Automated measurement of serum ferroxidase activity

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A method is described for automated measurement of serum ceruloplasmin ferroxidase activity. In this method, Fe^{2+} ions are used as the substrate. In addition, a new calibration system without ceruloplasmin is also presented. Optimum assay reaction conditions were determined. Maximal catalytic activity was obtained at 0.45 mol/L acetate buffer, pH 5.8. The reagents and calibrator are stable for at least 6 months. Significant correlations between serum ferroxidase and *p*-phenylenediamine oxidase activities ($r = 0.96$; $P < 0.0001$) and copper concentration ($r = 0.93$; $P < 0.0001$) were found. The range for serum ceruloplasmin ferroxidase activity in healthy persons was 198-1107 U/L, and in patients with bronchial asthma it was 601-1912 U/L.

The copper-containing blue oxidase protein ceruloplasmin (ferroxidase-I; EC 1.16.3.1) has oxidase activities towards polyamines, polyphenols, and inorganic ferrous ions. However, only the ferrous ion is considered as a biological substrate for ceruloplasmin. Ceruloplasmin has the highest affinity for Fe^{2+} among other substrates *(1)*, and the catalytic oxidation of ferrous ions or ferrous complexes to the ferric state by ceruloplasmin is termed as ferroxidase I activity.

Several methods for determining oxidase and ferroxidase activities of ceruloplasmin have been reported *(2–4)*. However, none of these methods are currently utilized in automated analyzers. Consequently, the only method currently used for determination of serum ceruloplasmin concentration is immunochemistry.

In this study, a new serum ceruloplasmin ferroxidase activity measurement method was developed and applied to an automated analyzer. In addition, a new calibration system without ceruloplasmin was also presented. Optimum reaction conditions for enzymatic activity were

indicated. Serum ferroxidase activities in healthy persons and in patients with disease were determined.

Materials and Methods

ASSAY PRINCIPLE

The serum sample was incubated with a known amount of ferrous ion in 0.45 mol/L acetate buffer (pH 5.8). During the catalytic oxidation of ceruloplasmin, ferrous ions are oxidized to ferric ions. The chromogen, 3-(2-pyridyl)-5,6-bis(2-[5-furylsulfonic acid])-1,2,4-triazine, forms a highly colored Fe^{2+} complex with ferrous ions *(5)* but does not form a colored complex with ferric ions. At the end of the incubation period, the chromogen was added, and the remaining nonoxidized ferrous ions were measured photometrically at 590–610 nm. The difference in the ferrous ion concentration before and after the enzymatic reaction indicated the amount of oxidized ferrous ion. The amount of enzyme that converted 1 μmol of substrate into product per minute was defined as one unit. Autooxidation of ferrous ions was inhibited during the assay and storage without altering the ferroxidase activity.

CALIBRATION PRINCIPLE

In this calibration system, ferroxidase activity was estimated by the endpoint measurement method. This system measures the changes of ferrous ion concentration in the reaction medium, which contains ferrous ions present in substrate solution. EDTA is a chelator of ferrous ions and prevents formation of the colored Fe^{2+} complex from ferrous ions. Because EDTA completely chelates all ferrous ions and no free ferrous ion is left in the reaction medium to form colored Fe^{2+} complex, usage of EDTA solution as a calibrator leads to zero absorbance. On the other hand, deionized water as an another calibrator gives maximal absorbance in the assay because all ferrous ions in the reaction medium are available to form colored Fe^{2+} complex. This calibration system was linear for the measurement of ferrous ion concentration. When this calibration system is used, the changes of ferrous ion concentration in the reaction medium can be estimated directly.

Because of the negative relationship between ferroxidase activity and absorbance value, a two-point inverse

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calibration was used. Deionized water was used as the first calibrator, and EDTA solution was the second calibrator. The first calibrator gave the absorbance of the total ferrous ion concentration in the reaction medium and reflected substrate concentration. The second calibrator gave zero absorbance and mimics that all of the substrate was oxidized. The incubation period of the enzyme and the concentration of the substrate are known. The values of calibrators were estimated as enzyme activity. Although this calibration was linear for the measurement of ferrous ion concentration, it was linear only on the first half of the calibration curve for the measurement of ferroxidase activity. The enzymatic activity measurements were performed in this linear region, and above this value, automated dilution was performed.

