IRON CONCENTRATION AND FEMALE PEOPLE, ALBANIA

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Abstract

The purpose of the research was the determination of iron content in blood serum of pregnant and non-pregnant women and to show the effectiveness of therapy with substitute of iron.

77 blood samples were collected during the period of February - March 2013 in accordance with the World Health Organization protocol. The serum samples were prepared through the high speed centrifugation (3600 rpm, in gel tubes) of blood samples diluted to 1:10 ratio with deionized water that contains 0.25% Triton X-100. The analysis of serum samples were performed by using Varian 10+ Atomic Absorption Spectrophotometer equipped with flame system. Through the cluster analysis, based on 70% of similarity and Euclidian distance, the samples under investigation were separated in three groups; the first one with 33 cases (non-pregnant females) the second with 28 cases (pregnant females in treatment with substitute of iron) and the third group with 16 cases (pregnant females without treatment with substitute of iron). During pregnancy iron level in the blood is reduced due to the growing baby and placenta, so pregnant female were in treatment with substitute of iron.

The results show normal levels of iron at pregnant females in comparison with those who were not pregnant. This study demonstrates the effectiveness of therapy with substitute of iron, during the first months of pregnancy.

***Key words**: Iron, Female, Pregnant female, Blood sample, Statistical treatment.*

1. Introduction

The iron requirements grow up significantly during pregnancy. Iron is essential for making hemoglobin, the protein in red blood cells that carries oxygen to other cells. During pregnancy, the amount of blood in the body increases until the female have almost 50 percent more than usual. And it needs more iron to make more hemoglobin for all that additional blood. Pregnant female also need extra iron for their growing baby and placenta. The fetus is relatively protected from the effects of iron deficiency by up-regulation of placental iron transport proteins [1] but evidence suggests that maternal iron depletion increases the risk of iron deficiency in the first 3 months of life, by a variety of mechanisms [2, 3]. There is some evidence for the association between maternal iron deficiency and preterm delivery, low birth weight, possibly placental abruption and increased peripartum blood loss [4, 5, and 6].

Normal ranges of iron in blood sample are:

- Men: 65 to 176 μg/dL
- Women: 50 to 170 μg/dL
- Newborns: 100 to 250 μg/dL
- Children: 50 to 120 μg/dL

Unfortunately, most women start pregnancy without sufficient stores of iron to meet their body's increased demands, particularly in the second and third trimesters so they become anemic. Iron deficiency in childbearing women increases maternal mortality, prenatal and perinatal infant loss, and prematurity. Forty percent of all maternal perinatal deaths are linked to anaemia. Favorable pregnancy outcomes occur 30-45% less often in anaemic mothers, and their infants have less than one-half of normal iron reserves

The risk is even higher:

- if they have sickness severe enough to cause frequent vomiting,
- if they've had two or more pregnancies close together,
- if they're pregnant with more than one baby,
- If they have an iron-poor diet, or if theirs pre-pregnancy menstrual flow were heavy [7].

To reduce the risk of adverse effects caused by iron deficiency during pregnancy, a suggested scheme proposed by World Health Organization guideline, for daily iron supplementation in pregnant women [8] is recommended to take the following daily supplements as is shown in Table 1:

Table 1 Suggested scheme for daily iron supplementation in pregnant women

Supplement composition	Iron: 30 - 60 mg of elemental iron
Frequency	One supplement daily
Duration	Throughout pregnancy, iron supplementation should begin as early as possible
Target group	All pregnant adolescents and adult women
Settings	All settings

**30 mg of elemental iron equals 150 mg of ferrous sulfate heptahydrate, 90 mg of ferrous fumarate or 250 mg of ferrous gluconate.

Objectives of this work were to:

• Determinate iron content in blood serum of pregnant and non-pregnant women.

• Show the effectiveness of therapy with substitute of iron.

2. Materials and Methods

2.1. Materials

Blood Sampling

77 blood samples were collected during the period of February - March 2013 in accordance with the World Health Organization protocol that has these rules:

• Extend the patient's arm and inspect the antecubital fossa or forearm

• Locate a vein of a good size that is visible, straight and clear.

• Apply the tourniquet about 4 - 5 finger widths above the venipuncture site and re-examine the vein

• Disinfect the site using 70% isopropyl alcohol for 30 seconds and allow to dry completely (30 seconds)

• Anchor the vein by holding the patient's arm and placing a thumb below the venipuncture site.

• Enter the vein swiftly at a 30 degree angle

• Once sufficient blood has been collected, release the tourniquet before withdrawing the needle.

• Withdraw the needle gently and then give the patient a clean gauze or dry cotton-wool ball to apply to the site with gentle pressure [9].

Blood Serum Preparation

The biological material used in this study to determine the concentration of Fe in women, is blood serum. To obtain blood serum is passed through this stages:

1. 5 ml of blood obtained by venous puncture is added to the tube with gel.

2. Blood is allowed to clot by leaving it undisturbed at room temperature.

3. Than we removed the clot by centrifuging at 3600rpm in a refrigerated centrifuge.

At the end of this process were obtained the blood serum with a pale yellow color [9].

