The Clinical Significance of Ferritinuria

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Urinary ferritin levels were measured by a "2-site" immunoradiometric assay in normal volunteers and in patients with various hematologic disorders. The mean urinary ferritin concentration in normal subjects averaged 2.2 μg/liter, only 3% of the serum ferritin level. Elevated urinary ferritin levels averaging 45 μg/liter were observed in patients with hematologic malignancies, but there was a proportional increase in serum ferritin so that the urinary level still averaged only 7% of the serum value. The highest urinary ferritin values (mean 170 μg/liter) were associated with chronic hemolytic anemia, and in these patients, urinary ferritin rose disproportionately in relation to the serum, averaging 82% of it. This higher urinary level apparently reflects increased ferritin in renal tubular cells due to glomerular filtration of unbound hemoglobin, a mechanism that is supported by a highly significant correlation between urinary ferritin and serum haptoglobin levels. In normal subjects and in patients with malignancy, the source of urinary ferritin appears different, since a highly significant correlation was observed between urinary ferritin and reticuloendothelial iron stores as measured by serum ferritin or total iron-binding capacity. In this setting, the most likely source of urinary ferritin is the iron contained in renal tubular cells, which is apparently in equilibrium with body iron stores.

Iron Loss from the urinary tract is normally less than 0.1 mg daily, a concentration that is below the sensitivity of colorimetric iron methods. In a clinical setting, large iron losses in the urine can occur in patients with paroxysmal nocturnal hemoglobinuria or prosthetic heart valves, this hemosiderinuria, which accompanies intravascular hemolysis, can be readily detected by examining an iron stain of the urinary sediment. Between these two extremes of normal iron loss and gross hemosiderinuria, measurements of urinary iron have not found a useful role in clinical diagnosis or management. However, reliable measurements have been difficult to obtain because of the ease of iron contamination during collection of urine specimens.

An alternative approach to measuring urinary iron that eliminates the problem of iron contamination has recently become available. Under physiologic conditions, nonheme urinary iron is presumably derived from intracellular stores in renal tubular epithelial cells. If true, greater precision in measuring urinary iron might be obtained by assaying for ferritin protein rather than iron. In the present study, a sensitive immunoradiometric assay (IRMA) recently developed for measurements of serum ferritin has been used to measure urinary iron loss in normal individuals and patients with hematologic disorders.

MATERIALS AND METHODS

Studies were performed in 11 normal subjects, 46 patients with various hematologic malignancies, and 15 patients with chronic hemolytic anemia. The 6 male and 5 female normal subjects were between 25 and 40 yr of age and gave no history of chronic or recent illness. The hematologic malignancies included 11 patients with Hodgkin disease, 14 with non-Hodgkin lymphoma, 12 with multiple myeloma, and 9 with acute myeloid leukemia. All patients had active disease at the time of their evaluation on the hematology service at University of Kansas Medical Center. Patients with chronic hemolytic anemia included 4 with autoimmune hemolytic anemia, 9 with sickle cell anemia, and 1 each with hemoglobin SC disease and intravascular hemolysis due to a prosthetic heart valve. All patients had a reticulocyte index greater than 3. Abnormal renal function was eliminated as a variable by excluding patients with a history of renal disease, abnormal urinalysis, or serum creatinine above 1.2 mg/dl.

Urinary ferritin was measured by a modification of the "2-site" IRMA for serum ferritin. Unlike the original method, which is inhibited by serum and therefore requires that standards contain the same concentration of serum as the unknowns, standards in the urine assay were diluted in bovine serum albumin (BSA) buffer (0.05 M Veronal, pH 8.0, containing 4.5 g/liter NaCl, 20 mg/liter sodium azide, 1 g/liter bovine serum albumin, and 1 ml/liter normal rabbit serum). Recovery of recrystallized ferritin added to fresh urine ranged in 5 separate studies from 88% to 126%, with a mean of 94%. Serial dilutions of urine containing high concentrations of ferritin produced a proportional decrease in the measured value.

To evaluate assay precision, variability in urine levels was measured both within and between assays. The within assay variability, as determined from the range in triplicate values for 100 urine samples measured at a 1:5 dilution was 6.2%. The between assay variability for a urine specimen with a mean ferritin of 5.4 μg/liter assayed on 9 separate occasions was 14.4%. This variability is similar to that for serum samples measured in the "2-site" IRMA.

Neither centrifugation nor ultrasonication of urine samples prior to analysis altered the ferritin level, indicating that at the time of voiding, all the measured ferritin is extracellular.

