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Araştırma Makalesi / Research Article

Determination of Iron in Blood Serum by Spectrophotometric and Atomic Absorption Methods as a Comparative Study

Kan Serumundaki Demir Miktarının Spektrofotometrik ve Atomik Absorpsiyon Metodlarıyla Karşılaştırmalı Olarak Tayini

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ABSTRACT

Purpose: Iron is an essential element for most life on Earth, including human beings and its accurate determination is extremely important. A simple and sensitive spectrophotometric method has been used for the determination of iron concentration in blood serum and compare with atomic absorption spectrophotometric method.

Material and Methods: To find the appropriate, simple and accurate method for iron determination in serum samples. A comparative study between two analytical methods was planned. The methods are based on the reaction of iron with ferene reagent at acidic condition. The method of determining iron in serum by flame atomic absorption spectrophotometry with slurry nebulization into an air-acetylene to various flame has been used.

Results: The results showed that distribution of iron content between serum samples are different, it was between 41 to 343.5 mcg/dL. Values of iron content measured in the serum samples with spectrophotometer method are in good agreement with the results obtained with atomic absorption method.

Conclusion: The spectrophotometric method with ferene reagent can be successfully and accurately applied to the determination of iron in serum and it can be used instead atomic absorption.

Key Words: İron, serum, determination, ferene, spectrophotometry, atomic absorption.

ÖZET

Amaç: Demir insanoğlu dâhil dünyada ki birçok yaşam formu için gerekli bir element olup onun canlıdaki miktarının doğru tayini son derece önemlidir. Çalışmamızdaki amacımız kolay ve hassas olan spektrofotometrik metod ile kan serumundaki demir konsantrasyonlarını belirleyerek bulunan değerleri atomik absorpsiyon spektrofotometrik yöntemi ile karşılaştırılmaktadır.

Materyal ve Metod: Serum örneklerinde demir tayini için kullanılabilecek en uygun, en basit ve en doğru yöntemi bulmak. İki analitik yöntem arasında karşılaştırmalı bir çalışma yapılması düşünüldü. Yöntemler asidik koşullarda ferene reaktifi ile demirin reaksiyonuna dayanır. Serum demir belirleme yöntemi olarak nebulizasyon ürününün bir hava-asetilen karışımı içerisinden geçirildiği alevli atomik absorpsiyon spektrofotometresi yöntemi ile yapıldı.

Bulgular: Elde edilen bulgular; serum örnekleri arasındaki demir içeriği dağılımının 41 ile 343.5 mcg/dl aralığında farklılık gösterdiğini ortaya koymuştur. Serum örneklerinde spektrofotometre yöntemi ile ölçülen demir içeriğine ait değerler atomik absorpsiyon yöntemi ile belirlenen sonuçlarla uyum göstermektedir.

Sonuç*:* Ferene reaktifinin kullanıldığı spektrofotometrik yöntem serum demir içeriğinin başarılı ve doğru olarak belirlenmesinde uygulanabilir ve ayrıca bu yöntem atomik absorpsiyon yöntemi yerine kullanılabilir.

Anahtar Kelimeler: Demir, serum, belirleme, ferene, spektrofotometre, atomik absorpsiyon

INTRODUCTION

Iron is an essential element for most life on Earth, including human beings. The control of this necessary but potentially toxic substance is an important part of many aspects of human health and disease[1]. Iron ions circulate bound to plasma transferrin and accumulate within cells in the form of ferritin. The subsequent development of practical clinical measurements of serum iron, transferrin saturation, plasma ferritin, and red cell protoporphyrin permitted the definition and detection of the body's iron store status and iron-deficient erythropoiesis[2]. The difference in iron absorption from various foods , meals or drugs depends, in part, on the chemical properties of iron[1]. The clinical effects of iron deficiency have been described in the medical literature dating back to the Middle Ages, in fascinating accounts of a disorder called chlorosis. Large amounts of ferrous salts are toxic, but fatalities are rare in adults[2]. Iron overload usually causes tissue damages, so studying concentration of serum iron content has its diagnostic and pathologic value.

