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Koji Nishiya, *Okayama University*  
Hideki Matsueda, *Okayama University*  
Toshiaki Shirakami, *Okayama University*  
Makoto Hatano, *Okayama University*  
Keisuke Yano, *Okayama University*  
Toshiro Ogura, *Okayama University*  
Michio Takaoka, *Okayama University*  
Yoshio Hiraki, *Okayama University*  
Kaname Aono, *Okayama University*  
Hidemitsu Ezawa, *Okayama University*  
Zensuke Ota, *Okayama University*

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## Abstract

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**KEYWORDS:** rheumatoid arthritis, serum ferritin, urinary ferritin, gold therapy

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## SERUM AND URINARY FERRITIN LEVELS IN PATIENTS WITH RHEUMATOID ARTHRITIS

Koji NISHIYA, Hideki MATSUEDA, Toshiaki SHIRAKAMI\*, Makoto HATANO, Keisuke YANO, Toshiro OGURA, Michio TAKAOKA, Yoshio HIRAKI\*, Kaname AONO\*, Hidemitsu EZAWA\*\* and Zensuke OTA

*Departments of Internal Medicine and \*Radiology, Okayama University Medical School and \*\*Kurashiki-Kosai Hospital, Okayama, 700 Japan*

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*Abstract.* The serum and urinary ferritin levels in 52 RA patients were measured by the 2-site immunoradiometric assay method. Serum ferritin levels in RA patients correlated with C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) but not with serum iron levels and hemoglobin concentrations, although they were within the normal range. High serum ferritin levels were associated with sera with hyper  $\gamma$ -globulin and rheumatoid factors. In sequential studies, serum ferritin changed in parallel with ESR, CRP and disease activity in a majority of the patients. The urinary ferritin levels and u/s ratios in some RA patients were higher than control values. Higher values were found particularly in the group of patients under gold therapy but not in groups under other treatments.

*Key words :* rheumatoid arthritis, serum ferritin, urinary ferritin, gold therapy.

Significant deposition of iron and ferritin, an iron storage protein, in synovial tissue of patients with rheumatoid arthritis (RA) has been described (1-4). Recently, the amount of iron and ferritin in synovial tissue has been reported to correlate with disease persistency and activity in RA patients (5). In normal subjects, the serum ferritin (s-ferritin) concentration correlated with the amount of iron stored in the body (6) and increased as acute phase reactants in inflammation (7) and infection (8). The interpretation for s-ferritin concentration in RA patients was complicated since the majority of those patients had anemia of unknown causes (9) and, coincidentally, chronic inflammation in joints. Two opposite papers have been presented. One proved the correlation of s-ferritin levels with the amount of iron in bone marrow of RA patients which reflected total iron storage in body (10-13) and the other showed s-ferritin concentration as an indicator of disease activity (14-16).

An increase in urinary ferritin (u-ferritin) concentration has been found in patients with hemolytic anemia (17, 18) and urinary tract malignancy (19). The local ferritin production in kidney could be stimulated by hemoglobin deposition containing iron in hemolytic anemia. Instead of iron, the cluster formation of ferritin in renal tubules after lead injection has been demonstrated in experimental

animals (20).

In this study, we examined whether s-ferritin levels correlate with anemia or disease activity and measured the u-ferritin concentrations in RA patients under gold therapy.

#### MATERIALS AND METHODS

*Patients.* Fifty-two patients (49 female, 3 males; average age, 50 years, ranging from 17 to 70) with definite or classical RA meeting the ARA criteria (21) were studied. The average of disease duration of these patients was 8.8 years, ranging from 0.5 to 26 years. At the time of investigation, 20 patients were receiving nonsteroidal anti-inflammatory drugs (NSAID) alone, 15 : gold therapy, 12 : D-penicillamine, and 5; NSAID with steroid hormone. The s- and u-ferritin levels in 23 healthy volunteers (11 males, 12 females; average age, 31 years, ranging from 24 to 43) were measured as controls.