APPARATUS
A Cecil 3000 spectrophotometer with a temperature-controlled cuvette holder (Cecil), a Hitachi 911 automated analyzer (Boehringer Mannheim), and a Varian SpectrAA 250 Plus atomic absorption spectrophotometer (Varian) were used.

REAGENTS AND CHEMICALS
All chemicals and the human ceruloplasmin calibrator were purchased from Sigma Chemical Co. Type I reagent grade deionized water was used.

SERUM SAMPLES
Serum samples of healthy persons from a blood bank, of patients with vivax malaria from the Malaria Eradication Center of Sanliurfa of Turkey, and from patients with Wilson disease or bronchial asthma from the Research Hospital of Harran University of Turkey were supplied. Individuals fasted for 12 h prior to sample collection. For the precision determination, a serum pool that had high ferroxidase activity was obtained from serum samples of asthmatic patients. The serum pool with medium ferroxidase activity was provided from healthy persons. The serum pool with low ferroxidase activity was set up by mixing the serum samples of a patient with Wilson disease and a healthy person.

PROCEDURES
p-Phenylenediamine oxidase activity measurement. Serum ceruloplasmin *p*-phenylenediamine (PPD) oxidase activity was measured according to Sunderman and Nomoto *(2)*. At pH 5.4, ceruloplasmin catalyzes the oxidation of PPD to yield a colored product. The rate of formation of the colored oxidation product is proportional to the concentration of serum ceruloplasmin if a correction is made for nonenzymatic oxidation of PPD.

FERROXIDASE ACTIVITY MEASUREMENT
Reagents. The 0.45 mol/L acetate buffer solution, pH 5.8 (reagent 1), was made as follows: 36.91g of CH_3COONa was dissolved in 1000 mL of deionized water (final concentration, 0.45 mol/L). Reagent grade glacial acetic acid (25.87 mL) was diluted to 1000 mL with deionized water (final concentration, 0.45 mol/L). The sodium acetate solution (940 mL) was mixed with 60 mL of the acetic acid solution under a pH meter; the pH of the acetic acid-sodium acetate buffer was 5.8. One milliliter of chloroform was added. The buffer solution is stable for at least 6 months at room temperature.

The substrate solution (reagent 2; 367 μmol/L) was made as follows: 9.90 g of thiourea was dissolved in 1000 mL of deionized water, and then 0.1440 g of $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ was dissolved in 1000 mL of this 130 mmol/L thiourea solution. This sequence is important, because ferrous ions are oxidized when ferrous compound is dissolved in water prior to thiourea addition. However, because the reduction of oxidized ferric ions to ferrous ions takes a long time, no ferrous ion is oxidized if the substrate solution is prepared according to the procedure described above. In addition, 0.2 mL of Brij 35 solution and 1.0 mL of chloroform were added. The substrate solution is stable for at least 6 months at 4 °C.

The chromogen solution (reagent 3; 18 mmol/L) was made as follows: 0.8899 g of 3-(2-pyridyl)-5,6-bis(2-[5-furylsulfonic acid])-1,2,4-triazine was dissolved in 100 mL of buffer solution. The chromogen solution is stable at least 6 months at 4 °C.

The first calibrator was type I reagent grade deionized water, which was prepared fresh. To make the second calibrator, 25 mmol/L EDTA, 0.8955 g of EDTA trisodium salt was dissolved in 100 mL of deionized water. This calibrator is stable for at least 6 months at room temperature.

Procedure. The main chemistry settings of the Hitachi 911 automated analyzer were adjusted according to following instructions. Three microliters of sample or calibrator was added to 350 μL of reagent 1. Then, 75 μL of reagent 2 was added after 1 min, and the mixture was incubated 3.8 min at 37 °C. At the end of the incubation period, 30 μL of reagent 3 was added, and the absorbance of the colored complex at 600 nm was recorded. The absorbance remains stable for at least 1 h. For the blank correction, a second (bichromatic) wavelength at 700 nm was used. In the calibration procedure, two-point inverse calibration was used. The first calibrator was 0.00 U/L, and the second calibrator was 2400 U/L.

