recent report that ALAD is also inhibited by heme (13).

The utility of ALAD and ferrochelatase as sensitive determinants of iron deficiency state is substantiated by highly significant correlation between hemoglobin, serum iron and percent saturation on one hand, and ALAD as U/ml blood ($r=-0.95$, -0.91 and -0.90, respectively, giving $P<0.001$) and ferrochelatase (U/10^6 reticulocytes) on the other hand ($r = +0.85$, $+0.82$, and $+0.81$, respectively, giving $P< 0.001$) in the pooled data of control as well as IDA group.

As can be seen in the Table–II, the value of urinary ALA was found to be significantly reduced. The earlier reports on ALA excretion by iron deficient persons were contradictory (6,7,9). The report of genetic regulation of synthesis of ALA synthase by iron (14) gives more convincing evidence that in iron deficiency ALA synthase activity is reduced which would result in decreased ALA synthesis.

We feel that out of all these parameters, activities of ALAD and ferrochelatase, deserve to be investigated further in iron deficiency and anemia for their utility for diagnosis and differential diagnosis. The former because of simple method of estimation and ferrochelatase because the enzyme protein is not only decreased *per se,* but iron is a cosubstrate of the reaction and the enzyme is inhibited by increased concentration of protoporphyrin (15).

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