INCREASING AND DECREASING PHASES OF FERRITIN AND HEMOSIDERIN IRON DETERMINED BY SERUM FERRITIN KINETICS

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ABSTRACT

We attempted to clarify the mechanism of the storage iron metabolism. A new program of serum ferritin kinetics was applied for studying the increasing and decreasing phases of ferritin and hemosiderin iron in iron addition and removal in patients with a normal level of iron stores or iron overload. The change of ferritin iron in response to iron addition and removal was rapid in the initial stage, but it was slow later. In contrast, the change of hemosiderin iron was slow in the initial stage, but it became rapid later. These changes of ferritin and hemosiderin iron suggest that the turnover of ferritin iron is preferential to that of hemosiderin iron, and that the initially existed ferritin iron is gradually replaced by the ferritin iron recovered by taking iron from hemosiderin in iron mobilization. The crossing of the increasing curves of ferritin and hemosiderin iron in iron addition indicates a switching of the principal storage iron from ferritin to hemosiderin. The crossing point shifted toward a higher storage iron level in the increase of iron deposition. Iron storing capacity can be increased not only by the transformation of ferritin into hemosiderin, but also by the expansion of cell space as seen by hepatomegaly in hereditary hemochromatosis. The amounts of hemosiderin iron exceeded ferritin iron in all 10 patients with chronic hepatitis C even though they had normal storage iron levels. This suggests it is difficult to store iron in the form of ferritin in chronic hepatitis C.

Key Words: Serum ferritin kinetics, Recovery of ferritin iron, Transformation of ferritin into hemosiderin, Increasing and decreasing phases of iron stores, Iron in chronic hepatitis C

INTRODUCTION

Recent advances in medicine clarified the importance of storage iron metabolism in relation to the etiology of various disorders in iron deficiency and overload, especially in the latter.

Shoden et al.[1] pioneered the biochemical studies of ferritin and hemosiderin iron metabolism

in 1953, and a revolutionary advance in the clinical study of iron metabolism came with the introduction of the radioimmunoassay of serum ferritin by Addison *et al.*[2] in 1972. However, the clinical determination of ferritin and hemosiderin iron was impossible thereafter for 40 years due to the lack of a method for doing so.

The serum ferritin level before iron addition or removal reflects a static level of tissue ferritin iron. However, a static single value of serum ferritin cannot reveal the dynamic behaviors of ferritin and hemosiderin iron. Therefore, we followed up the increasing and decreasing curves of serum ferritin to observe storage iron turnover. After an analysis of the serum ferritin decrease curve, we came to assume that it was composed of the two elements; the decreasing and increasing (recovering) component. This reflected the recovery of tissue ferritin by removing iron from hemosiderin. The results of the study for determining ferritin iron and hemosiderin iron from the serum ferritin decrease curve were thought to verify our assumptions because no evidence contradicting the above described assumptions was encountered.

By the computer-assisted simulation of the serum ferritin decrease curve, we confirmed two iron-pathways[3]: one from ferritin to hemosiderin in iron deposition and the other from hemosiderin to ferritin in iron mobilization out of 9 iron-pathways as proposed by Shoden *et al.*[1] Furthermore, we measured the efficacy of iron removal by the iron chelating agent deferasirox in iron overloaded patients with transfusion-dependent anemia (TD) from serum ferritin balance in iron addition by transfusion.

Although the serum ferritin kinetics[3] as mentioned above brought about a marked advance in the understanding of ferritin and hemosiderin iron metabolism, the increasing and decreasing phases of ferritin and hemosiderin iron remained unclear.

Therefore, we attempted to clarify the increasing and decreasing phases of ferritin and hemosiderin iron during iron addition and removal with a new method for measuring serum ferritin kinetics.

Here we explain our terminology.

We classified patients into three groups by the amount of storage iron: iron deficiency, iron normal, and iron overload, (Table 1).

"Normal iron stores" defines the level of iron storage between iron deficiency and iron overload unrelated to the disease and body iron distribution.[3] Generally, it is recognized that iron stores in normal males are around 0.5 to 1.0 g and those in females are 1/3 to 1/4 times smaller than males.

The border between the state of iron decrease within the normal iron stores and iron deficiency is clear cut, but that between the state of iron increase within normal iron stores and iron overload is not. Therefore, we made a transitional zone between normal iron increase and iron overload.