*Measurement of serum and urinary ferritin concentration.* The serum ferritin concentration was measured by the 2-site immunoradiometric assay (22), using SPAC Ferritin Kits (Daiichi Radioisotope Corporation). Briefly, tubes coated with rabbit anti-human liver ferritin antibody were incubated with 25  $\mu$ l of the test serum and 0.5 ml of phosphate buffered saline (PBS) for 2-3 hours at room temperature. The tubes were washed with physiological saline.  $^{125}$ I-labelled anti-ferritin antibody was added to the tubes and incubated overnight at room temperature. The tubes were washed with physiological saline and counted with a  $\beta$ -counter. Normal values of serum ferritin from a company survey were  $59.0 \pm 63.0$  ng/ml (Mean  $\pm$  SD, n = 43) for males and  $31.1 \pm 50.0$  ng/ml (n = 48) for females.

Urinary ferritin levels were measured by the same method as s-ferritin. The urine from one-time urination was used according to ref. 19 since there is no significant difference in u-ferritin levels between urine samples from one-time urination and from 24 h total urine.

*Laboratory tests.* Hemoglobin, serum iron levels, erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP) were measured when patients visited the clinic according to routine practices.

*Statistical analysis.* The statistical significance among the groups was analysed by Student's t test.

#### RESULTS

*Correlation between serum ferritin levels and CRP, ESR.* The average serum ferritin level of the 52 patients was  $36.7 \pm 28.9$  ng/ml (Mean  $\pm$  SD, n = 165). This value was not significantly different from that for the controls of the company survey ( $31.1 \pm 50.0$  ng/ml, n = 48). The serum ferritin concentration was compared with CRP and ESR. There was a close correlation between s-ferritin levels and the amount of CRP and ESR, as shown in Figs 1 and 2.

*Correlation between serum ferritin levels and RA test,  $\gamma$ -globulin.* Hyper- $\gamma$ -globulinemia and positive rheumatoid factor were commonly found in RA patients. Ferritin levels in sera with more than 2 g/dl of  $\gamma$ -globulin ( $65.5 \pm 48.3$  ng/ml, n = 11) were higher than in sera with less than 2 g/dl of  $\gamma$ -globulin ( $29.8 \pm 24.8$  ng/ml, n = 24) (Table 1.).

Thirty-eight out of 161 samples showed a negative RA test during the clinical

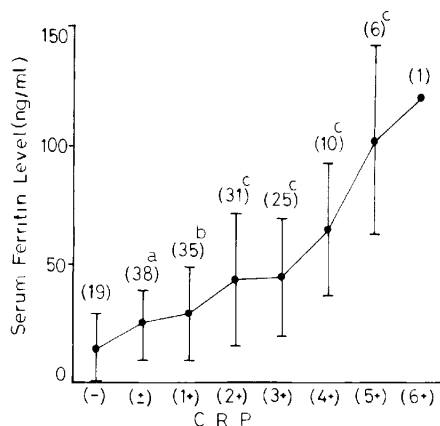


Fig. 1. Correlation between serum ferritin levels and C-reactive protein in RA patients. Each point represents the mean  $\pm$  SD. The s-ferritin levels in each CRP-positive group compared with the values of the CRP-negative group by Student's t test. (a :  $p < 0.05$ , b :  $p < 0.01$ , c :  $p < 0.001$ ). ( ) : number of tests.

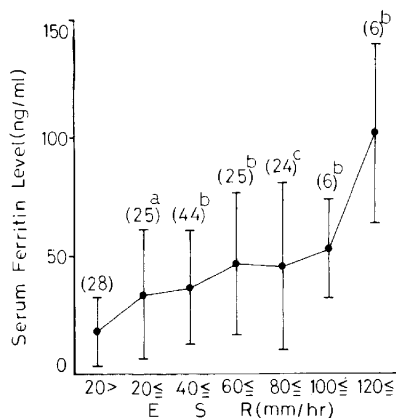


Fig. 2. Correlation between serum ferritin levels and ESR in RA patients. Each point represents the mean  $\pm$  SD. The s-ferritin levels in each group compared with values of the group with less than 20 mm/h of ESR by Student's t test. (a :  $p < 0.02$ , b :  $p < 0.001$ , c :  $p < 0.005$ ). ( ) : number of tests.

