ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 7, Issue, 10, pp.21741-21749, October, 2015

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

TRACE METALS CONCENTRATRION IN BLOOD SERUM OF FEMALE PEOPLE IN ALBANIA

***[1]Marjena BIXHI, [2]Pranvera LAZO and [2]Joana GJIPALAJ**

[1]Clinic of Gynecology, New Life, Durrës, Albania
[2]Department of Chemistry, Faculty of Natural Sciences, University of Tirana, Albania

ARTICLE INFO

Article History:
Received 01st July, 2015
Received in revised form
15th August, 2015
Accepted 30th September, 2015
Published online 31st October, 2015

Key words:

Trace metals, Female,
Pregnant female,
Blood sample,
Flame AAS,
Electro-thermic atomiser AAS,
Flame AES,
Statistical treatment.

ABSTRACT

The aim of present study is: i) to determine trace metals content in blood serum of pregnant and non-pregnant female, ii) to find a possible correlation between the metals under investigation, iii) to show the effectiveness of therapy with substitute of some elements during pregnancy. 77 blood samples were collected from February to March 2013 using the procedure described in World Health Organization (WHO) protocol. The serum samples were prepared using the high speed centrifuge (3600 rpm, in gel tubes) and than were diluted to 1:10 ratio with de-ionized water that contains 0.25% Triton X-100. The analysis of blood serum samples were carried out via i) Atomic Absorption Spectroscopy (AAS) with acetylene-air flame measuring absorbance of magnesium, calcium and zinc, ii) flame Atomic Emission Spectroscopy by measuring emission intensity of sodium, potassium and lithium, iii) graphite furnace Atomic Absorption Spectroscopy is used for lead, iron, copper, chrome, nickel, manganese content in serum samples. Results reported show that: 1) the range of concentration of Na, K, Ca, Mg, Zn, Cu, Mn, Ni were within the normal concentration range of each element. 2) The concentration of most of elements were higher in pregnant female who were treated with substitutes demonstrating the effectiveness of the substitutes therapy, 2) the female under investigation have normal concentration values of Li, an element related to the nervous system, and low concentration values of Pb, a toxic element.

Citation: Marjena BIXHI, Pranvera LAZO and Joana GJIPALAJ, 2015. "Trace metals Concentration in blood serum of female people in Albania", *International Journal of Current Research*, 7, (10), 21741-21749.

INTRODUCTION

Trace metals can be present in animal and plant cells and tissue and are a necessary part of nutrition and physiology. The ingestion of and/or exposure to excessive quantities of these metals can be toxic. However, insufficient plasma or tissue levels of certain trace metals can cause pathology. Trace metals are metals needed by living organisms to function properly and are depleted through the expenditure of energy by various metabolic processes of living organisms. They are replenished in animals through diet as well as environmental exposure, and in plants through the uptake of nutrients from the soil in which the plant grows. Human vitamin pills and plant fertilizers can be a source of trace metals.

Calcium

Calcium is the most important mineral substance for the organism. On average, an individual adult body contains about 1200 g calcium. About 1% of the amount of calcium in the blood is in the plasmatic water space. The rest of about 99% is deposited in bone tissue and located in a dynamic balance with the amount of calcium present in the plasmatic water space (Barnett *et al.,* 1973). Chemically, calcium in the blood plasma could be in three different forms: i) connected to plasma proteins, mainly with albumins in an average content of 40-50% of the total amount of calcium present in blood plasma; ii) in free ionic form, that represents the chemically active calcium able to exert physiological actions and participate in biochemical processes, iii) chemical complexes with citrate and phosphates, that represents 5-10% of the total amount of calcium contained in the blood plasma (Gindler and King, 1972; Bagrnski *et al.,*1973; Brommer and Coburn, 1981; Robertson and Marshall, 1981).

The daily average calcium intake for adults is 700 mg, while during pregnancy and the period of lactation, the daily calcium intake goes up to 1000-1500 mg.

It has been reported that the normal values for calcium are 9 - 11 mg/dl or 2.25 - 2.75 mMol/L for children and 9.0 - 10.7 mg/dl or 2.25 - 2.67 for adults (WHO, WHA 2012).