The clinical symptom of iron overload appears when serum ferritin exceeds 1000 ng/ml.

Table 1 Classification of iron status by the level of iron stores

Iron deficiency ± symptom		< decrease	Normal iron stores	increase <		Iron overload ± symptom	
Serum ferritin	ng/ml	< 12		250 to 500 <			
Iron stores	g	< 0.1		2.5 to 5.0 <			
Hemosiderin*		−	−	±	+	++	+++

* Microscopically detectable hemosiderin granules

PATIENTS AND METHODS

Permission by Ethics Committee

Study programs of iron removal therapy for hepatitis and for the transfusional iron overload were permitted by the Ethics Committee of Nagoya University Hospital.

Patients

We studied 10 patients with chronic hepatitis C (CHC), 1 patient with iron deficiency anemia (IDA) after treatment by intravenous iron injection (TIDA), 8 patients with hereditary hemochromatosis (HH),[4 - 6] and 2 patients with transfusion-dependent anemia (TD): patient #1 with myelodysplastic syndrome (MDS) and myelofibrosis, and patient #2 with aplastic anemia. The patient with IDA, after treatment by intravenous iron injection (TIDA), was losing iron by constant intestinal bleeding. Except for this case, all other patients with blood loss were excluded from the present study.

Patient data other than HH were offered from co-authors at Nagoya University Hospital and affiliated Institutes. We selected patients whose iron stores were determined and followed up with a period sufficient for determining serum ferritin decrease or increase curve.

Authors[4, 5, 6] did not refer to gene examination of HH. Hemochromatosis-related genes were not examined for TD and IDA with normal storage iron level before they became ill. Such genes were examined but not detected in patients with CHC.

Methods

Iron stores

Iron stores were calculated from the ratio of iron to hemoglobin in the phlebotomized[7] or transfused blood. The information on the iron content in the injected colloidal iron preparation was offered from its manufacturer. During the period of iron removal, patients were instructed to take a diet low in iron.

Serum ferritin[2, 7 - 9]

Serum ferritin was determined by enzyme-immunoassays using commercially available assay kits. The Fujirevio Incorporated (Tokyo, Japan) kit was used at Nagoya University Hospital, and the Denka Seiken (Tokyo, Japan) kit at the National Hospital Organization Nagoya Medical Center. Inter-assay correction was performed for the above assay systems.

Ferritin iron and hemosiderin iron

Ferritin iron and hemosiderin iron were determined by serum ferritin kinetics following the method introduced by Saito *et al.*[3] Serum ferritin kinetics is based on the series of serum ferritin assay data obtained in the course of iron addition or iron removal.

Iron removal

Patients with HH[4 - 6] and CHC were treated by phlebotomy.[4 - 7, 10, 11] and patients with TD were treated orally with the iron chelating agent deferasirox (Exjade),[12] a product of Novartis Pharma (Basel, Switzerland).

Iron administration

One patient with IDA was treated by intravenous iron injection.

Hiroshi Saito *et al.*

Intestinal blood loss

Intestinal blood loss was detected by fecal immunochemical occult blood tests and macroscopic findings. The blood loss[13] of the patient with IDA was calculated by dividing the amount of intravenously injected iron using the term of duration of non-iron deficiency state following the patient after intravenous iron injection.

Determination of increasing and decreasing phases of ferritin iron and hemosiderin iron

The crossing point of a straight serum ferritin decrease curve and a horizontal axis scaled for iron stores indicates the amount of ferritin iron.[3] However, this measure cannot display the decreasing phases in ferritin iron and hemosiderin iron. Therefore, we introduced a new program for determining the increasing and decreasing phases of ferritin iron and hemosiderin iron in this study.

To find the best curve to fit the assay dots of serum ferritin increase or decrease and to obtain the increasing and decreasing phases of serum ferritin, we used a spreadsheet program.

