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Ferritin Concentrations in Plasma from Capillary (Finger Prick) Blood and Venous Blood Compared

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Investigating the feasibility and validity of determining plasma ferritin concentration in blood obtained by finger prick, we studied 29 adults (ages 21–49 years) and 35 children (ages 14–66 months). Blood was sampled simultaneously in the same subject from both the antecubital vein (venous blood) and by finger pricking (capillary blood). The plasma was obtained by centrifugation. Ferritin concentration was determined by immunoradiometric analysis. Ferritin concentration in plasma from capillary blood was significantly higher than in venous plasma ($p < 0.01$). This difference was more marked in children. The correlation between ferritin from the two blood sources was highly significant ($r^2 = 0.945$ and 0.994 for samples from adults and children, respectively), and the slopes of the respective regression lines in both children and adults were significantly different from 1 ($p < 0.0001$). We conclude that, despite the close association between the two procedures, the determination of ferritin concentration in capillary blood plasma overestimates the concentration of ferritin in venous blood plasma.

Additional Keyphrases: *sample collection · variation, source of · age-related effects · pediatric chemistry*

Ferritin, a ubiquitous iron-containing protein (*1*), acts primarily as an iron storage compound (*2*). Its concentration in plasma or blood serum has been significantly correlated with iron absorption (*3*), iron administration (*4*), and blood transfusion (*5, 6*). As one may expect, therefore, there is also a positive correlation between concentrations of ferritin in blood and stored iron (*7*), ferritin being low in iron deficiency and high in iron overload (*8*). Thus, data on serum or plasma concentrations of ferritin are now successfully used clinically to estimate iron stores in various disease states (*9*) and to evaluate iron reserves of populations (*10–12*).

Ferritin is commonly measured in plasma or serum of venous blood by radioimmunoassay (*9*). In population studies, however, venous samples are not always easy to obtain. Especially under field conditions, there may be some resistance on the part of subjects to venipuncture. Furthermore, in infants and small children, obtaining venous blood represents a difficult and often traumatic task.

To overcome these problems, we have investigated the feasibility and validity of using capillary blood obtained by finger prick, instead of venous blood, to measure ferritin concentrations. We report here the results of this study.

Materials and Methods

Experiment 1. In a first experiment, blood was drawn in the field from 29 rural adult Guatemalan subjects (ages 21–49 years) by both venipuncture and finger prick. Venous blood, collected from the antecubital vein with a syringe, was placed in heparinized test tubes, centrifuged, and the resulting plasma was used for ferritin analysis.

Capillary blood was sampled in triplicate by pricking the finger tip with a lancet and using heparinized hematocrit capillary tubes for its collection. The capillary tubes were then centrifuged as when measuring hematocrit, broken just above the cells–plasma interphase, and the plasma blown out into a small vial to be analyzed for ferritin. We usually obtained 70 to 100 μL of plasma by this procedure.

Experiment 2. The purpose of this experiment was to measure variations of ferritin concentration in the same subject, determined in plasma obtained as follows: (V) from venous blood collected and centrifuged as usual; (VC) from the same venous blood, after drawing it from the test tube into hematocrit capillary tubes and handling exactly the same as the finger prick samples (to evaluate the effect of the capillary blood methodology); and (C) from capillary blood taken from the finger and treated as described above.

For this experiment we studied 35 children (ages 14–66 months), temporary residents of the Children's Convalescent Home in Guatemala City. Blood was collected by both venipuncture and by finger prick as in Experiment 1, and treated by the three different collection and separation procedures described in this section. For the venous blood capillary tube plasma sample, however, we used plain capillaries, to avoid any effects of excess heparin.

Ferritin assay. We determined plasma ferritin by the two-site immunoradiometric assay described by Addison et al. (*13*), as modified by Miles et al. (*14*), using a custom-made "fer-Iron" radioimmunoassay kit (Ramco Laboratories, Inc., Houston, TX 77098). It differed from the regular Ramco Kit, designed for clinical use, in two aspects. First, the specific activity (at least 10^8 cpm/L) of the ^{125}I-labeled antibody was much higher, for improved sensitivity of the assay in the low range of ferritin values. Secondly, the spleen ferritin standard provided by the company was a stock solution of highly purified ferritin (1.000 mg/L); we confirmed its protein content by the method of Lowry et al. (*15*).

The procedure for using this kit was also modified as follows:

1. The standard curve was prepared from analysis of the following ferritin concentrations: 0.00, 0.25, 0.50, 1.00, 2.00, 4.00, 5.00, 10.00, and 100.00 μg/L, prepared by diluting the stock ferritin standard with the standard-diluting buffer in the kit. The 0 concentration standard (buffer only) was used to determine the "nonspecific" binding, and the 100.00 μg/L concentration, on the other hand, was used to calculate maximum binding. Each standard was run in quadruplicate.