The serum copper concentration was measured by atomic absorption spectrophotometer *(6)*.

STATISTICAL ANALYSES
ANOVA, correlation analyses, and linear regression analyses were performed by SPSS for Windows, Release 6.0, computer program (SPSS Inc.).

Results

COLOR DEVELOPMENT
Ferrous ions formed a highly colored Fe^{2+}-complex with 3-(2-pyridyl)-5,6-bis(2-[5-furylsulfonic acid])-1,2,4-

triazine within a few seconds. Color development was complete within 5 min. Maximal absorbance was measured between 590–610 nm. In this assay, the chromogen was present in excess, and the absorbance of the colored complex is dependent on the amount of ferrous ion. The absorbance of the colored Fe^{2+} complex was found to be linear up to 100 μmol/L ferrous ion concentration. The maximal absorbance value was obtained above 300 μmol/L chromogen concentration in the reaction medium.

OPTIMUM CONDITIONS

Acetate buffer solutions ranging from pH 4.5 to 5.9 and sodium phosphate buffer solutions ranging from pH 5.8 to 6.8 were used for ferroxidase activity measurement. The optimum pH of the acetate buffer was adjusted to 5.8, and the buffer concentration was 0.45 mol/L. Buffers containing phosphate increase the rate of spontaneous oxidation of ferrous ions. Chloroform (1 mL/L) was added to the buffer solution as microbicidal agent. Sodium azide was avoided because it completely inhibited ceruloplasmin ferroxidase activity.

Ferrous ion concentrations were increased to the maximal limit of the linearity of the colored Fe^{2+} complex at 100 μmol/L. However, at this point the absorbance value obtained was near the upper limit of spectrophotometric measurements. Therefore, 60 μmol/L substrate was used in the assay. The K_m of serum ferroxidase for Fe^{2+} was found to be 4.5 μmol/L. To prevent spontaneous oxidation of ferrous ions, 130 mmol/L thiourea was used in the substrate solution. This additive protected the substrate against autooxidation during the assay and storage. Thiourea, a weak reducing agent, completely protected the substrate against autooxidation. However, thiourea did not markedly reduce the ferric ions formed during assay.

Other antioxidants, ascorbate and hydroxylamine, reduced large amounts of ferric ions and also inhibited ferroxidase activity. For these reasons, these antioxidants were not included in the reagents. Sodium azide was avoided; chloroform (1 mL/L) and Brij-35 (0.2 mL/L) were added. Oxidation and deterioration of substrate solution did not occur for at least 6 months.

The serum ferroxidase activity rate at 37 °C was linear with respect to duration of an incubation period of 0–12 min. The lag phase period of enzymatic reaction was found to be <10 s.

CALIBRATION OF THE ASSAY WITHOUT CERULOPLASMIN

In the calibration process in which deionized water and EDTA were used, the measurement of ferrous ion concentration had indeed been calibrated. The measurement of ferrous ion concentration was linear from 0.0 to 100 μmol/L; however, in this assay the range from 0.0 to 60 μmol/L was used.

In the first calibration point, ferrous ion concentration was 60 μmol/L and gave an absorbance value of 1.20A. In the second calibration point, the ferrous ion concentration was 0.0 μmol/L and gave ~0.00A. The decreased (oxidized) amount of ferrous ion within the 0–60 μmol/L range could be measured by this calibration. The corresponding ferroxidase activity for 1 μmol/L ferrous ion oxidation was calculated:

$$Enzyme\ activity = (C_1 - C_2) \cdot t^{-1} \cdot df$$

Where C_1 is the substrate concentration at the beginning of the enzymatic reaction (60 μmol/L), and C_2 is the substrate concentration at the end of the enzymatic reaction:

$$C_1 - C_2 = \Delta C_s$$

t is the incubation time of the enzyme with substrate (3.8 min), and d_f is the dilution rate of the sample (total volume/sample volume; 458/3):

$$Enzyme\ activity$$
$$= (\Delta C_s/3.8) \cdot (458/3) = \Delta C_s \cdot 40\ U/L$$

If ΔC_s is 1.0 μmol/L, the corresponding ferroxidase activity is 40 U/L. Because the total ΔC_s is 60 μmol/L, the value of EDTA calibration is (60 · 40), or 2400 U/L.