The increasing phases of ferritin and hemosiderin iron were determined by the following formula of proportional allotment:

Hemosiderin iron (g) = Total iron stores (g) x Transformed serum ferritin* (ng/ml) / {Last value of serum ferritin (ng/ml) + Last value of transformed serum ferritin* (ng/ml)}

Ferritin iron (g) = Total iron stores (g) x Serum ferritin (ng/ml) / {Last value of serum ferritin (ng/ml) + Last value of transformed serum ferritin* (ng/ml)}

*Transformed serum ferritin reflects the expelled amount of tissue ferritin iron from the ferritin iron pool to the hemosiderin iron depot in iron addition.

The decreasing phases of ferritin and hemosiderin iron were determined by the following formula of proportional allotment:

Hemosiderin iron (g) = Total iron stores (g) x Cumulative serum ferritin recovery (ng/ml) / {Initial value of serum ferritin (ng/ml) + Last value of cumulative serum ferritin recovery[3] (ng/ml)}

Ferritin iron (g) = Total iron stores (g) x Serum ferritin (ng/ml) / {Initial value of serum ferritin (ng/ml) + Last value of cumulative serum ferritin recovery (ng/ml)}

Cumulative serum ferritin recovery reflects the cumulative tissue ferritin iron increased (recovered) by removing iron from hemosiderin. Therefore, the cumulative tissue ferritin recovered corresponds to the cumulative hemosiderin iron removed.

RESULTS

Increasing phases of ferritin iron and hemosiderin iron

The increase of serum ferritin was prompt and large initially, but the increase rate was gradually reduced along with the increase of storage iron in iron addition (Fig. 1).

Letsky *et al.*[14] observed 24 children with thalassaemia undergoing regular blood transfusion and examined the relationship between serum ferritin and units of transfused blood. We calculated the transfused blood iron from their data of 23 patients, and produced a fitted curve to their data points. Then, we obtained the increasing curves of ferritin iron and hemosiderin iron which were the same as those of patient #1 with TD (Fig. 2). The crossing point of the increasing curves was located at 25 g of iron stores (Fig. 2) (Table 2).

Decreasing phases of ferritin and hemosiderin iron

The curves of serum ferritin decrease and cumulative recovery in the course of iron removal
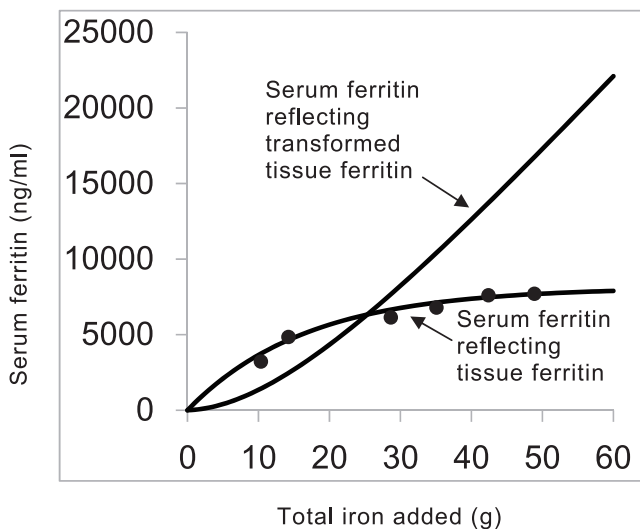
FERRITIN AND HEMOSIDERIN IRON METABOLISM

Fig. 1 Increasing curves of serum ferritin determined in the course of transfusional iron addition in patient #1 (Table 2) with transfusion-dependent anemia (TD) with iron overload.
The convex increasing curve of serum ferritin reflects that of tissue ferritin iron. The concave increasing curve of serum ferritin reflects the cumulative transformed tissue ferritin. Black dots show assayed values of serum ferritin.
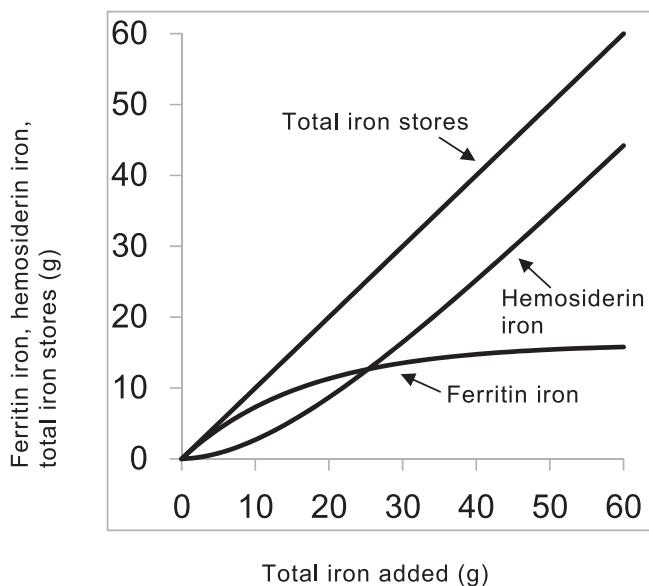

