The Seoul Journal of Medicine
Vol. 34, No. 3: 173-180, September 1993

Evaluation of Erythrocyte Zinc Protoporphyrin/Heme Ratio for Iron-Deficiency Anemia in Pregnant Women

Bum Suk Tchai, Seok Woon Kwon[1] and Jung Ho Han[1]

Departments of Biochemistry and Clinical Pathology[1]
Seoul National University College of Medicine, Seoul 110-744, Korea

=Abstract=**To evaluate the diagnostic values for detecting iron depletion and for predicting anemia, the erythrocyte zinc protoporphyrin(ZPP) and ZPP/heme ratio were measured using a hematofluorometer and compared with the serum ferritin and blood hemoglobin levels in 136 randomly selected pregnant Korean women. A significant decline in mean hemoglobin and serum ferritin levels and an increase in ZPP/heme ratio level were observed as pregnancy advanced. During the third trimester, 41.7 % of pregnant women were found to have hemoglobin concentrations below 11 g/100ml. 61.0 %, 58.5 % and 48.8 % of the third trimester group demonstrated an abnormal ZPP/heme ratio, serum ferritin and ZPP levels, respectively. The ZPP/heme ratio showed good negative logarithmic correlation with the serum ferritin (r = -0.51). The diagnostic sensitivity and positive predictive value of ZPP/heme ratio for evaluating iron depletion and the risk of anemia compared favorably to those of serum ferritin. It was concluded that the operational simplicity and low cost of erythrocyte ZPP/heme ratio measurement in addition to good diagnostic efficiency offer a procedure for the primary screening test of iron depletion in pregnancy or other risk groups.**

Key Words: *Erythrocyte zinc protoporphyrin(ZPP), ZPP/heme ratio, Serum ferritin, Iron deficiency, Pregnancy*

INTRODUCTION

Iron deficiency has been considered the most common cause of anemia in women, especially in pregnancy. During pregnancy an appreciable amount of iron is transferred to the developing fetus and placenta, and there is abundant evidence that iron deficiency frequently ensues (Kaneshige 1981; Park *et al.* 1987). However, iron stores must be depleted before overt symptoms, and nonhematologic manifestations of iron deficiency such as impaired muscle function and neurologic changes are now important health issues(Dallman 1986). Interest increasingly has turned to detection of the latent subclinical iron deficiency or assessment of the iron status periodically by laboratory tests before hemoglobin

Received May 1993, and in final form August 1993
서울대학교 의과대학 생화학교실: 채범석
서울대학교 의과대학 임상병리학교실: 권석운, 한정호

level decreases in these risk groups, and effective intervention in screening programs would be greatly aided if the results of the reliable primary screening test could be made available inexpensively, simply and immediately at the screening site.

But earlier routine laboratory tests for detecting iron deficiency lack specificity and do not accurately reflect iron status until the iron deficiency is advanced to the point of anemia (Brittenham *et al.* 1981; Thompson *et al.* 1988). So preanemic iron deficiency poses a greater diagnostic problem, with usually poor correlation among laboratory results. Serum ferritin and the examination of stainable bone marrow iron are considered the useful tests for the status of iron storage(Zanella *et al.* 1989; Milne *et al.* 1990), however, both tests must be performed in clinical laboratories, therefore they are expensive and commonly entail a delay of several days between sample collection and the availability of results.

The erythrocyte protoporphyrin level also reflects the disparity between iron supply and the requirement of individual cells(Holly 1953; Dagg *et al.* 1966). When iron supply is restricted, erythrocyte protoporphyrin rises and, accordingly, high levels are found in true iron deficiency. A new test for iron status that recently has become available is the erythrocyte zinc protoporphyrin (ZPP) and ZPP/heme ratio. A zinc ion is incorporated to form ZPP when there is insufficient iron to saturate the active site on ferrochelatase during heme synthesis, and increased amounts of ZPP bind to globin(Lamola *et al.* 1975). Zinc protoporphyrin is fluorescent and the amount of ZPP or ratio to heme can readily be measured in a relatively inexpensive hematofluorometer (Blumberg *et al.* 1977; Stanton *et al.* 1989). This test is analytically more suitable than serum ferritin measurement for field and office testing because ZPP or ZPP/heme ratio can be quickly and inexpensively measured from a drop of whole blood by hematofluorometry.