Although the absorbance of the colored product was linear from 1.20 to 0.00, the assay of enzymatic activity measurement was linear from 1.20 to 0.47 (from 0.0 to 1380 U/L). Above this activity limit, automated dilution of serum was performed. Disodium, tetrasodium, and tripotassium salts of EDTA caused zero absorbance. Any form of EDTA salt that causes zero absorbance can be used in the second calibrator.

INHIBITION

Heparin did not inhibit the assay, but citrate did. Sodium azide completely inhibited ferroxidase activity of commercial ceruloplasmin samples; it inhibited 95–99% of serum ferroxidase activity. Commercial human ceruloplasmin samples gave a mean recovery of 98.8% (range, 96.7–99.8%) when added to grossly hemolyzed, jaundiced, or lipemic sera. The method is not susceptible to interference by hemolysis, jaundice, or lipemia. No significant difference was observed between fresh and nonfresh serum ferroxidase activities. The within-day CV was 2.1%.

LINEARITY

Serial dilutions of commercial human ceruloplasmin with acetate buffer were made. The upper limit of linearity in the assay was 1380 U/L. In the regression analyses, the r value was 0.99, the slope was 0.5974 ($S_{y|x} = 0.002$; $P < 0.0001$), the intercept was -3.5329 ($S_{y|x} = 1.2791$; $P = 0.003$) and $SD_{y|x}$ was 19.83, respectively.

ANALYTICAL SENSITIVITY

As the slope of the calibration curve, the analytical sensitivity was found to be 0.0005 absorbance units $\cdot$ $U^{-1} \cdot L^{-1}$.

DETECTION LIMIT

The detection limit of the method was determined by evaluating the zero calibrator (deionized water) 10 times. The result (mean ± SD) was 15.0 ± 3.6 U/L. The detection limit, defined as the mean ferroxidase activity of the zero calibrator + 3 SD, was 25.8 U/L.

PRECISION AND STORAGE

Within- and between-batch precision were calculated for each of three sera and are shown in Table 1. Serum ferroxidase activity was not affected by storage at 4 °C for 1 week and at −20 °C for 1 month.

RECOVERY

The percent recovery was calculated according to the formula:

$$\% \text{ recovery} = \frac{\text{Amount recovered}}{\text{Amount added}} \times 100$$

where the amount added was the amount of commercial ceruloplasmin added to the sample

$$\times \frac{\text{volume ceruloplasmin}}{\text{total volume}}$$

and the amount recovered was the value for the sample with added ceruloplasmin minus the baseline value. Two pools of sera were used as baseline samples, and quadruplicate assays were run. The mean percent recovery of commercial human ceruloplasmin was 101% (range, 96.1–105.4%).

RELATIONSHIP BETWEEN FERROXIDASE AND PPD OXIDASE ACTIVITIES OF CERULOPLASMIN

We set up 20 different samples of ceruloplasmin, using serial dilutions of commercial human ceruloplasmin in acetate buffer. The ceruloplasmin PPD oxidase activity of samples was measured by spectrophotometer. The ferroxidase activity of the same ceruloplasmin samples was measured by automated analyzer. The most significant correlation was found between ceruloplasmin PPD oxidase activity and ferroxidase activity values ($r = 0.99$; P