Fig. 2 Increasing phases of ferritin iron, hemosiderin iron and total iron stores determined in the course of iron addition in patient #1 (Table 2) with transfusion-dependent anemia (TD) with iron overload.

in patient #1 with CHC are shown in Fig. 3. Then, we obtained the decreasing curves of tissue ferritin iron and hemosiderin iron in the course of iron removal in patient #1 with CHC (Fig. 4). Fig. 4 was produced using the data of Fig. 3.

Table 2 Cases with a crossing point on the increasing (↑) or decreasing (↓) curves of ferritin iron and hemo-siderin iron in patients with transfusion-dependent anemia (TD) and hereditary hemochromatosis (HH) Serum ferritin, ferritin iron and hemosiderin iron are the values before iron removal.

Disease	Patient No.	Sex	Serum ferritin ng/ml	Ferritin iron g	Hemosiderin iron g	Crossing point g
TD	1	F	8000	11	<15	25↑, 28↓
TD[14]	a group of 23 cases					25↑
TD	2	M	1700	3	> 1.5	3↓
HH[4]	3	M	3000	7	> 6	12↓
HH[4]	4	M	4000	6.5	= 6.5	13↓
HH[4]	5	M	5000	8	> 7	12↓

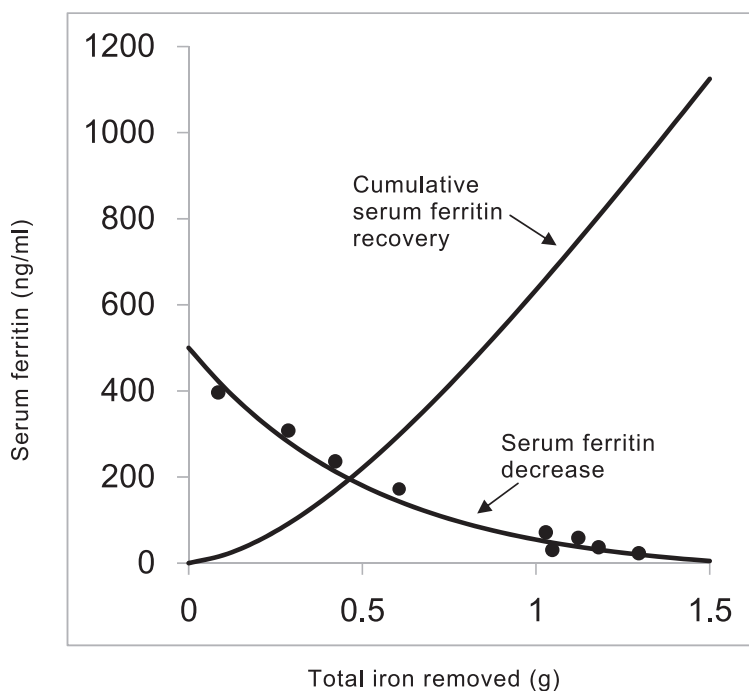Reference numbers are shown as disease name superscript nos.

Fig. 3 A concave serum ferritin decreasing curve and a convex cumulative serum ferritin recovering curve determined in the course of iron removal by phlebotomy in patient #1 (Table 4) with chronic hepatitis C (CHC)
Black dots show assayed values of serum ferritin.

A large difference was observed between the decreasing curves of ferritin iron and hemosiderin iron (Fig. 4). The decreasing curves did not cross in all 10 cases with CHC.

The decrease of tissue ferritin iron was greater than that of hemosiderin iron initially, but it became smaller later. The removal of hemosiderin iron (recovery of tissue ferritin iron) was accelerated progressively and became largest before the exhaustion of hemosiderin iron (Fig. 4 to Fig. 6).