Poor maternal, newborn health and nutrition remain significant contributors to the burden of disease and mortality. Calcium supplementation has the potential to reduce adverse gestational

Corresponding author: Marjena BIXHI
Clinic of Gynecology, New Life, Durrës, Albania.

outcomes, in particular by decreasing the risk of developing hypertensive disorders during pregnancy, which are associated with a significant number of maternal deaths and considerable risk of preterm birth, the leading cause of early neonatal and infant mortality (WHO, 2012).

Table 1. The suggested scheme for calcium supplementation in pregnant women (WHO, WHA, 2012)

Dosage	1.5–2.0 g elemental calcium/day (1 g of elemental calcium equals 2.5 g of calcium carbonate or 4 g of calcium citrate)
Frequency	Daily, with the total daily dosage divided into three doses (preferably taken at mealtimes)
Duration	From 20 weeks' gestation until the end of pregnancy
Target group	All pregnant women, particularly those at higher risk of gestational hypertension
Settings	Areas with low calcium intake

Magnesium

Magnesium is an important mineral element of the human body. This element is part of the most important group of cation ions that are in the human body, preceded by: sodium, potassium, calcium.

The plasmatic concentration of magnesium is 1.7- 2.1 mg / dL (0.6- 15.1 mMol / L). 55% of magnesium content should be found in the skeleton with the remainder located in the space within the cell. Magnesium and calcium are the most important cations of the body. Only 1% of magnesium content in the body is actually found in blood. In blood serum, approximately 55% of the magnesium is in the form of free Mg^{2+} ions, 30% is associated with proteins (mainly with albumins) and 15% forms complexes with phosphates ($PO4^{3-}$), citrate and other anions (Elin, 1988).

The reduction of magnesium in the blood is caused by renal losses, gastrointestinal disorders and certain therapeutic treatments. The absence of magnesium in the body causes neuromuscular hyper-excitation, disorders cardiac rhythm, etc.

The increasing of magnesium content beyond the permitted values is caused by renal insufficiency, by hemolytic anemia, and certain therapy which contain magnesium. One of clinical signs of increased values of magnesium is the neuromuscular depression (Fawcett *et al.,* 1999; Farrell, 1984).

The most part of Mg and K are concentrated in the areas within the cells. 30% of magnesium is coming through the diet absorbed in the intestinal level. 70% of plasmatic magnesium, is filtered at the glomerular space, re-absorbed in proximal tube and then actively secreted in the distal level of tubule. A large number of studies have shown that the absence of Mg is related to Ca disorders, phosphates and potassium during cardiac disorders, particularly in the ventricular arrhythmias which is resistant to the classic treatment (Farrell, 1984; Gambling *et.al.,* 2001).

Iron

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 by 50% of usual blood content that needs more iron to make more hemoglobin for the additional blood content. Pregnant female need also extra iron content 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 (Puolakka J.,1980) but the evidence suggests that maternal iron depletion increases the risk of iron deficiency in the first 3 months of life, by a variety of mechanisms (Colomer J *et al.,* 1990; Scholl TO and Hediger ML., 1994). There are some evidences for the association between maternal iron deficiency and preterm delivery, low birth weight, possible placental abruption and the increased of peripartum blood loss (Cogswell Mary E *et al.,*2003; Arnold DL *et al*., 2009; WHO,2001).

The normal ranges of iron in blood of human body (WHO, 2001):

- 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 of women start pregnancy without sufficient stores of iron to meet their body's increased demands, particularly in the second and third trimesters of their pregnancy 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 anemia. Favorable pregnancy outcomes occur at 30-45% less often in anemic mothers, and their infants have less than one-half of normal iron reserves

The risk is even higher (WHO,2012)

- if they have morning 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.

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 (Bonardi R *et al.,* 1999) is recommended to take the following daily supplements as is shown in Table 2:

Table 2. 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.