2. The first incubation, binding the human plasma ferritin to the solid-phase antihuman ferritin, was for 4 h at room temperature, with the reaction trays on a vibrating tray. The second incubation, binding the purified radiolabeled antihuman ferritin with the insoluble antihuman ferritin complex, was for 48 h at room temperature, without vibration.

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3. The unknown samples were diluted 20-fold with sample-diluting buffer (same composition as standard-diluting buffer but different color code) and run in triplicate.

4. We used a Hewlett-Packard 9830-A computer to calculate the standard curve and the ferritin concentrations of the unknown samples. To compensate for the "high-dose hook effect" (*14*) and attain a linearization of the standard curve, we used a computer program designed to generate different possible regressions, to define the fitted maximum and the best fitted regression line (*16*).

To dilute the standards and unknown samples and to deliver the labeled antibody, we used an automatic "high-speed" pipette (Model 25005; Micromedics Systems, Horsham, PA 19004). By using this instrument, we needed only 10 μL of plasma for the analysis. We were careful to wash the delivery tip of the instrument with sample-diluting buffer after each sampling, to avoid carryover of ferritin from one sample to the next.

We measured the radioactivity with a Gamma 300 radiation counter (Beckman Instruments, Inc., Irvine, CA 92664).

We analyzed ferritin in venous and capillary blood as specimen pairs—that is, both samples from the same subject were measured in the same assay run.

Statistical analyses. The data were analyzed as follows. The ferritin values from the adult group (Experiment 1) were compared by a paired *t*-test (*17*). For the children (Experiment 2), we first compared the results by analysis of variance for repeated measurements (*17*) and then determined the specific differences between procedures, by using Bonferroni's multiple comparison (*18*). Linear regression analyses (intercept and no-intercept models) were also performed to correlate the different analytical procedures for determining ferritin (*18*).

Results

Table 1 shows the mean values for plasma ferritin concentration by the different procedures. In the adult group, the plasma ferritin concentration in capillary blood was significantly greater than that in venous plasma ($p < 0.0180$, paired *t*-test).

In the values from the children, the analysis of variance for repeated measurements showed that results by the three procedures were significantly different [$F = 27.23$; $(1, 34)$ *df*; $p < 0.0001$].

We then determined that the specific statistical differences between procedures in this experiment (by Bonferroni's multiple comparison test) and the results were as follows: (*a*) plasma ferritin in capillary (finger prick) blood was significantly greater than plasma ferritin in venous blood obtained and handled as common in blood-collection tubes [mean difference (C − V) = 8.06 μg/L, $p < 0.01$]; (*b*) ferritin concentration in plasma obtained by centrifugation of venous blood in capillary tubes was significantly greater than plasma ferritin concentration from the same venous blood but handled in blood-collection tubes [mean difference (VC − V) = 4.86 μg/L, $p < 0.01$]; (*c*) concentrations of plasma ferritin in capillary blood were significantly greater than that in plasma obtained after centrifugation of venous blood in capillary tubes [mean difference (C − VC) = 3.20 μg/L, $p < 0.05$]. On the average, the difference between ferritin in plasma from capillary blood and that in plasma from venous blood handled in blood-collection tubes (C vs V) was greater in children (18.6%) than in adults (8.3%).

Figure 1 illustrates the results obtained by linear regression analyses when comparing plasma ferritin concentration in capillary blood with that in venous blood (not treated in capillary tubes) for both adults and children. For the adult group, the original regression line obtained with an intercept regression model was $y = 0.85380x + 1.22549$ ($r^2 = 0.99490$). Because the intercept of this line was not statistically different from 0 ($t = 1.39$), we used a no-intercept regression model for the results from the adults (Figure 1). In both adults and children, the correlations between plasma ferritin from capillary blood and that from venous blood were highly significant, the coefficients of determination (r^2) being 0.94536 and 0.99412 for adults and children, respectively. Moreover, the slopes of each of these regression lines were statistically different from 1: $t = 4.62$ ($p < 0.0001$) and 6.21 ($p < 0.0001$) for adults and children, respectively.

Discussion

Our results indicate that although it is feasible to measure plasma ferritin concentration in capillary blood obtained by pricking the finger, the resulting values exceed those found in plasma from venous blood. This difference was larger in children, the concentration of plasma ferritin from capillary blood being 18.6% greater than that found in plasma from venous blood, while in adults, the difference was only 8.9%. These results are outside the variability range of the within-day (CV = 4.9%) and between-day (CV = 7.8%) precision of the ferritin assay in our laboratory.