When measurements were performed repeatedly on fresh undiluted urine allowed to stand at room temperature for 24 hr, a continuous decrease in values was observed. However, constant ferritin values could be obtained for at least 5 days if urine was diluted 1:5 in BSA buffer within 4 hr of collection and stored at 4°C; urine specimens were handled accordingly. All urine was assayed initially at dilutions of 1:5 and 1:20 in BSA buffer, while urine
containing more than 250 µg/liter ferritin was assayed at progressively higher dilutions.

Preliminary studies were also performed in normal subjects to determine the most appropriate expression for urinary ferritin levels. Quantitative 24 hr urine collections in 7 of these subjects did not significantly reduce the variability in urine ferritin values as compared with random samples. To determine whether variability in urine ferritin could be reduced by expressing the level in relation to a nonabsorbable solute, such as creatinine, urinary ferritin was measured in 6–10 random samples collected in each of 7 normal volunteer subjects over 24 hr and expressed both as per milliliter urine and per milligram creatinine measured as described by Boutwell5 (Fig. 1). The coefficient of variation averaged 77% (range, 46%–107%) when expressed in relation to urine volume as compared with a mean of 67% (range, 49%–100%) when expressed in relation to creatinine. All urine ferritin levels were therefore determined on random urine samples and expressed as µg ferritin/liter urine.

In the majority of clinical studies, a blood sample was obtained at the time of urine collection for measurements of serum ferritin, serum iron, and total iron-binding capacity (TIBC). Serum haptoglobin levels were measured in patients with hemolytic anemia using the method of Lionetti et al.8

RESULTS

Urine and serum ferritin levels in normal individuals and hematologic disorders are shown in Fig. 2. The urine values, like serum ferritin, are lognormally distributed, as is the ratio of urine:serum ferritin. In normal subjects, identical geometric means of 2.2 µg/liter for urine ferritin were observed in 6 men and 5 women with a range in the composite group of 0.4–9.9 µg/liter. The serum ferritin in normal subjects averaged 65 µg/liter giving a mean ratio for urine:serum ferritin of 0.03 (range, 0.01–0.13).

In patients with hematologic malignancies, urine and serum ferritin were both significantly higher than in normal subjects. In the total group of 46 patients, urine ferritin averaged 45 µg/liter (± 1 SD, 11–192 µg/liter) with a range of 3–1723 µg/liter. Very similar levels were observed in the various solid malignancies: Hodgkin disease, 30 µg/liter; non-Hodgkin lymphoma, 32 µg/liter; and multiple myeloma, 43 µg/liter. A significantly higher mean of 151 µg/liter was observed in patients with acute myeloid leukemia. In the composite group of hematologic malignancies, serum ferritin increased in parallel with the urine level: the average serum ferritin was 600 µg/liter (range 35–16,000 µg/liter). This increase in serum ferritin is comparable to the elevated levels seen in patients with infection or inflammation.9 The mean ratio of 0.07 (± 1 SD, 0.02–0.25) for urine:serum ferritin in patients with hematologic malignancy was slightly higher than in normal subjects, but the difference was not statistically significant (p > 0.10).

The highest concentration of urine ferritin was
observed in patients with hemolytic anemia. The geometric mean of 170 μg/liter (range, 36–1521 μg/liter) was significantly higher than in either normal subjects (p < 0.001) or patients with malignant disease (p < 0.001). The mean serum ferritin of 207 μg/liter in these patients was not increased in parallel with urine values and as a result, the mean ratio for urine:serum ferritin of 0.82 (±1 SD, 0.15–4.23) was significantly higher than in either normal subjects or patients with hematologic malignancy (p < 0.001 respectively) (Fig. 2).

The highest level of urine ferritin, 1521 μg/liter, was observed in a patient with severe intravascular hemolysis due to an aortic valve prosthesis. Gross hemosiderinuria was observed in this patient but could not be detected in other patients with active hemolysis. Nevertheless, it is likely that the higher levels of urine ferritin in these patients reflects filtration by the kidney of small amounts of unbound hemoglobin and subsequent uptake by renal tubular cells. Thus, even though hemoglobinuria could not be detected by conventional laboratory methods, a highly significant correlation coefficient of −0.858 (p < 0.001) was observed between urine ferritin and serum haptoglobin (Fig. 3).

In the combined group of normal subjects and patients with hematologic malignancies, correlations were examined between urine ferritin and other parameters of iron status. A significant correlation was observed between urine and serum ferritin (r = −0.798, p < 0.001), and the relationship was similar in the normal and patient groups as indicated by similar regression slopes of 0.379 and 0.429, respectively (Fig. 4). Urine ferritin showed no significant correlation with the serum iron (r = 0.231, p > 0.10), but showed a strong inverse correlation with TIBC (r = −0.639, p < 0.001). As a result, the highest correlation with urine ferritin was observed with the ratio of plasma iron: TIBC or transferrin saturation (r = −0.693, p < 0.001) (Fig. 5). The correlation between urine ferritin and TIBC is similar to that observed previously between serum ferritin and TIBC, both in normal subjects and in various clinical disorders. In the present study as well, serum ferritin did not correlate with serum iron (r = −0.395, p > 0.10) but correlated well with both the TIBC (r = −0.577, p < 0.001) and transferrin saturation (r = −0.655, p < 0.001).