But little data is available defining the clinical significance of ZPP and ZPP/heme ratio tests in iron deficient state. This study evaluated the diagnostic value of erythrocyte ZPP and ZPP/heme ratio tests by comparison with serum ferritin for assessing iron status and anemic risk in pregnant women where iron deficiency is the most common cause of anemia.

MATERIALS AND METHODS

One hundred and thirty-six pregnant women aged from 18 to 41 years(mean 27.1) at various stages of pregnancy attending the outpatient obstetric clinic of a general hospital in Seoul were randomly selected for this study. They were considered normal without any diseases except iron deficiency anemia by various laboratory tests.

Venous blood samples were collected from study participants. Hemoglobin was measured using the Technicon H-1^{TM} System. Serum ferritin concentration was determined with a radioimmunoassay kit(DPC, Los Angeles, CA, U.S.A.). The ZPP was measured from a drop of capillary blood by a ZP Hematofluorometer Model 206(Aviv Biomedical Inc., Lakewood, NJ, U.S.A.)(Blumberg *et al.* 1977). The hematofluorometer was calibrated to measure the ZPP in micrograms per 100 ml of blood. The ZPP/heme ratio was calculated from blood ZPP and hemoglobin levels by the equation below (Stanton *et al.* 1989).

$$\mu\text{mole of ZPP/mole of Heme} = \frac{\mu\text{g/100ml of ZPP}}{\text{g/100ml of Hb}} \times 25.8$$

The within-run precision of this method was 1.7 and 3.3 % at the levels of 142 and 42 μg/100ml, respectively, and the between-run precision was 5.6 % at the level of 33 μg/100ml.

Our laboratory's reference ranges were less than 48 g/100mL for ZPP and 94 μmol/mol for ZPP/heme ratio in Korean women(Tchai *et al.* 1992). Reference ranges for other tests are $\geqq$ 11 g/100ml for hemoglobin and $\geqq$ 10 ng/ml for serum ferritin.

Various statistical evaluations of the data were performed using the SPSS/PC^{+} computer program, and Bayes' theorem was used to calculate the sensitivity and predictive values of the test results(Galen and Gambino 1975).

RESULTS

The means and standard deviations of the test results in each trimester period are shown in Table 1. As pregnancy advanced, the mean hemoglobin and serum ferritin levels were significantly decreased until the third trimester of pregnancy($p < 0.05$ and $p < 0.001$, respectively), and the mean ZPP/heme ratio was increased ($p < 0.05$). However, the mean ZPP levels did not show significant differences.

The incidence of test results below or above cut-off values for anemia was also remarkably increased in the second and third trimesters of pregnancy(Table 2), and the highest incidence was observed in ZPP/heme ratio(53.7 % for total subjects) and then serum ferritin(48.5 % for total subjects) tests. In third trimester pregnant women, 41.7 % of 82 women demonstrated hemoglobin concentrations below cut-off value and 58.5 % for serum ferritin. For ZPP and ZPP/heme ratio, 48.8 % and 61.0 % of women showed values above cut-off values, respectively.

The Kolmogorov-Smirnov Goodness of Fit statistical test showed that the distribution of hemoglobin values for the total 136 subjects was Normal, but those of serum ferritin, ZPP and ZPP/heme ratio values were skewed(statistical data are not shown). So log-transformed data of these three tests were used in the correlation and regression analyses(Fig 1). The ZPP/heme ratio showed good semi-logarithmic correlation with hemoglobin($r = -0.51$, Fig 1-A) and logarithmic correlation with serum ferritin($r = -0.51$, Fig. 1-C). Serum ferritin showed weak semi-logarithmic and logarithmic correlations with hemoglobin ($r = 0.40$, Fig 1-B) and ZPP($r = -0.44$, Fig 1-D). The ZPP and hemoglobin showed lower semi-logarithmic correlation($r = -0.27$).