<0.0001; n = 20). In the regression analyses, the slope was 0.5826 ($S_{y|x} = 0.0033$; $P < 0.0001$), the intercept was 3.2947 ($S_{y|x} = 2.3859$; $P = 0.1842$), and $SD_{y|x}$ was 27.38, respectively. The relationship between ferroxidase and PPD oxidase activities of serum samples was also significant ($r = 0.96$; $P < 0.0001$; n = 200; Fig. 1). In the regression analyses, the slope was 0.5829 ($S_{y|x} = 0.0128$; $P < 0.0001$), the intercept was 2.2657 ($S_{y|x} = 9.1801$; $P = 0.8053$), and $SD_{y|x}$ was 978.24, respectively.
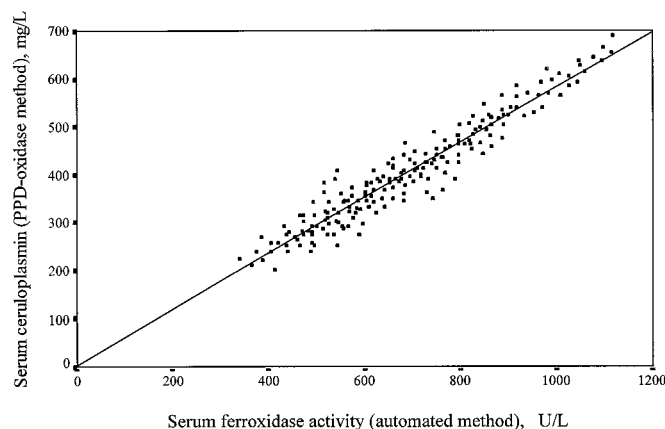

Fig. 1. Correlation between serum ferroxidase and PPD oxidase activities.

CORRELATION BETWEEN SERUM FERROXIDASE ACTIVITY AND COPPER CONCENTRATION

Serum ferroxidase activity was determined by automated analyzer, and serum copper concentration was determined by atomic absorption spectrophotometer. A significant correlation ($r = 0.99$; $P < 0.0001$; n = 50; Fig. 2) was found between serum ferroxidase activity and serum copper concentration of patients with bronchial asthma. In the total group, which contains patients and healthy persons, the correlation coefficient value was lower than that of the asthmatic patient group ($r = 0.93$; $P < 0.0001$; n = 350).
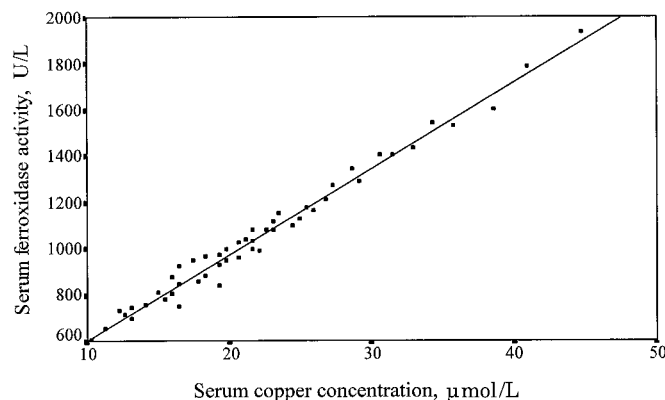
Table 1. Within- and between-batch precision data obtained in the measurements of serum ferroxidase activity.

		Activity, U/L	
	Number	Mean	SD
Within-batch measurements			
High	30	1282	20
Medium	30	521	8
Low	30	353	6
Between-batch days			
High	30	1320	35
Medium	30	512	8
Low	30	320	5

Fig. 2. Relationship between serum copper concentration and ferroxidase activity of patients with bronchial asthma.

The serum ferroxidase activity and copper concentration in healthy persons and patients with Wilson disease, malaria, and bronchial asthma are shown in Table 2. Serum ceruloplasmin concentrations calculated from ferroxidase activities are shown as milligrams per liter in Table 2.