Serum iron determinations have made important contributions to the diagnostic process for several decades. Despite the importance of iron measurements, the accuracy of present routine methods is suspect; speed and convenience of methods have taken the place of accuracy[3]. Main sources of error in iron methods are incomplete dissociation of iron from binding proteins, loss of iron during protein precipitation, incomplete reduction of iron(III) to iron(II), copper and hemoglobin interferences, spectrophotometric interferences by compounds present in the serum matrix (e.g., bilirubin and lipids)[4] and proteins may produce turbidity because of the high proportion of serum that must be used and because the chromogenic reaction is usually performed at pH's that are near the isoelectric point of many serum proteins[5]. Usually, surfactants have been used to eliminate turbidity, but not always with complete success, owing to the variable protein composition of sera[5,6]. In the field of clinical diagnostics, the amount of iron bound to transferrin is commonly referred to as the serum iron, There is essentially no difference between plasma and serum iron. Serum is usually used for the iron assay for reasons of technical capability. Normally the amount of iron in serum/plasma (not including hemoglobin) is about 100 micrograms per l00 milliliters of blood[7]. In the clinical laboratory, the amount of iron measured in serum can be done by known methods. In one method, serum iron is assayed by adding a serum sample to a reagent buffered at an acid pH. At this acid pH, ferric ion dissociates from transferrin. The reagent includes a reducing agent, which aids in the dissociation process and reduces ferric ion to ferrous ion. A chromogenic reagent is then added and the chromogen complexes with ferrous iron to form a colored complex. The colored complex is measured spectrophotometrically[7]. Results were obtained by the use of o-phenanthroline[8], protocatechuic acid[9], 4: 7-diphenyl-1: 10-phenanthroline[10], ethanolic solution of bathophenanthroline[11], 2,4,6-Tripyridyl-*s*-triazine (TPTZ)[12], Catalytic photometric method[13], N-ethyl-2-methyl-3-hydroxypyridin-4-on(EMHP)[14] and many other chelating reagents. Ferene was synthesized in 1980 and reacts with iron(II) to form a stable, deep blue complex which is also very soluble in water[15]. Iron content was determined by atomic absorption spectrophotometry "AAS" in undiluted plasma or a 1 :1 dilution of plasma and deionized water[16]. Flame Atomic Absorption Spectrophotometry (AAS) was used for determination of Fe(II) and Fe(III) in water after their separation with Aspergillus niger immobilized on sepiolite[17].

Iron is traditionally measured in unhaemolysed serum by colorimetric assay. The two major approaches are colored complex formation followed by spectrophotometry or AAS. Colorimetric procedures to quantify serum iron are usually available in hospital pathology departments

and are suitable for rapid emergency analyses, although they suffer from at least one of three undesirable features:[1] low sensitivity of the color reaction employed,[2] turbidity in the final color solution, and[3] nonspecific background absorbance in the color solution. More sensitive and specific AAS " Atomic Absorption Spectrometry" procedures can be applied to the analysis of iron in serum, plasma, whole blood and urine.

Therefore this study was carried out to estimate the appropriate method for iron analysis in blood serum. So determination of iron concentration in blood serum with two different analytical methods was done.

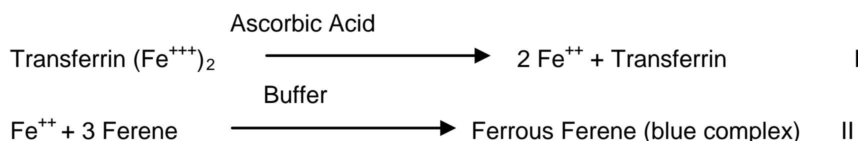
MATERIALS and METHODS

Ninety seven (97) serum samples were obtained from healthy volunteers. Serum samples were kept at -20^0C till analysis.

Analytical Procedure

1. *Spectrophotometry*

Principle:- In an acidic medium transferrin bound iron dissociates into ferric ions which are reduced to ferrous ions in the presence of ascorbic acid. The ferrous iron reacts with the chromogen Ferene to form a blue chromophore which absorbs at 595 nm. The absorbance is directly proportional to the serum iron concentration.



Reagents

Components and concentrations

R1: Acetate buffer		pH 4.5	800 mmol/l
Thiourea			90 mmol/l
R2: Ascorbic Acid			45 mmol/l
Ferene			0.6 mmol/l
Thiourea			20 mmol/l
Standard	Ferrous sulfate	100 mcg/dl	(17.9 mmol/l)

Assay procedure

Wavelength 595nm
Optical path 1cm
Temperature 20 – 25°C, 37°C
Measurement Against Blank

	Blank	Sample or standard
Sample or standard	-	0.1 ml
Dist. Water	0.1 ml	-
Reagent 1	1 ml	1 ml
Mix, read absorbance A1 after 1 – 5 min., then add:		
Reagent 2	0. 25 ml	0.25 ml

Mix, read absorbance A2 after 2 min.

ΔA = [(A2 -0.82 A1) sample "standard"] – [(A2 – 0.82 A1) blank]

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The factor 0.82 compensates the decrease of the absorbance by addition of reagent 2. The factor is calculated as follows: (Sample + R1)/Total volume. This compensation was necessary as a high sample volume was used.

Calculation

With standard.

$$\text{Iron [mcg / dl]} = \frac{\Delta A \text{ Sample}}{\Delta A \text{ Standard}} \times \text{Conc. Std[mcg / dl] "100 mcg/dl"}$$

2. *Atomic Absorption Spectrophotometer*

The same samples were reanalyzed for iron contents by Perkin-Elmer Atomic Absorption Spectrophotometer at a flow rate 6-8 ml/min, operated at 248.3 nm wavelength.

Statistical Methods

The statistical significance between iron content in the samples by two used methods was evaluated with Wilcoxon Signed Ranks Test, p-values less than 0,05 was considered to be statistically significant.