Table 1. Concentrations(Mean ± SD) of hemoglobin, erythrocyte zinc protoporphyrin(ZPP), ZPP/heme ratio and serum ferritin in pregnant women

	Trimester			Total
	1	2	3	
Number of subjects	14	40	82	136
Hemoglobin (g/100 ml)*	12.2 ± 0.9	11.6 ± 1.4	11.2± 1.3	11.4 ± 1.3
ZPP (μg/100 ml)	41.4 ± 18.8	45.6 ± 19.2	51.2 ± 18.8	48.5 ± 19.1
ZPP/heme (μmol/mol heme)*	88.1 ± 39.8	103.0 ± 45.4	121.4 ± 53.6	112.5 ± 51.1
Ferritin (ng/ml)**	22.1 ± 13.4	15.3 ± 10.1	11.7 ± 8.8	13.8 ± 10.2

* : $P < 0.05$ between trimester groups(one-way ANOVA test).
** : $P < 0.001$ between trimester groups(one-way ANOVA test).

Table 2. Incidence(%) of hematological values below or above cut-off values in 136 pregnant women

Hematologic tests	Trimester			Total
	1(N = 14)	2(N = 40)	3(N = 82)	
Hemoglobin	7.1	35.0	41.7	36.8
ZPP	28.6	32.5	48.8	41.9
ZPP/heme	35.7	45.0	61.0	53.7
Serum Ferritin	14.3	40.0	58.5	48.5

Cut-off values: Hb < 11 g/100ml; Ferritin < 10 ng/ml; ZPP ≧ 48 g/100ml; ZPP/heme ratio ≧ 94 μmol/mol heme.

The mean hematological values between the anemic and non-anemic subjects(Fig. 2-A) and-between the iron-deficient and normal subjects (Fig 2-B) were compared. The differences in mean values of each test were statistically significant at the level of $p<0.01$ for ZPP between anemic and non-anemic groups and $p<0.001$ for others.

Table 3 shows the sensitivity and positive predictive values(PV) of serum ferritin, erythrocyte ZPP, ZPP/heme ratio and hemoglobin tests for