Hemosiderin iron co-existed with ferritin iron from the initial to the last stage at a storage iron level near zero (Fig. 4 to Fig. 6).
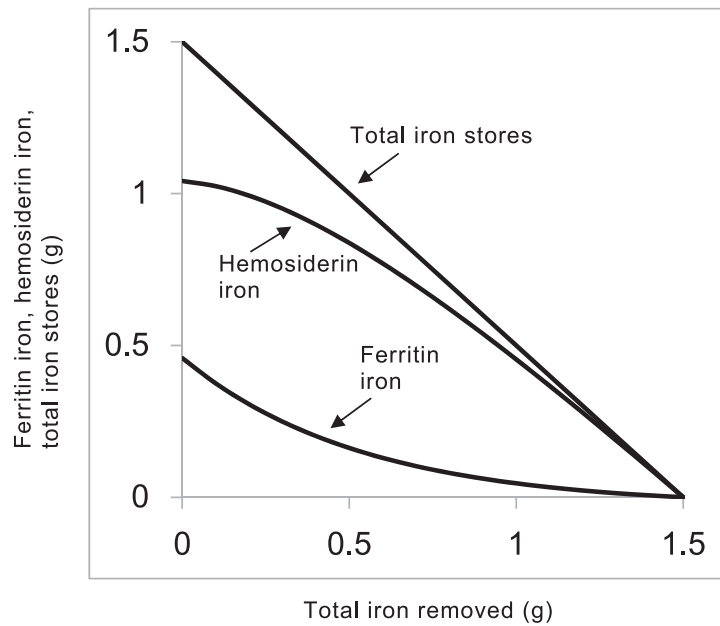
Fig. 4 Decreasing phases of ferritin iron, hemosiderin iron and total iron stores in the course of iron removal in patient #1 (Table 4) with chronic hepatitis C (CHC).
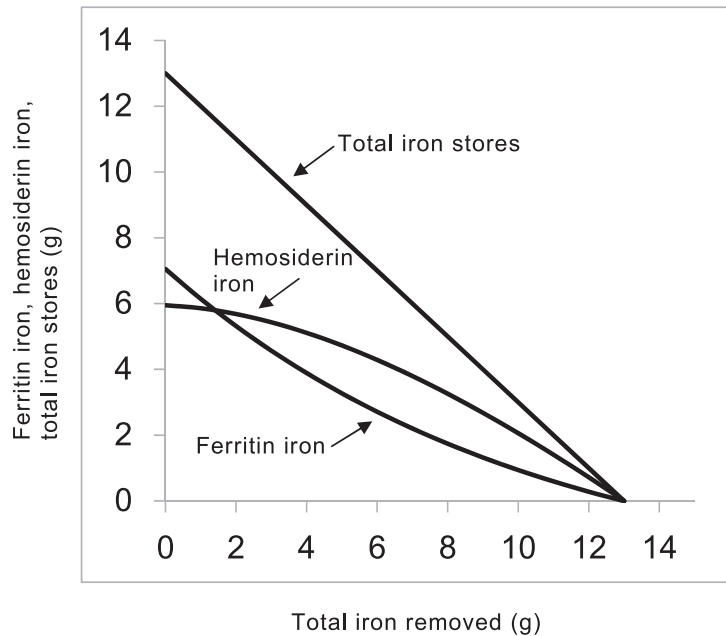

Fig. 5 Decreasing phases of ferritin iron, hemosiderin iron and total iron stores determined in the course of iron removal by phlebotomy in patient #3 (Table 2) with hereditary hemochromatosis (HH).
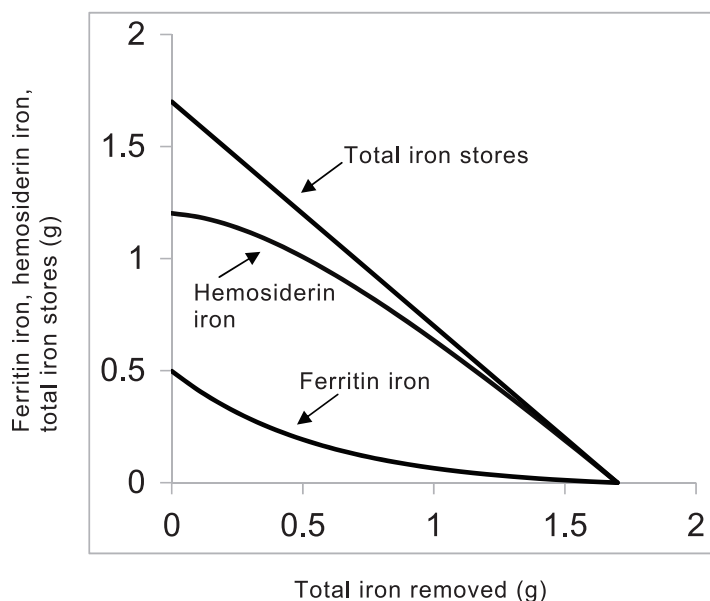
Hiroshi Saito *et al.*