TABLE 1. CORRELATION BETWEEN SERUM FERRITIN LEVEL AND AMOUNT OF  $\gamma$ -GLOBULIN IN RA PATIENTS

$\gamma$ -gl. (g/dl)	No. of tests	Serum ferritin (ng/ml)
2.0 >	24	29.8 $\pm$ 24.8
2.0 $\leq$	11	65.5 $\pm$ 48.3*

Each value represents the mean  $\pm$  SD.

\*  $p < 0.01$

TABLE 2. CORRELATION BETWEEN SERUM FERRITIN LEVEL AND RA TEST IN RA PATIENTS

RA test	No. of tests	Serum ferritin (ng/ml)
(-)	38	32.4 ± 23.2
(+)	52	36.9 ± 33.5
(++)	71	40.9 ± 29.4*

Each value represents the mean ± SD. \*p<0.05.

TABLE 3. CORRELATION BETWEEN SERUM FERRITIN LEVEL AND HEMOGLOBIN CONCENTRATION IN RA PATIENTS

Hb (g/dl)	No. of tests	Serum ferritin (ng/ml)
10 >	39	40.4 ± 37.0
10 ≤	122	36.1 ± 25.7

Each value represents the mean ± SD.

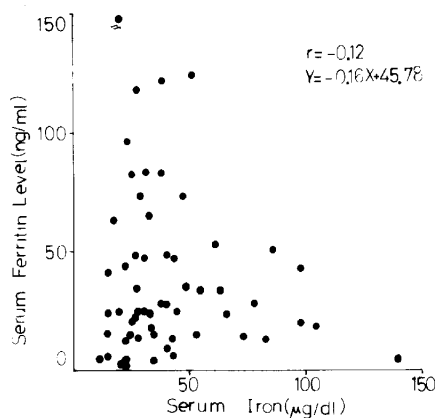


Fig. 3. No correlation between serum ferritin and serum iron levels.

course. These samples had an s-ferritin concentration of  $32.4 \pm 23.2$  ng/ml ( $n = 38$ ). The s-ferritin levels in patients with positive RA test results were  $36.9 \pm 33.5$  ng/ml ( $n = 52$ ) for + patients and  $40.9 \pm 29.4$  ng/ml ( $n = 71$ ) for ++ patients, which were higher than that of patients with negative RA test results (Table 2).

*No correlation between s-ferritin level and serum iron, hemoglobin concentration.* Thirty-nine out of 161 samples showed less than 10 g/dl of hemoglobin when tested for s-ferritin levels. There was no significant difference in s-ferritin levels between samples with more and those with less than 10 g/dl hemoglobin (Table 3).

No correlation between s-ferritin levels and serum iron was found (Fig. 3). The samples with more than 50 ng/ml of s-ferritin tended to have less than 50  $\mu$ g/dl of serum iron and vice versa.

*Sequential studies of hemoglobin concentration, ESR and s-ferritin levels in four RA*