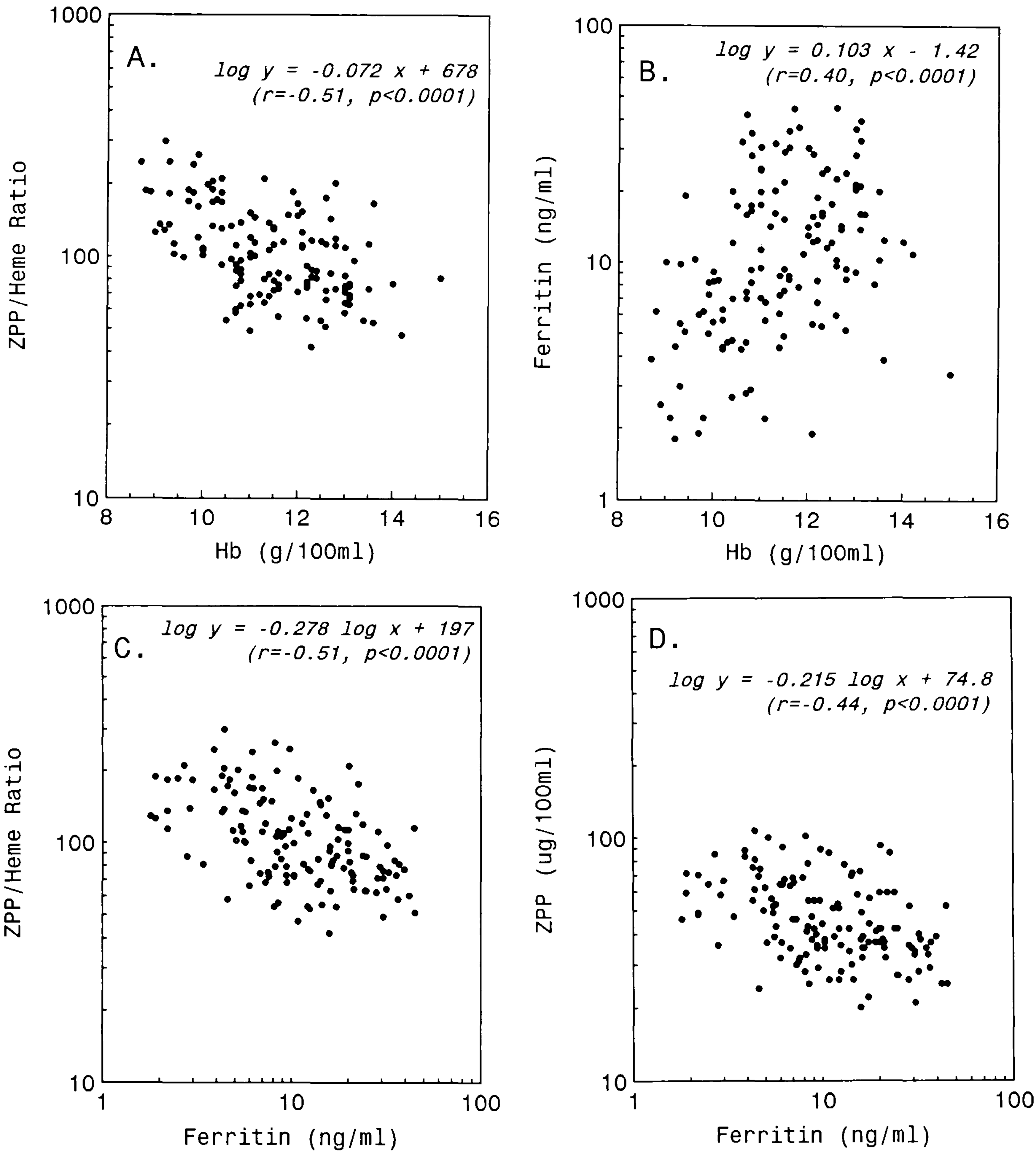

Fig 1. Correlation between hematological values in 136 pregnant women. A: hemoglobin vs. ZPP/heme ratio; B: hemoglobin vs. ZPP; C: ferritin vs. ZPP/heme ratio; D: ferritin vs. ZPP.

accessing anemia and iron depletion. In accessing anemia, serum ferritin and ZPP/heme ratio showed similar diagnostic efficiency in both test parameters(74 and 76 % for sensitivity, and 56 and 52 % for positive PV), but ZPP showed lower efficiency. In accessing iron depletion, the highest sensitivity was observed in the ZPP/heme ratio(74 %), but hemoglobin showed higher positive PV than those of the ZPP or ZPP/heme ratio. The diagnostic efficiencies of the ZPP and ZPP/heme ratio for detecting iron deficiency in each trimester period are shown in Table 4. The test parameter values were highest in the third trimester and very low in the first trimester.

DISCUSSION

The final step in heme synthesis is the incorporation of iron into protoporphyrin IX(PPN) to form heme, and free erythrocyte protoporphyrin (FEP) refers to the non-heme PPN within red blood cells. The concentration of FEP has been known for more than 40 years to increase in persons with disorders of heme synthesis(Holly 1953; Dagg *et al.* 1966; Tchai *et al.* 1974). But studies in the middle of the 1970's demonstrated that much of the non-heme PPN in erythrocyte is in the form of zinc protoporphyrin(ZPP) and the zinc ion was removed from the porphyrin in earlier assay procedures during extraction process (Lamola *et al.* 1975). They showed that the fluorescence spectrum of the ZPP is quite different from that obtained from the free protoporphyrin IX. Thus by avoiding acid extraction of the blood, the fluorometric measurement of ZPP allows one to differentiate easily between iron-deficient anemia and porphyria. In 1977, a small, inexpensive portable filter fluorometer, the hematofluorometer was developed for the rapid assay of ZPP in unprocessed blood(Blumberg *et al.* 1977), and evaluated for its performance in screening iron deficiency.