Discussion

Holmberg and Laurell *(7)* named the blue protein from serum ceruloplasmin. The physiological function of ceruloplasmin has been the subject of considerable investigation. Multiple biochemical activities of ceruloplasmin have been described, including copper transport, oxidation of various amines, oxidation of Fe^{2+} to Fe^{3+} for subsequent uptake by transferrin and ferritin, antioxidant activity against lipid peroxidation, and endogenous modulation of the inflammatory response. Reported cellular actions of ceruloplasmin include stimulation of cell proliferation and angiogenesis *(8)*. Ceruloplasmin is an acute phase protein, with a response of intermediate magnitude compared with other acute phase proteins; the plasma concentration is increased two- to threefold during inflammation and pregnancy and after traumatic injury, including surgery. The serum concentration of ceruloplasmin has been considered as a useful indicator in various clinical studies such as those on Wilson disease and Menkes syndrome. Ceruloplasmin contains six or seven copper atoms per molecule and represents 90–95% of serum copper content *(9)*.

Ceruloplasmin has oxidase activity toward several physiological and nonphysiological substrates. However, the oxidase activity of ceruloplasmin is the greatest toward Fe^{2+} *(1)*. A few original methods using Fe^{2+} as substrate have been described. One method uses an ion chromatography system *(4)*, which is impractical for routine analyses. The others have been based on the kinetic method *(3)* in which oxidized iron ions are bound to apotransferrin, and the change of absorbance of the Fe^{3+}-transferrin complex at 450 nm is followed. In these methods turbidity, autooxidation of substrate, and the stability of reagents are obstacles.

Although ceruloplasmin is an enzyme that has high affinity to its physiological substrate, ferrous ions, it is most frequently assayed by immunochemical techniques.

Because the protein is labile and pronounced modification of its immunochemical properties can occur, nephelometric measurements of ceruloplasmin must be interpreted with care.

An important difficulty in various ferroxidase activity measurement methods is to prevent autooxidation of the substrate. Some processes, such as degassing, applied to prevent autooxidation do not give satisfactory results *(4)*. In this study, thiourea completely prevented autooxidation of substrate during the assay and storage without inhibition of ferroxidase activity. Thiourea is a weak reducer and makes weak complexes with metals. It may prevent autooxidation by its weak reductive effect; additionally, it may also make a weak thiourea-ferrous ion complex.

The use of EDTA solution instead of ceruloplasmin as a calibrator was the second main point of this study. EDTA has more advantages than human commercial ceruloplasmin samples. Ceruloplasmin, which has protein structure, may lose its enzymatic activity with time; an EDTA solution, on the other hand, maintains its chelator effect for at least 6 months at room temperature. Another advantage of EDTA solution is that it is less expensive than human ceruloplasmin samples.

In both spectrophotometric and automated analyzer experiments, EDTA solution completely chelated all ferrous ions and prevented the formation of the colored Fe^{2+} complex. This calibration point gave no absorbance (absorbance = 0.00A). Because EDTA is present only in the second calibrator and is not present in the buffer, substrate, and chromogen solution, it does not effect serum ferroxidase activity.

In this method, human ceruloplasmin sample can be used as direct calibrator(s); however, in this condition the results are expressed as mg/L. If the results are to be expressed as enzyme activity, the specific ferroxidase activity of the ceruloplasmin sample must be known.

I found serum ferroxidase activity in healthy subjects to be 537 ± 201 U/L by the automated measurement method. Swain et al. *(10)* reported the activity as 780 ± 75 U/L, and Winkles et al. *(3)* found the activity to be 2030 ± 490 U/L. Because the measurement of enzyme activity depends on the absorbance of the ferric ion-apotransferrin

Table 2. Serum ferroxidase activities, ceruloplasmin concentrations, and copper concentrations in healthy persons and in patients with Wilson disease, vivax malaria, and bronchial asthma.

	Subject, n	Ferroxidase,[a] U/L	Ceruloplasmin,[a] mg/L	Copper,[a] µmol/L
Healthy persons	250	537 ± 201	315 ± 119	15.82 ± 4.15
Wilson disease[b]	1	25	17	2.63
Vivax malaria	50	749 ± 214[c]	440 ± 125[c]	25.17 ± 7.19[c]
Bronchial asthma	50	1057 ± 292[c,d]	618 ± 172[c,d]	24.50 ± 9.64[c]

[a] Mean $\pm$ 1 SD.

[b] The values of the patient with Wilson disease were withdrawn from statistical analysis because this group has only one data point.