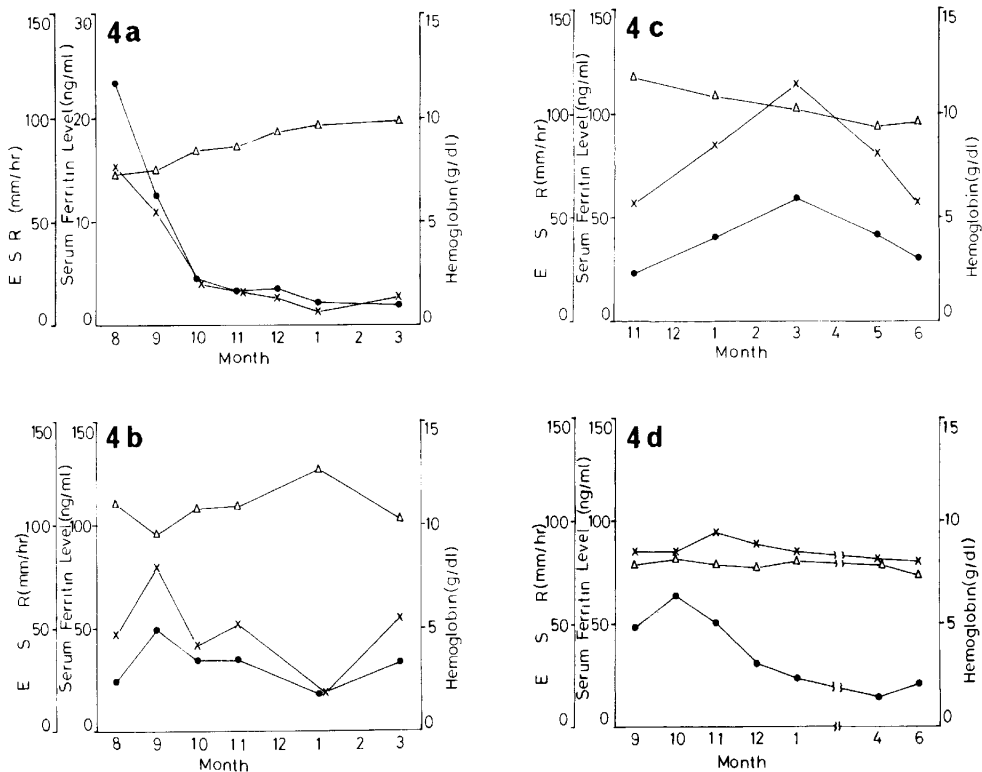


Fig. 4a-4d. ESR, hemoglobin concentration and s-ferritin levels in four representative RA patients. (●—●: s-ferritin, ×—×: ESR, △—△: hemoglobin concentration)

patients. Serum ferritin levels were measured and compared with ESR and hemoglobin concentrations in each RA patient. The data of four representative RA patients are shown in Figs. 4a-4d.

Case 1 (Fig. 4a). A 38-year-old female (Class I, Stage III; disease duration : 5 years) was treated with a total dose of over 3000 mg gold sodium thiomalate and became refractory to gold therapy. One hundred mg per day of D-penicillamine was given orally starting from August of 1983. The s-ferritin levels fell from the initial value of 23.4 ng/ml to 2.0 ng/ml in March of 1984 with improvement in disease activity. The value of ESR changed in parallel from 77 mm/1h to 14 mm/1h. In contrast, the hemoglobin concentration rose from 7.4 g/dl to 9.9 g/dl.

Case 2 (Fig. 4b). A 32-year-old female (Class II, Stage I; disease duration : 2.5 years) was treated with weekly injections of 10 mg of gold sodium thiomalate. The total dose of gold was 1265 mg by March of 1984. Serum ferritin levels fluctuated along with the disease activity and ESR, oppositely from hemoglobin concentrations during the period.

Case 3 (Fig. 4c). A 17-year-old female (Class I, Stage I; disease duration : 2

TABLE 4. SERUM AND URINARY FERRITIN LEVELS IN RA PATIENTS

Subjects	Treatment	s-Ferritin (ng/ml)	u-Ferritin (ng/ml)	U/S ratio
Controls	—	56.3 ± 53.4 (23)	5.1 ± 4.1	0.12 ± 0.10
RA	—	33.9 ± 26.3 (45)	8.7 ± 12.7	0.28 ± 0.25
	NSAID	35.5 ± 30.5 (16)	5.3 ± 4.9	0.22 ± 0.24
	Gold	40.3 ± 28.4 (15)	14.1 ± 20.2*	0.33 ± 0.22
	D-peni.	21.8 ± 9.1 (10)	6.7 ± 7.0	0.30 ± 0.27
	Steroid	37.9 ± 30.5 (4)	6.2 ± 4.7	0.21 ± 0.23

Each value represents the mean ± SD. ( ) number of patients. \*  $p < 0.05$  compared with controls.

years) received gold therapy from April of 1984 with weekly injections of 25 mg of gold sodium thiomalate. The total dose of gold was 300 mg by June of 1984. Serum ferritin levels changed with ESR, in parallel with clinical improvement. The hemoglobin concentration gradually decreased from 11.9 g/dl to 9.8 g/dl without correlation with s-ferritin concentrations.