Simultaneously, some investigators had indicated that the rise in the ratio of FEP to hemoglobin or heme increased exponentially with a decrease of hemoglobin levels, and thus a small decrease in hemoglobin resulted in a relatively large increase in the ratio providing the greatest sensitivity to iron deficiency anemia (Piomelli *et al.* 1976; Labbe *et al.* 1979). Based

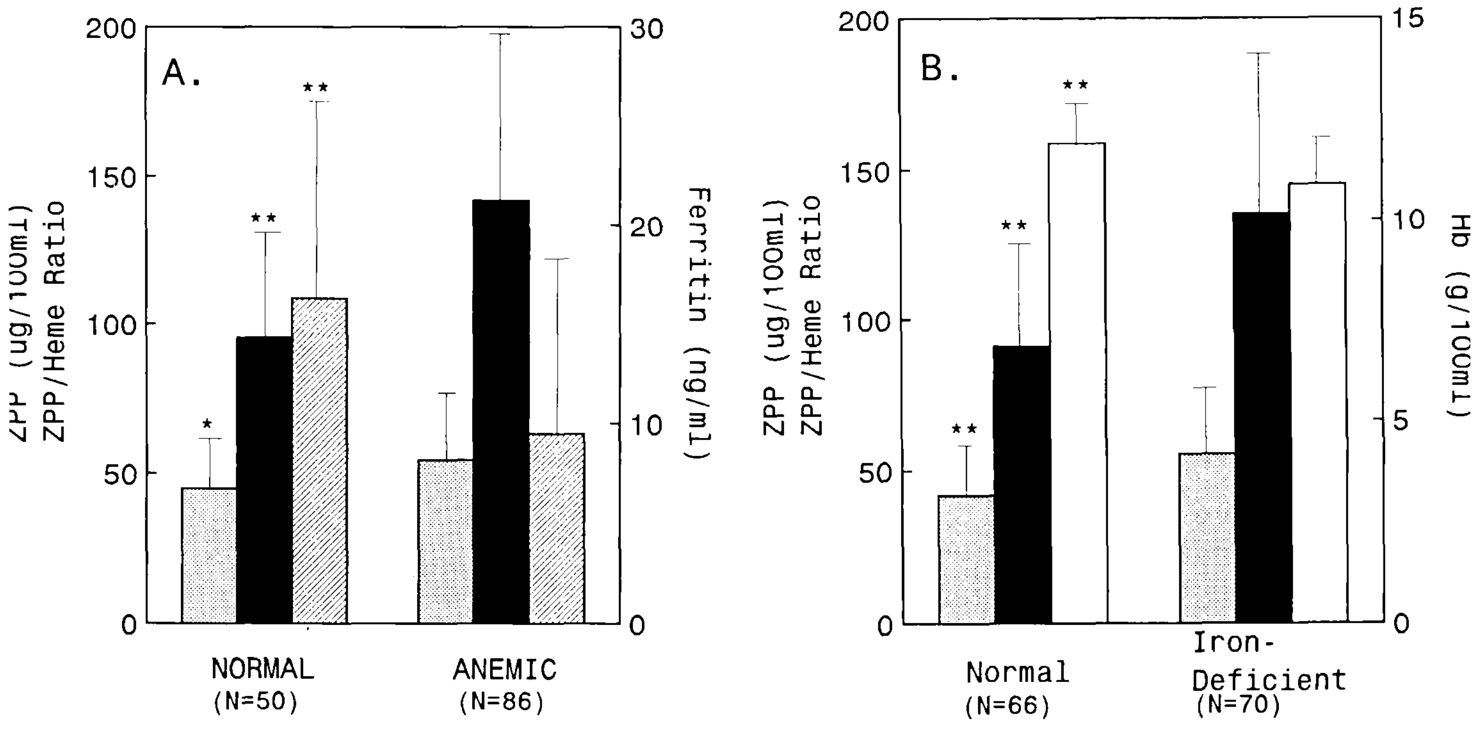

Fig 2. Differences of mean(with SD) hematological values between anemic (Hb < 11 g/100ml) and non-anemic groups(A), and iron-deficient (serum ferritin < 10 ng/ml) and normal groups(B). Asterisks:significantly different two groups(unpaired t-test, *:p < 0.01, **:p < 0.001); Stippled bars:ZPP; Closed bars:ZPP/heme ratio; Hatched bars:serum ferritin; Open bars:hemoglobin.

Table 3. Evaluation of sensitivity and predictive value of hemoglobin, ZPP, ZPP/heme ratio and serum ferritin for detecting anemia(Hb < 11 g/100ml) and iron deficiency(ferritin < 10 ng/ml) in 136 pregnant women

	Anemia			Iron-deficiency		
	ZPP	ZPP/heme	Ferritin	Hb	ZPP	ZPP/heme
Sensitivity(%)[1]	52	76	74	56	59	74
Predictive value(%) of positive(abnormal) test[2]	46	52	56	74	68	67

[1]: % of subjects with abnormal test results in anemic(Hb < 11 g/100ml) or iron-deficient(serum ferritin < 10 ng/ml) women.

[2]: % of anemic or iron-deficient subjects among those with abnormal test results.Cut-off values : Hb < 11 g/100ml; Ferritin < 10 ng/ml; ZPP ≧ 48μg/100ml; ZPP/heme ratio ≧ 94μmol/mol heme.

Table 4. Evaluation of sensitivity and predictive value of the ZPP and ZPP/heme ratio for detecting iron deficiency (ferritin < 10 ng/ml) in each trimester of 136 pregnant women

		Trimester		
		1(N = 14)	2(N = 40)	3(N = 82)
Sensitivity(%)[1]	ZPP	0	50.0	64.6
	ZPP/heme	50.0	62.5	79.2