The serum samples were diluted to 1:10 ratio with deionized water that contains 0.25% Triton X-100.

2.2 Methods

The analysis of serum samples were performed by using Varian 10+ Atomic Absorption Spectrophotometer equipped with flame system. Initially we optimized the measurement conditions. The measurements were done at 248.2 nm and deuterium lamp was used for background correction. Peak height was measured for 3 replicates.

In order to realize the procedure of the control analysis in the absence of Certified Reference Materials (CRMs), we have used the calibration method, measuring the blank sample during measurement, the discontinuation of the apparatus after each measurement, the control of one of the standard solution in every 10 measurements, and analysis of several parallel samples (5% of the total number of samples). The standard addition method is used to check the recovery of the analysis. The calibration plot obtained by linear regression of the absorbance against the absolute content of Fe^{3+} concentration is shown in Figure 1.

$$y = 0.0365x + 0.0098$$
$$R^2 = 0.9994$$
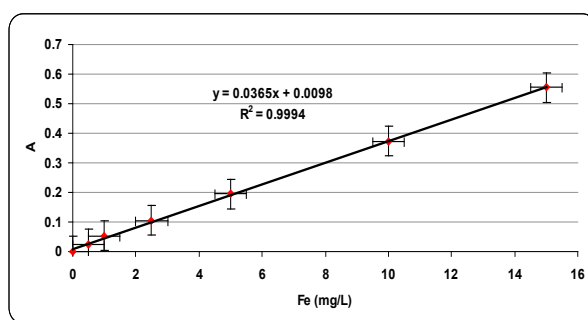
Figure 1. Calibration curve obtained from measurements performed

The calibration curve demonstrates the dependence of the absorbance's measured by the method of atomic absorption spectroscopy, by the concentration of iron for each sample taken in study. The most important parameters of the performance of the calibration line (limit of detection, limit of quantification, RSD% for N = 3 and the recovery tested by standard addition method) are shown in Table 2.

Table 2. The parameters of the performance of the analysis

Element	Σ	AL*	CL	Cp	RSD% mes	Rec% mes
Fe	0.000816	0.0034	0.14	0.42	2.69	102.4

AL* is minimum signal value of absorbance obtained in this study, using Atomic Absorption Spectroscopy method.

CL is the smallest concentration obtained during the measurements, which is reliably detected.

CP is the minimum level of analyte that can be determined quantitatively. RSD % is the relative standard deviation expressed as a percentage and Rec % is the recovery of this method also expressed as a percentage.

The calibration curve obtained through the AAS measurements of the serial of standard solution show a good linearity and high sensitivity. The range of the linear calibration curve was 0.5 - 15.0 mg Fe/L). The equation of the linear regression line was Abs = 0.037x + 0.0098 with high value of the coefficient of linearity ($R^2 = 0.9994$). The relative standard deviation (RSD) calculated from 3 successive measurements of each standard solution was 2.7%. The detection limit (LOD) calculated on the basis of three times the standard deviation (3σ) of ten repetitive measurements of the blank solution divided by the slope of the calibration curve is 0.14 mg/L and LOQ = 3LOD = 0.42 mg/L Fe.

3. Results and Discussion

The pregnant female included in this study were in treatment with substitute of iron. The objectives of this research were to determine the iron content in blood serum of pregnant and non-pregnant women and to show the effectiveness of therapy with substitute of iron.

The measurements obtained by this study have been subjected to the Descriptive Statistics and the results are shown in the Table 3 and in the Figure 2.

The mean interval (101.04 ± 25.59) differ from the median interval (81.25 ± 16.74), by indicating that the data are not normally distributed. On other hand, the mean interval (101.04 ± 25.59) shows that most of measurements are within the confidence interval.

Table 3. The parameters obtained by the Descriptive Statistics of the measurements