Sodium

Sodium is the main electrolyte of the extra cellular environment. There are approximately 4,000 mMol sodium in the body. Sodium located in the organism is spread in different ways:

- The extra cellular fluids, which contain 45% of the total quantity of sodium or about 1,800 mMol;
- The intracellular water spaces, which contain approximately 10% of the total quantity of sodium or about 400 mMol / L;
- The rest of sodium or 45% (1.800 mMol / L) is located in bone tissue, in the form of chemical bonds that is not affected by normal metabolic changes;

The human body receives from the external environment 4-6 grams of sodium per day, mainly in the form of NaCl. After its dissociation in Na+ and Cl- ions, sodium ions are absorbed in the digestive tract. Absorption occurs without any active control, through a mechanism similar to the sodium-potassium pump-ATP-aze.

The content of sodium in the body is kept at relatively constant through the renal regulation.

In the kidney through the mechanism of re-absorption of sodium Na + ions, the level of renal tubes checked and regulates the concentration of sodium in the water space plasma.

These mechanisms ensure the concentration of sodium in the normal organism in a concentration range of 135 to 145mMol/L (Kruse *et al.,* 1984; Fraser *et al.,* 1989).

The concentration of sodium in the blood below 135 mMol /L, is called the hipo-sodium condition. If plasma sodium values reach 120 mMol /L is an indicator for intensive therapy. The increasing of the concentration of sodium in the blood over 145 mMol / l, causes hiper-sodium.

Potassium

Potassium is the most essential cation ion of intracellular fluids. The average concentration of potassium inside the cell environment is 140 mMol/L and in extra cellular environment it is about 4 mMol /L. Intracellular concentration of potassium differs significantly in function to its metabolic activity. Reserves of potassium in the body are much smaller than those of sodium and this can change, leading to serious pathology (Walmsley RN *et al.,* 1999). Potassium important for the exercise and metabolic functions, such as:

- The activation of many enzymatic processes;
- Regulation of the fibro- cells conductivity in general and those of myocarditis in particular;
- Transmission of nerve impulses;

The content of potassium in the body is approximately 3.200 mMol /L. Through the food feeding, the body takes 26 mMol of potassium for 24 hours. Normal plasma potassium concentration is generally 3.5-5.3 mMol / L. Its low concentration compared to the sodium, make it passive and effect ness to the water balance. An important mechanism in the displacement of potassium through cell the membranes, is the acid-alkaline balance.

Hipo-potassium condition is the clinical situation in which the amount of plasmatic potassium is below the lower limit of normal values (Fewtrell L *et al.,* 2003).

Hiper-potassium condition is the clinical situation in which the amount of potassium plasma is above the upper limit of normal values.

Lead

Lead is a toxic metal. The environmental lead pollution is a global problem that may several health problems in many parts of the world. It is a cumulative toxicant that affects multiple body systems, including the neurological, hematological, gastrointestinal, cardiovascular, and renal systems. Children are particularly vulnerable to the neurotoxic effects of lead, and even in relatively low levels of exposure it can cause serious and in some cases irreversible neurological damage (IPCS, 1995; WHO, 2007).

Lead enters the body through the respiratory and digestive systems that is associated with red blood cells and stored in the liver, kidney and furthermore into the bone. This element is eliminated through the stool and in smaller amounts through the urine. The source of lead pollution related to long-term professional exposure, urban pollution, or from the lead containing equipments used for water transportation. Absorption of lead from the intestine followed with the decrease of: Fe, Ca, Zn, Mg, P or vitamin D content. The use of the above micro-elements supplement will serve as the antidote in cases of intoxication with Pb. The maximum level of lead in the blood should be smaller than 100 mg / L that is an indication level to normal growing of children. Adults are less affected by neurological symptoms caused by Pb than children, but values above 300 mg / L should be considered for adverse effect in adults.