Predictive value(%) of positive(abnormal) test[2]	ZPP	0	61.5	77.5
	ZPP/heme	20.0	55.6	76.0

[1]: % of subjects with abnormal test results in iron-deficient(serum ferritin < 10 ng/ml) women.

[2]: % of iron-deficient subjects among those with abnormal test results.Cut-off values : ZPP ≧ 48μg/100ml; ZPP/heme ratio ≧ 94μmol/mol heme.

on these observations, Labbe *et al.*(1987) had suggested that ZPP/heme ratio is perhaps the most sensitive response to declining iron reserves, and recently a new hematofluorometer, the Protofluor-Z, which reports results as the micromoles of ZPP per mole of heme was introduced(Stanton *et al.* 1989).

These new hematofluorometric methods make it possible to measure the erythrocyte ZPP and ZPP/heme ratio in blood from a finger puncture, which cannot readily be done in cases of serum ferritin and other tests for iron deficiency. The simplicity, rapidity and low-cost of this method warrant its serious consideration as a primary screening test or survey tool for detection of iron deficiency in various risk groups such as pregnancy and blood donors(Schifman *et al.* 1982; Labbe and Rettmer 1989; Jensen *et al.* 1990).

But the usefulness of ZPP and ZPP/heme ratio tests in assessing iron deficiency is uncertain, because their responses to changes in iron status have not been rigorously tested. Furthermore, values for laboratory tests for anemia and iron deficiency are known to vary to some degree according to various factors including environmental and racial differences (Yip *et al.* 1984), and interlaboratory discrepancies in erythrocyte protoporphyrin tests have also been observed(Jackson 1978). Zanella *et al.*(1989) reported that serum ferritin was a more sensitive predictor of iron deficiency in the absence of anemia than was the erythrocyte protoporphyrin.