Element	Mean	StDev	Variance	CoefVar	Min.	Median	Max.	Skewness	Kurtosis
Fe	101.04	75.22	5657.36	74.44	25.59	81.25	400.39	1.67	3.37
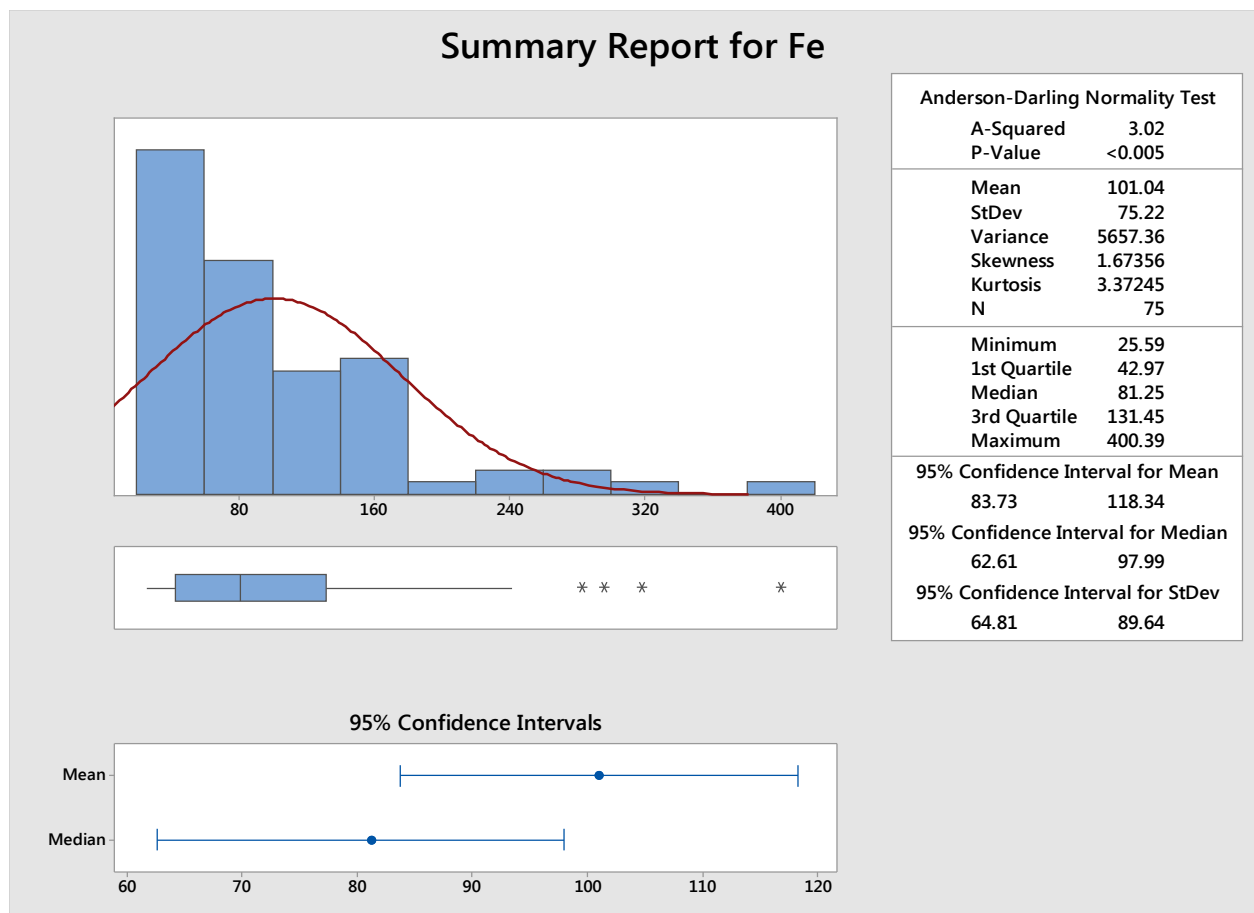
Figure 2. The Descriptive Statistics of measurements obtained by this study

The high positive values of skewness (> 0) and kurtosis (> 3) indicate that the data are positively skewed. The data are characterized by high variation (CV% = 74%) varied moderately. RSD% presented in the Table 1 indicates that the values are repeated and systematic errors are negligible.

Through the cluster analysis [10] based on 70% of similarity and Euclidian distance the samples under investigation were classified in three different groups:

4. The first one with 33 cases includes the non-pregnant female

5. The second with 28 cases includes the pregnant female in treatment with substitute of iron

6. The third group with 16 cases includes the pregnant female not in treatment with substitute of iron.

Figure 3 presents the diagram of iron concentration in the blood samples and the permitted levels.
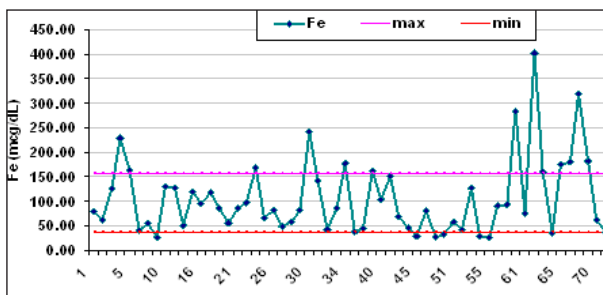
Figure 3. The diagram of iron concentration in the blood samples and the permitted levels

The most part of samples show normal values of iron content. The concentrations of the iron of pregnant females that we have studied belong to the normal range of the concentration and are comparable with iron concentration in blood samples of non-pregnant female. Only 5 cases show higher concentration values than the permitted levels and in 5 other cases the iron concentration level is just in the border between normal and higher concentration level, which shows that the therapy with substitute of iron is effective.

4. Conclusions

The purpose of the research was the determination of iron content in blood serum of pregnant and non-pregnant women and to show the effectiveness of therapy with substitute of iron. During pregnancy iron level in the blood is reduced due to the growing baby and placenta.

- The pregnant female were separated in two groups:

7. Pregnant female in treatment with substitute of iron.

8. Pregnant female not in treatment with substitute of iron.

- The results show normal levels of iron at pregnant female in comparison with those not pregnant.

- This study demonstrates the effectiveness of therapy with substitute of iron, during the first months of pregnancy.

5. References

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