[c] $P < 0.05$ vs healthy persons.

[d] $P < 0.05$ vs vivax malaria.

complex, nonenzymatic oxidation of ferrous ions to ferric ions may lead to falsely increased activity because the substrate is not protected against to autooxidation. In addition, apotransferrin, which is a ferric ion acceptor, may potentiate ferroxidase activity. These characteristics may explain the difference between the results of this and previous methods.

The most significant correlation ($r = 0.99$; $P < 0.0001$) was found between the PPD oxidase and ferroxidase activities of commercial human ceruloplasmin samples; however, the correlation coefficient of serum samples was lower ($r = 0.96$; $P < 0.0001$) than that of commercial human ceruloplasmin samples. In the oxidase activity determination, various lag phase periods for different serum samples have been reported (11). In the new method the lag phase period was found to be shorter than 10 s. In this study, serum ceruloplasmin ferroxidase activity was found to be responsible for 95–99% of the measured total serum ferroxidase activity. On the other hand, serum ceruloplasmin PPD oxidase activity is known to be responsible for 90–95% of measured serum PPD oxidase activity. These characteristics may explain the difference between correlation coefficients of PPD oxidase and ferroxidase activities of commercial human ceruloplasmin and serum ceruloplasmin samples.

Sodium azide binds copper and inhibits oxidase and ferroxidase activities of ceruloplasmin. In this study, sodium azide completely inhibited ferroxidase activity of commercial ceruloplasmin; however, it inhibited 95–99% of serum ferroxidase activity. Serum ferroxidase activity that cannot be inhibited is termed as ferroxidase II activity. Subtracting ferroxidase II activity from total ferroxidase activity gives net ceruloplasmin ferroxidase activity, which is termed as ferroxidase I. Because the ferroxidase II activity was a very low percentage of the total ferroxidase activity, the subtraction procedure was neglected in this study. In the same manner, it has also been shown that sodium azide completely inhibits the oxidase activity of commercial human ceruloplasmin, whereas it inhibits 90–95% of the oxidase activity of serum ceruloplasmin.

Recent studies of experimental copper depletion in humans suggest that specific enzymatic activity, which is the ratio of enzymatic activity to the enzymatic mass concentration of ceruloplasmin, may be a more sensitive indicator of copper status than either plasma copper and erythrocyte superoxide dismutase (12). Presently, serum ceruloplasmin is measured by immunometric methods. The specific enzymatic activity of ceruloplasmin may be calculated automatically, and the copper status can be evaluated sensitively by an automated assay system for serum ceruloplasmin ferroxidase activity.

Because ceruloplasmin contains 95% of the copper in human blood and is the major copper-transporting protein, a high correlation between serum copper concentration and ceruloplasmin concentrations has been reported (2). In the asthmatic patient group, the correlation coefficient between serum ferroxidase activity and copper concentration was the most important ($r = 0.99$; $P < 0.0001$); however, in the total group, which contains patients and healthy persons, the correlation coefficient was lower ($r = 0.93$; $P < 0.0001$) than asthmatic patients. This difference may originate from non-ceruloplasmin-bound copper, which is increased in some diseases, and inactivation of ceruloplasmin by oxidative stress (13).

Although the liver is the major site of ceruloplasmin synthesis, some studies have demonstrated that the lung is the predominant extrahepatic site of ceruloplasmin gene expression in both the rat and human (14). In this study, serum ferroxidase activity and copper concentration were found to be increased. Increased serum ceruloplasmin activity in asthma might also originate from the lung itself in addition to synthesis in the liver.

The increased serum ferroxidase activity and copper concentration of patients with vivax malaria may be dependent on the acute phase reaction of the host.

Serum ferroxidase activity in the patient with Wilson disease was 25 U/L. Because some patients with Wilson disease have serum ceruloplasmin protein that has immunological antigenic properties but is functionally inactive (15), enzymatic measurement methods of ceruloplasmin may be preferred to immunochemical determination methods in the screening of Wilson disease (16).

The described method can easily be automated, is less expensive, and may be more appropriate than alternative methods for measurement of ceruloplasmin.

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