We previously reported the normal erythrocyte ZPP and ZPP/heme ratio values in Koreans (Tchai *et al.* 1992), but the observed normal cut-off values were slightly higher than those reported by other investigator. So possible environmental and racial difference in performance of the ZPP and ZPP/heme ratio tests using the hematofluorometer must be taken into consideration in order to optimize the identification of iron deficiency. Thus, we performed a study to determine the diagnostic efficiency of these indices in comparison with serum ferritin values in pregnant Korean women.

In the present study, the mean value of serum ferritin level underwent marked changes as pregnancy advanced to the second trimester and during the last trimester its level fell further to 11.7 ng/ml(Table 1). This value as compared with the mean(30 ng/ml) of normal non-pregnant Korean women(Park *et al.* 1987) was a decrease of 61 %, and also high numbers(35 % to 58.5 %) exhibited serum ferritin and hemoglobin concentrations below cut-off values during the same periods (Table 2). These results confirm that maternal body iron storage is depleted during the second trimester in pregnant women who are not receiving supplemental iron in agreement with a previous investigation (Kaneshige 1981). Although the mean of ZPP did not show any significant change, the mean of ZPP/heme ratio and the incidence of these test results above cut-off value was consistently increased during the second and third trimesters of pregnancy. Among the four hematologic parameters, the highest incidence was observed in ZPP/heme ratio(61.0 % for third trimester and 53.7 % for total subjects).

During the first trimester, the incidences of the ZPP and ZPP/heme ratio above the cut-off values were higher than those in hemoglobin and serum ferritin(Table 2), and the sensitivities and positive predictive values(PVs) of these tests in the first trimester group were lower than those in the second and third trimester groups(Table 4). Although the number of subjects studied in the first trimester group was relatively small, these findings suggest that the diagnostic efficiencies of these tests have some limitation and agree well with other investigations that showed the diagnostic efficiencies of the ZPP and ZPP/heme ratio varied with the hemoglobin concentration or prevalence of iron deficiency (Schifman *et al.* 1982; Zanella *et al.* 1989; Jesen *et al.* 1990).

But, in a total of 136 women, the ZPP/heme ratio has a high specificity(about 75 %) with moderate PVs of positive test for detecting anemia and iron deficiency (low serum ferritin) in comparison with the ZPP and hemoglobin concentration (Table 3), and a much better correlation was found between the ZPP/heme ratio and serum ferritin(r = –0.51) or blood hemoglobin(r = –0.51) than between the ZPP, hemoglobin and serum ferritin(Fig 1). The ZPP and ZPP/heme ratio consistently demonstrated significantly different mean values between anemia and non-anemic or iron-deficient and normal groups(Fig. 2).

In spite of the low diagnostic efficiency of the ZPP/heme ratio in the first trimester group with low prevalence of iron deficiency, our results in this study suggest that the ZPP/heme ratio is superior to that of blood hemoglobin and ZPP in identifying iron deficiency, and has a similar diagnostic efficiency with the serum ferritin measurement. So the erythrocyte ZPP/heme ratio test is shown to be an effective field or office method in iron-depletion screening because of its speed, convenience and low cost, and will reduce the number of serum ferritin analyses needed in the high risk groups for iron deficiency.