Fig. 6 Decreasing phases of ferritin iron, hemosiderin iron and total iron stores determined in the course of iron removal by blood loss in patient with treated iron deficiency anemia (TIDA).

Table 3 Cases with hereditary hemochromatosis (HH) without a crossing point on the decreasing curves of ferritin iron and hemosiderin iron
Serum ferritin, ferritin iron and hemosiderin iron are the values before iron removal.

Disease	Patient No.	Sex	Serum ferritin ng/ml	Ferritin iron g	Hemosiderin iron g	Crossing point g
HH[4]	1	M	10000	8	<22	none
HH[4]	2	M	6000	8	< 9	none
HH[5]	6	M	1800	1	< 3	none
HH[5]	7	M	4000	4	<10	none
HH[6]	8	M	2200	2	< 5	none

Reference numbers are shown as disease name superscript nos.

    The decreasing phases of tissue ferritin iron and hemosiderin iron in patient with TIDA are shown in Fig. 6. A large difference between the decreasing curves of ferritin iron and hemosiderin iron similar to Fig. 4 was observed (Fig. 6).
    The crossing point on the decreasing curves of ferritin iron and hemosiderin iron was located at 3 g in patient #2 with TD, and at 12, 12 and 13 g in patient #3 to 5 with HH.
    The crossing point of the decreasing curves appeared in the cases when the amount of initial ferritin iron was larger or equal to that of initial hemosiderin iron before starting iron removal (Table 2).
    The crossing point did not appear on the decreasing curves of ferritin iron and hemosiderin iron in 5 patients with HH, nor in any patient with TD and the 10 patients with CHC, whose hemosiderin iron content was larger than ferritin iron before starting iron removal (Fig. 2, 4, 5 and 6) (Table 3) (Table 4).
    A linear correlation was observed between the crossing points and total iron stores.
    The increasing and decreasing curves of ferritin and hemosiderin iron did not show a mirror

FERRITIN AND HEMOSIDERIN IRON METABOLISM

Table 4 Cases with chronic hepatitis C (CHC) without a crossing point on the decreasing curves of ferritin iron and hemosiderin iron

Serum ferritin, ferritin iron and hemosiderin iron are the values before iron removal.

Disease	Patient No.	Sex	Serum ferritin ng/ml	Ferritin iron g	Hemosiderin iron g	Crossing point g
CHC	1	M	500	0.5	< 1.0	none
CHC	2	M	225	0.3	< 0.8	none
CHC	3	M	216	0.3	< 0.7	none
CHC	4	M	200	0.2	< 0.7	none
CHC	5	M	152	0.4	< 0.7	none
CHC	6	M	145	0.4	< 0.6	none
CHC	7	M	132	0.3	< 0.4	none
CHC	8	M	128	0.3	< 0.4	none
CHC	9	M	81	0.1	< 0.3	none
CHC	10	M	28	0.1	< 0.3	none
Mean ± SD			181 ± 58	0.3 ± 0.1	0.6 ± 0.2	
Log Mean			144			
Range			28 to 500	0.1 to 0.5	0.2 to 1.0	

image, but rather an image reversed upside down in the opposite side; they showed convex ferritin and concave hemosiderin iron increasing curves versus concave ferritin and convex hemosiderin iron decreasing curve (Fig. 2) (Fig. 4 to Fig. 6).

