IRON STORE AND FREE RADICALS IN THALASSEMIA

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ABSTRACT

Hyper-ferrous state is the result of multiple transfusions, whereby the iron excess in such conditions may be toxic to the organism. Patients suffering from thalassemia receive multiple transfusions. In the eastern part of India, both β^0 -thalassemia and heterozygous E β -thalassemia occur. This paper expresses the hyper-ferrous state, leading to iron overload in these two conditions and also reports the increased generation of free radicals, consequent to the iron overload.

KEY WORDS

Thalassemia; Ferritin; Malondialdehyde.

INTRODUCTION

All metals including iron are toxic to the organism. But iron is also an important nutritional metal for many physiological functions (1). Therefore, iron status is tightly controlled so that the physiological functions are not disturbed and excess iron is protein bound. Since there is virtually no excretion of iron in the urine, and very little iron is present in the stools the regulation of iron status is achieved by varying the iron absorption from the gut (2). However, persons receiving multiple transfusions as part of the treatment for thalassemia, are faced with problem of iron overload and consequent metabolic derangements. Under normal circumstances there is virtually no free iron. All irons are protein bound. Since, there is a need for iron in all tissues, some of the protein bound iron is taken up by receptors for the plasma transferrins, which eventually are internalised and the iron is made available to the tissues. In patients receiving multiple transfusion e.g., in thalassemia, the iron liberated from the haemoglobin saturate the transferrins, and the transferrins transfer the iron on to a storage iron protein called apoferritin. The ferritin iron is available to the organism in times of need. When the ferritin is saturated, another storage iron protein called hemosiderin is formed. The formation of hemosiderin is eventually controlled. During the course of metabolism, a superoxide anion is produced. Normally the superoxide anion is converted by the ubiquitous enzyme superoxide

dismutase to produce H_2O_2 (3). The H_2O_2 is converted to innocuous compounds by the action of catalase and peroxidase. But if free ferrous iron is available it reacts with H_2O_2 to produce hydroxyl radical which is an extremely reactive species leading to depolymerisation of polysaccharide (4), DNA strand breaks (5) inactivation of enzymes and initiation of peroxidation (6). The availability of Fe^{2+} is made possible by the reaction of superoxide radical entering the ferritin core through the hydrophobic channel causing reduction of Fe^{3+} to Fe^{2+} (7, 8).

Excess ferritin is present in multiple transfused thalassemic patients. All the above phenomena can be shown to occur in these patients. Since there are two syndromes (β^0 -thalassemia and heterozygous HbE, β -thalassemia), it was of interest to see whether quantitatively, same metabolic derangements are present in the two kinds of thalassemia. We report that the metabolic derangements are the same and related to the number of transfusions in the two conditions.

MATERIALS AND METHODS

Twenty normal subjects, 25 E β -thalassemia and 22 β^0 -thalassemia patients were the subjects of the investigations. Hemoglobin was measured by cyanmethaemoglobin method (9, 10). Blood was analyzed for serum lipid peroxide by quantitative assay of MDA as thiobarbituric acid reactive substances (11). The serum ferritin quantitative test is based on a solid phase enzyme-linked immuno-sorbent assay, using a kit supplied by Monobind Inc, CA, USA. Results were analyzed by Student's t-test (12). Results were deemed statistically significant where $p < 0.05$.

RESULTS AND DISCUSSION

The results are summarized in Table 1.

Hemoglobin : E β -thalassemia patients had received

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Table 1. Biochemical investigations in blood of thalassemia patients and control group

Status (No. of subjects)	Hemoglobin (in gm/100 ml)	Ferritin (in µg/L)	Malondialdehyde (in nmol/ml)
Control (20)	14.8 ± 1.23	144 ± 126	0.86 ± 0.36
Eβ-thalassemia (25)	7.9 ± 2.10	436 ± 28	2.46 ± 1.03
β ⁰ -thalassemia (22)	8.1 ± 1.63	854 ± 186	2.05 ± 1.17

Values represent mean ± SD.

an average of 7.5 annual transfusions. But hemoglobin content was much lower than an ideal of 12 gm/100 ml. Thalassemia patients had received 12-14 transfusions annually but still the hemoglobin content was lower than 12 gm/100 ml.

Ferritin : Serum ferritin content varied widely in normal persons. In EB-thalassemia patients the ferritin level was three times the normal and the standard deviations were much lower than normal. In β⁰-thalassemias the serum ferritin content was 6 times the normal.

Malondialdehyde : In EB-thalassemia the malondialdehyde contents were also three times the normal as the serum ferritin concentrations. In β⁰-thalassemia, however, the malondialdehyde contents were 2.5 times that of normal, although ferritin content was 6-times that of normal. In thalassemia it is possible that the very high ferritin had resulted in saturation of the apoferritin level and concomitant production of hemosiderin, where the iron was protein bound and therefore could not result in increased production of free oxygen radicals.

That thalassemic patients who had multiple transfusions had increased serum iron and ferritin levels, are well known (13). This is true for both β⁰-thalassemia and Eβ⁰-thalassemia. Compared amongst themselves, β-thalassemics had higher ferritin contents than Eβ⁰-thalassemics. An apoferritin molecule can bind about 100 atoms of iron. When the iron contents are very high, perhaps the total apoferritin levels approach a saturated condition and hemosiderin takes care of the excess iron. In Eβ-thalassemia the normal amount of superoxide ions can draw iron from the ferritin molecule and the free iron can induce the formation of OH⁰ radicals by Fenton and Haver Weiss reactions, which can induce further toxic damage. However, since the number of transfusions were more in β⁰-thalassemia than in heterozygous Eβ-thalassemia, the production of ferritin and malondialdehyde were more in the β⁰-

thalassemia.

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