Case 4 (Fig. 4d). A 58-year-old female (Class II, Stage III; disease duration: 14 years) was treated only with NSAID during the period of this study since she previously had side effect with second line therapy. Serum ferritin levels decreased from 63.6 ng/ml to 14.4 ng/ml with an unchanged ESR value and hemoglobin concentration. This case is unusual in terms of correlation of s-ferritin levels with disease activity which most RA patients revealed in this study.

*Serum, urinary ferritin concentrations and u/s ratio in 45 RA patients.* The serum and urine samples were collected from the patients during their visit at clinics. The control values were  $5.1 \pm 4.1$  ng/ml ( $n = 23$ ) for u-ferritin concentrations and  $0.12 \pm 0.10$  for the u/s ratio. All of the RA patients studied had no clinical renal damage. No significant difference between the RA patients and controls in u-ferritin levels and the u/s ratio was found. The RA patients were divided into four groups according to therapy when tested as shown in Table 4. S-ferritin levels among the groups were not statistically different. U-ferritin levels in the gold therapy group ( $14.1 \pm 20.2$  ng/ml,  $n = 15$ ) were significantly higher than in the control group ( $p < 0.05$ ). The same trend was found in the u/s ratio although there was no significant difference ( $p < 0.2$ ).

#### DISCUSSION

It is still controversial whether serum ferritin levels in RA patients correlate with the amount of body iron storage (10-13) or with disease activity as acute phase reactants in inflammation (14-16). Our study demonstrated that s-ferritin levels in RA patients were within the normal range, fluctuated along with disease activity and correlated with CRP and ESR (Figs. 1 and 2). Moreover, serum ferritin concentrations tended to be higher in RA patients with hyper- $\gamma$ -globulinemia



and positive RA test results (Tables 1 and 2). On the other hand, s-ferritin levels did not correlate with serum iron levels and hemoglobin concentrations (Fig. 3 and Table 3). These results are consistent with the report of Rothwell and Davis (16) that serum ferritin levels correlated with disease activity and behaved independently of hemoglobin concentrations.

In sequential studies, they also demonstrated that serum ferritin levels fell in patients whose disease activity improved after institution of appropriate therapy. In most of the patients we studied clinically, serum ferritin levels changed in parallel with CRP and ESR. There was, however, one exception (Fig. 4d). Blake and Bacon (15) have previously described that eight out of ten patients with high initial ferritin concentrations ( $\geq 300 \mu\text{g/l}$ ) had, or developed, systemic manifestations and severe disease, and that patients with low initial ferritin concentrations ( $\leq 15 \mu\text{g/l}$ ) had mild disease and were often seronegative. We also showed in this study that the patients who had hyper- $\gamma$ -globulinemia and were seropositive had higher serum ferritin levels than those who had normal  $\gamma$ -globulin and were seronegative. In terms of association of high serum ferritin concentrations with systemic manifestations, it is intriguing to compare RA patients with lupus patients with systemic involvement in some of whom we recently found high serum ferritin levels (unpublished data).

Urinary ferritin concentrations have been studied in patients with genitourinary malignancy (19) and some hematological disorders (17, 18). High u-ferritin levels might be caused by ferritin synthesis in tumor cells or in renal tubules with hemoglobin deposition. The cause of high u-ferritin levels in RA patients under gold therapy is not known. It seems to be unlikely that high u-ferritin levels are caused by secretion of high serum ferritin into urine, since u/s ratios of patients under gold therapy also tend to be high. Gold particles like those found in RA patients with gold-induced nephropathy (23) may stimulate ferritin synthesis in renal tissues. High urinary ferritin levels in SLE patients with high u/s ratios were, however, found to have no connection with heavy metal ions (unpublished data). The sites of ferritin deposition in renal tissues are under investigation in our laboratory.

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