DISCUSSION

Relationship between serum ferritin and iron stores

Serum ferritin may render a value higher than the actual storage iron level in patients with various inflammations, malignancies and hyperferritinemia cataract syndromes. Therefore, we excluded such suspected overestimation cases by clinical symptoms and examinations, such as C-reactive protein, transaminase and others. Despite such disadvantages, serum ferritin has been evaluated as an index superior to transferrin saturation and total iron-binding capacity[15] not only for the differential diagnosis of iron deficiency anemia from other hypochromic anemias, but also for the diagnosis of iron overload.

Iron stores are composed of ferritin iron and hemosiderin iron. Serum ferritin is originated from tissue ferritin. Therefore, serum ferritin before iron addition or removal reflects tissue ferritin iron, but not hemosiderin iron.

In iron addition, the serum ferritin increase curve may be taken to be the curve composed of a single component, considering that the expulsion of overproduced tissue ferritin iron is a one-way process with no return in iron addition. However, the serum ferritin increase curve is composed of the sum of two elements: an increasing component and a decreasing (dumping) component. In iron addition, reduction in the increasing pace of serum ferritin, along with the increase of iron stores, reflects the increased transformation of tissue ferritin iron into hemosiderin iron.[3, 16, 17] In iron removal, the serum ferritin decrease curve is also composed of the sum of two elements: a decreasing component and a recovering (increasing) component.[3] Cumulative serum ferritin recovery reflects the sum of tissue ferritin iron increased (recovered) by removing iron from hemosiderin; i.e., the amount of cumulative hemosiderin iron removed.[3]

The mixing of pre-existing ferritin with recovered ferritin occurs during the process of iron removal. However, such a mixing does not occur in iron addition.

Increasing phases of ferritin iron and hemosiderin iron

A rapid increase of serum ferritin in the early stage of iron addition indicates active synthesis of ferritin iron. The reduction of the increasing speed of serum ferritin found later in iron addition is caused by the transformation of ferritin iron into hemosiderin iron.[16, 17] This means that there is an expulsion of overproduced and denatured tissue ferritin iron arising through the limitation of iron stored in the form of ferritin.[1, 3] and keeping a dynamic balance between ferritin iron and hemosiderin iron.

Although the iron storage in the form of ferritin is limited, the storage of iron in the form of hemosiderin are practically limitless as shown by the gradual declination of increase in ferritin iron and a nearly linear increase in hemosiderin iron in the iron overload region in iron deposition.[1, 3]

Iron storing capacity can be expanded by increasing the iron storing cell space as seen by hepatomegaly in iron overloaded HH.[18] The shift of the crossing point to a higher storage iron level indicates an increase in iron storing capacity in response to the increase in iron deposition.

The crossing point of the increasing curves of ferritin iron and hemosiderin iron indicates that there is a switching of the principal storage iron from ferritin to hemosiderin.

Decreasing phases of ferritin and hemosiderin iron

The decrease of ferritin iron in the early stage of iron removal was rapid, whereas that of hemosiderin iron was slow. This means that removing ferritin iron is easier than removing hemosiderin iron. Rapid ferritin iron decrease suggests the direct iron removal of pre-existing tissue ferritin iron, and the slow decrease of hemosiderin iron suggests the indirect removal of hemosiderin iron.

In iron removal, hemosiderin iron decreased faster in the late stage than in the early one. This corresponds to the slower depletion of tissue ferritin iron in the late stage than the early one. Pre-existing tissue ferritin iron seems to be replaced with the ferritin iron recovered by removing iron from hemosiderin in the late stage.

The high proportion of hemosiderin iron in the patient with TIDA seems to be related to the increased concentration of colloidal iron in the phagocytes.

The hemosiderin iron level was higher than ferritin iron in all patients with CHC, although ferritin iron was slightly higher than the hemosiderin iron in the normal level of iron stores.[1, 3] The above-described data suggest the difficulty of storing iron in the form of ferritin under the milieu interne of CHC.

The increasing curves of ferritin and hemosiderin iron in iron deposition were reversed to the decreasing curves of those in iron removal. Such a reversion corresponds to the direction of iron flow from ferritin to hemosiderin in iron deposition, and to that from hemosiderin to ferritin in iron mobilization.[3]

To our knowledge, this is the first report to disclose the increasing and decreasing phases of ferritin iron and hemosiderin iron in iron addition and removal.

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