**DISCUSSION**

Modern studies have established an upper limit for urinary iron loss in normal subjects of about 0.1 mg daily. Higher levels were reported in earlier studies, but these were presumably the result of extraneous iron contamination. The source and nature of the iron excreted in normal urine has not been adequately identified. Some relationship may exist between iron loss and iron status in certain disorders, as for example

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Fig. 3. Correlation between urine ferritin and haptoglobin in 13 patients with chronic hemolytic anemia.

Fig. 4. Correlation between urine ferritin and serum ferritin in normals (■) and patients with hematologic malignancies (●).
in patients with idiopathic hemochromatosis in whom hemosiderinuria and increased urine iron excretion have been described.\textsuperscript{11,12} However, there is no evidence of an excretory mechanism that can modify urine losses of iron in response to body iron requirements.

The highest concentration of urinary iron occurs in patients with intravascular hemolysis,\textsuperscript{2} and the mechanism of this iron loss is now well defined.\textsuperscript{3} Small amounts of hemoglobin that enter the circulation are rapidly bound by haptoglobin, which prevents renal loss. When haptoglobin is fully saturated, unbound plasma hemoglobin is readily filtered by the renal glomerulus and is subsequently reabsorbed by renal tubular cells. Hemoglobin is then catabolized, and the liberated iron is incorporated into the ferritin storage compartment. This iron is then lost from the body when tubular epithelium is shed into the urine. Massive iron loss by this mechanism is readily detected by staining the urinary sediment for iron. This mechanism presumably explains the observation in the present study that the highest ratio of urine:serum ferritin was observed in patients with chronic hemolysis.\textsuperscript{13} The highly significant inverse correlation between serum haptoglobin and urine ferritin provides additional evidence that the latter reflects intravascular hemolysis, although it is generally believed that free hemoglobin does not circulate in the plasma until haptoglobin is fully saturated. For instance, in the absence of other acute disease, electrophoretic techniques have failed to detect haptoglobin in patients with sickle cell disease, thalassemia, hereditary spherocytosis, or immune hemolytic anemia.\textsuperscript{14,15} Perhaps because of methodological differences, some hemoglobin binding capacity could be detected in all hemolytic patients in the present study. Since the urinary ferritin correlates with the haptoglobin, it seems likely that despite unbound haptoglobin, some hemoglobin is delivered to the renal glomerulus in proportion to the degree of hemolysis. This unbound hemoglobin readily passes the glomerulus and accounts for the increased urinary iron excretion. Although the amount of iron lost in this manner may be too small to be detected histologically, the immunologic assay for ferritin employed in this study could readily detect it.

Urine ferritin in normal individuals and patients with hematologic malignancies was apparently derived by a different mechanism than in patients with chronic hemolysis. If the latter are excluded, a high correlation was observed in the remaining subjects between urine ferritin and body iron stores as measured by the serum ferritin. The origin of this urine ferritin was not established, but it is probably also derived from the iron storage compartment in renal tubular cells. This is true, it is surprising that in the absence of hemoglobinuria, the urine ferritin correlated better with serum ferritin, a measure of reticuloendothelial stores,\textsuperscript{9} than with the serum iron, a measure of parenchymal iron supply. There was a good correlation between urine ferritin and transferrin saturation, but the latter correlation was explained more by the TIBC than the serum iron. The significant relationship with TIBC is consistent with previous studies in normal subjects\textsuperscript{10} and clinical disorders\textsuperscript{9} where high correlations have been observed between the serum ferritin and TIBC but not serum iron.

Another possible source of urine ferritin in normal subjects is macrophages or leukocytes. The intracellular content of ferritin in both these cell lines is at least a hundredfold greater than the concentration in serum or mature erythrocytes.\textsuperscript{16,17} The ferritin content of these cells should also correlate with serum ferritin, while no relationship with serum iron would be expected. Further studies are needed to determine the relative contribution of these possible sources of urine ferritin.

While there does appear to be a relationship between the serum and urine ferritin, the relatively
low values in normal individuals and the overlap seen
between males and females indicate that the test will
be of limited value for evaluating iron stores in either
normal individuals or in patients with abnormalities in
iron balance. However, urine ferritin or the ratio of
urine to serum ferritin taken together with the hapto-
globin level may be useful in evaluating the severity of
intravascular hemolysis.

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