When we correlated both procedures, the intercept of the regression line for the group of children was significantly different from zero, and this was not the case for the adult group. This means that if ferritin concentrations obtained by

Table 1. Ferritin Concentrations in Plasma Obtained by the Different Procedures

Plasma source	Mean ferritin concentration (and SD), μg/L: Adults (n = 29)	Children (n = 35)
Venous blood (V)	17.50 (11.90)	43.25 (65.72)
Venous blood centrifuged in capillary tubes (VC)	— —	48.11 (67.08)
Finger prick blood (C)	18.95 (13.58)	51.31 (70.98)

Values are significantly different from others in the same column at $p < 0.05$ or better (adults, paired *t*-test; children, Bonferroni's multiple comparison procedure).

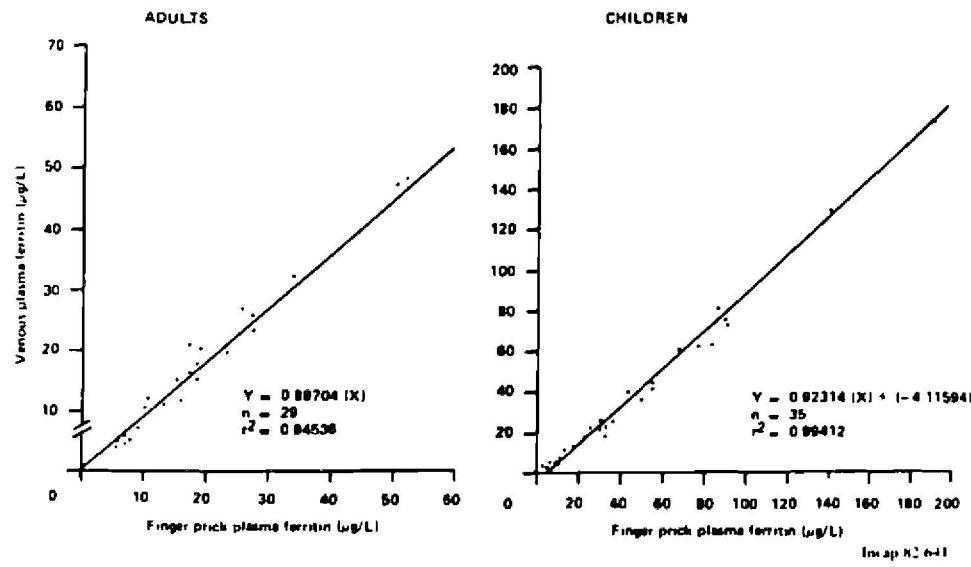

Fig. 1. Linear regression comparison between ferritin in plasma from blood obtained by finger pricking and in venous plasma obtained by common sample-handling procedures

A no-intercept regression model was used for the data from the adults

finger prick are to be compared with those from venous blood, children and adults should be considered separately.

It is clear, as shown in Table 1, that the differences between ferritin concentration from finger prick blood and that from venous blood is due in part to the procedure used for separating the plasma. When we introduced and centrifuged venous blood in capillary tubes in the same manner as when the plasma was obtained by pricking the finger, significantly greater concentrations of ferritin were observed than when we used plasma from the same blood separated by test tube centrifugation.

Although no hemolysis was evident in the capillary tubes, it is possible that the friction of packing blood cells in a narrow tube such as the hematocrit capillary tube may cause leaching of ferritin, particularly from leukocytes. Other investigators have found that leukocytes in general, and particularly monocytes, contain very high amounts of ferritin. The ferritin concentration of leukocytes amounts to about 24 mg/L (*19*) and that of monocytes to about 7.5 times the concentration found in other leukocytes (*20*). Release of ferritin from these cells thus would contaminate the plasma and increase its ferritin concentration. The plasma ferritin concentration in capillary blood, however, was still higher than that we measured in plasma from venous blood centrifuged in capillary tubes (see Table 1), implying that in addition to the effect of the procedure, something else, as yet unidentified, increases the concentration of ferritin in plasma measured in capillary blood. It is tempting to speculate that the increase may be due to mitochondrial ferritin released by tissue rupture.

The high coefficient of determinations (r^2) between ferritin in plasma from blood obtained by finger pricking and that from venous blood in both children and adults indicates that both procedures are closely associated (Figure 1). The fact that the slopes of the regression lines were different from 1, however, indicates that measuring ferritin in capillary plasma does not correspond quantitatively to the values found in venous plasma. That is, the finger prick procedure overestimates the concentration of ferritin. Considering the limited number of subjects and the narrow range of ferritin values (adults: 2.3–48.0 μg/L; children: 2.2–174.4 μg/L), the present investigation does not constitute a reference-range study. Nevertheless, the close association between both procedures leads us to believe that by increasing the number of subjects we could define regression equations that could be used to convert ferritin concentrations in blood taken from the finger to the corresponding values in venous blood. This conversion, however, may not always be necessary, as when one compares ferritin concentration using the same procedure, or when only relative values are required in clinical practice to define severe iron deficiency, iron overload, or malignancies.

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