REFERENCES

Beutler E, Robson MJ, Buttenwieser E. A comparison of the plasma iron, iron- binding capacity, sternal marrow and other methods in the clinical evaluation of iron status. Ann Intern Med 1958; 48:60-6

Blumnerg WE, Eisinger J, Lamola AA, Zuckerman DM. The hematofluorometer. Clin Chem 1977; 23:270-4

Brittenham GM, Danish EH, Harris JW. Assessment of bone marrow and body iron stores: Old techniques and new technologies. Semin Hematol 1981; 18:194-

221

Cook JD, Lynch SR. The liabilities of iron deficiency. Blood 1986; 68:803-9

Dagg JH, Goldberg A, Lockhead A. Value of erythrocyte protoporphyrin in the diagnosis of latent iron deficiency(sideropenia). Br J Haematol 1966; 12: 326-30

Dallman PR. Biochemical basis for the manifestations of iron deficiency. Annu Rev Nutr 1986; 6:13-40

Galen RS, Gambino SR. Beyond normality: the predictive value and efficiency of medical diagnosis. John Wiley, New York, 1975

Holly RG. The iron and iron-binding capacity of serum and the erythrocyte protoporphyrin in pregnancy. Obstet Gynecol 1953; 2:119-26

Jackson KW. Interlaboratory comparison of results of erythrocyte protoporphyrin analysis. Clin Chem 1978; 24:2135-8

Jensen BM, Sand SH, Grandjean P, WIggers P, Dalh j J. Screening with zinc protoporphyrin for iron deficiency in non-anemic female blood donors. Clin Chem 1990; 36:846-8

Kaneshige E. Serum ferritin as an assessment of iron stores and other hematologic parameters during pregnancy. Obstet Gynecol 1981; 57:238-42

Labbe RF, Finch CA, Smith NJ, Doan RN, Sood SK, Madan N. Erythrocyte protoporphyrin/heme ratio in the assessment of iron status. Clin Chem 1979; 25: 87-92

Labbe RF, Rettmer RL. Zinc protoporphyrin: A product of iron deficient erythropoiesis. Semin Hematol 1989; 26:40-6

Labbe RF, Rettmer RL, Shah AG, Turnlund JR. Zinc protoporphyrin: Past, present and future. Ann NY Acad Sci 1987; 514:7-14

Lamola AA, Joselow M, Yamane T. Zinc protoporphyrin (ZPP): A simple, sensitive, fluorometric screening test for lead poisoning. Clin Chem 1975; 21:93-7

Milne DB, Gallagher SK, Nielsen FH. Response of various indices of iron status to acute iron depletion produced in menstruating women by low iron intake and phlebotomy. Clin Chem 1990; 36:487-91

Park JW, Lee MY, Park HM, Kim WK, Bae DH. The changes of iron dynamics during pregnancy. Kor J Obstet Gynecol 1987; 30:819-27

Piomelli S, Brickman A, Carlos E. Rapid diagnosis of iron deficiency by measurement of free erythrocyte porphyrins and hemoglobin: the FEP/hemoglobin ratio. Pediatrics 1976; 57:136-41

Schifman RB, Rivers SL, Finley PR, Thies C. RBC zinc protoporphyrin to screen blood donors for iron deficiency anemia. JAMA 1982; 248:2012-5

Stanton NV, Gunter EW, Parsons PJ, Field PH. Empirically determined lead-poisoning screening cutoff for the Protofluor-Z hematofluorometer. Clin Chem 1989; 35:2104-7

Tchai BS, Cho KH, Lee KY. A study on red cell protoporphyrin concentration and iron metabolism. Korean J Nutrition 1974; 7:1-13

Tchai BS, Suh DH, Han JH. Study on the erythrocyte zinc protoporphyrin and ZPP/heme ratio in normal Korean. Korean J Nutrition 1992; 25:351-9

Thompson WG, Meola T, Lipkin M Jr, Freedman ML. Red cell distribution width, mean corpuscular volume, and transferrin saturation in the diagnosis of iron deficiency. Arch Intern Med 1988; 148:2128-30

Yip R, Johnson C, Dallman PR. Age-related changes in laboratory values used in the diagnosis of anemia and iron deficiency. Am J Clin Nutr 1984; 39:427-36

Zanella A, Gridelli L, Berzuini A, Colotti MT, Mozzi F, Milani S, Sirchia G. Sensitivity and predictive values of serum ferritin and free erythrocyte protoporphyrin for iron deficiency. J Lab Clin Med 1989; 113:73-8