RESULTS

Iron content of 97 serum samples was determined by spectrophotmetric method and compared with atomic absorption method. Quantification of the same serum samples that assayed with spectrophotmetric method was determined by atomic absorption. The linear regression equation was y= 0.015x + 0.002 with R^2 0,999 for atomic absorption. (Figure 1).

Iron concentration in the samples was summarized in table1 as correlation was done by: concentrations obtained by Spectrophotometry/ conc. obtained by AAS

Correlation mean for all samples was 1.00378 ± 0.064.

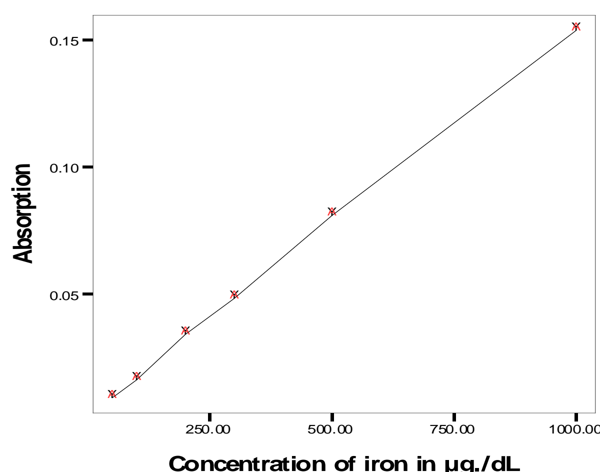

Figure 1. Linear regression of iron concentration in samples to absorption spectroscopy

Table 1. Correlation mean for all samples in different concentrations concentrations obtained by Spectrophotometry/ conc. obtained by AAS.

Conc. Range of samples	Number of samples	Correlation mean
Values < 100 μg/dL	18	1.01586 ± 0.067
Values between 100 μg/dL and 200 μg/dL	47	1.00389 ± 0.065
Values between 200 μg/dL and 300 μg/dL	28	0.99877 ± 0.064
Values between > 300 μg/dL	4	0.98316 ± 0.03

DISCUSSION

From our findings and comparisons of the values obtained from the two analytical methods for the same samples we found that the simple method (spectrophotmetric reading) produced accurate results that there were no significantly different from the atomic absorption method for the serum samples tested. Understanding the effects of trace metals on human health is as complex as it is fascinating. The high concentrations may prove toxic, as also, depletion in the concentration of the essential trace elements may cause various metabolic instabilities due to enzyme dysfunction. In the era of rapid industrialization and technological advances, it is imperative to watch keenly for contamination of the environment and its vital composition from heavy metal wastes emanating out of industries. Many metabolic disorders in man are accompanied by alterations in the concentration of one or more trace elements in some body fluid, especially blood serum or plasma. It is thus important to update ourselves with various techniques available for such determinations, their operational aspects, advantages / disadvantages etc[18]. An element, which is required in amounts smaller than 0.01% of the mass of the organism, is called a trace element. Trace metals function mostly as catalysts for enzymatic activity in human bodies. However, all essential trace metals become toxic when their concentration becomes excessive. Usually this happens when the levels exceed by 40-200 fold those required for correct nutritional response[18]. Interest in trace-element research in clinical medicine, biology, environmental studies, toxicology, and nutrition has become an exciting frontier, and during the last two decades the number of publications on this subject has progressively increased. Recent developments in instrumentation have lowered the limits for determining many trace elements to the low nanogram or even picogram range, thus enabling determination of parts per billion (ng/g) and, in some cases, even less[18,19]. Because most essential trace metals are present in biological specimens in very low concentrations[20], precise and accurate analysis is most essential if meaningful results are to be obtained. Trace-element supplementation is becoming widely used for patients undergoing total parenteral nutrition therapy. Monitoring these patients for the elements has been recommended, but resource restrictions and analytical problems, particularly those related to contamination, prevent adoption of such programs.

A method of determining iron in serum is flame atomic absorption spectrophotometry with slurry nebulization into an air-acetylene to various flame has been developed. This method has been applied to various kinds of trace elements in blood[18]. This method required skills personal and expensive maintenance[21].

This study showed that spectrophotometric method used to determine iron content in serum has no significantly differences with atomic absorption. Ferene was used as a color reagent. The spectrophotometric method is based on

reaction of ferene with iron(II) to form a stable, deep blue complex which is also very soluble in water showing maximum absorbance at 595 nm.

This method is inexpensive and safely compared to atomic absorption. The results showed that distribution of iron content between serum samples are different, it was between 41 to 343.5 mcg/dl. The simple method (spectrophotometric method) is practical, perfect and not very skilled personal for determination of iron in serum for all laboratories that has simple equipment such as spectrophotometer. Values of iron content measured in the serum samples with spectrophotometer method are in good agreement with the results obtained with atomic absorption method.

So we can conclude that the spectrophotometric method with ferene reagent can be successfully and accurately applied to the determination of iron in serum and it can be used instead atomic absorption.

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