Young children absorb lead about 4–5 times more than adults (apart from pregnant women). Infants, young children (particularly those less than 5 years of age) and pregnant women are most susceptible to the adverse effects of lead. The potential for adverse effects of lead exposure is greater for children than for adults, because in children 1) the intake of lead per unit body weight is higher, 2) more dust may be ingested, 3) lead absorption in the gastrointestinal tract is higher, 4) the blood – brain barrier is not yet fully developed and 5) neurological effects occur at lower Pb levels than in adults (WHO, in preparation). The most critical effect of lead in young children occur in the developing nervous system. Subtle effects on intelligence quotient (IQ) are expected from Pb blood level at least lower than 5 µg/dl, and the effects gradually increase with the increasing level of lead in blood (IPCS, 1995). A 2010 review of the latest scientific evidence indicating effects at lower Pb level did not provide any indication of a threshold for the key adverse effects of lead (IARC, 2006). Lead exposure has also been linked epidemiologically to attention deficit disorder and aggression

(Food and Agriculture Organization of the United Nations, 2002). Exposure of pregnant women to high levels of lead can cause miscarriage, stillbirth, premature birth and low birth weight, as well as minor malformations (WHO, 2007).

Zinc

Zinc is an essential trace element and has a number of roles and functions in the human body, like: 1) as an essential component/cofactor for more than 300 enzymes involved in the synthesis and metabolism of carbohydrates, lipids, proteins, nucleic acids and other micro-nutrients; 2) It stabilizes cellular components and membranes and so, it is important for cell, organ structure and integrity; 3) It is essential for cell division and is needed for normal growth and development during pregnancy, childhood and adolescence. Zinc is found in all body tissues and fluids and the total body zinc content is estimated at 2 g and/or 30 mMol/L (Beattie, 2012). The Food Standards Agency and the Department of Health in the UK advise that the Zn intake should not exceed 25 mg per day (National Institutes of Health, 2013).

A zinc deficiency can cause: 1) Inadequate diet; 2) Gastrointestinal diseases including ulcerative colitis, Crohn's disease, short bowel syndrome and chronic diarrhoea; 3) Chronic liver disease; 4) Chronic kidney disease; 5) Alcoholism (decreases zinc absorption and increases urinary zinc excretion); 6) Sickle cell disease; 7) Diabetes; 8) Pregnancy and breast-feeding problems; 9) adverse effect at the people taking large amounts of iron supplementation (iron can interfere with zinc absorption) (Mena *et al.,* 1981).

Manganese

Manganese is both an activator and a constituent of several enzymes. Those activated by manganese are numerous and include hydrolase's, kinases, decarbox-lases and transferases, but most of these enzymes can also be activated by other metals.

Manganese is often considered to be among the least toxic of the trace elements when administered orally. Thus, reported cases of human toxicity caused by oral ingestion of large amounts of manganese are few. The most common form of manganese toxicity is the result of chronic inhalation of large amounts of airborne manganese in mines, steel mills and some chemical industries (Keen CL *et al.,* 1984).

Keen *et al*. (Keen *et al.,* 1983; Cotzias *et al.,*1966) have suggested that the blood manganese concentration may be useful in assessing status because low blood concentrations in manganese deficient rats reflected low concentrations of manganese in soft tissue. This suggestion is supported by the finding that whole-blood manganese concentrations are elevated with excessive manganese intake in humans (Friedman BJ *et al.,* 1987).Normal whole-blood manganese in humans is more or less 8.4 pg/L (Friedman BJ *et al.,* 1987). Average basal or normative requirements for manganese cannot be established because the data required for this purpose are not available. The deficiency attempts to produce manganese by feeding diets providing as little as 0.74 mg/day (Johnson, Lykken, 1989) or 1.0 mg/day (Cox, Moore, 2002) resulted in neither conclusive nor marked effects on the health of adults. The threshold toxicity level is also unknown. Thus, a safe range of mean population intakes for manganese cannot be proposed.

Copper

Copper is one among the metal ions that are required for essential nutrient for body functions, but it is toxic in excess. Copper is a trace element present in all tissues and is required for cellular respiration, peptide amidation, neurotransmitter biosynthesis, pigment formation, and connective tissue strength. Copper is a cofactor for numerous enzymes and plays an important role in central nervous system development; low concentrations of copper may result in incomplete development, whereas excess copper maybe injurious (Cox, Moore, 2002; Madsen and Gitlin, 2007). Evidence of abnormal copper transport and aberrant copper-protein interactions in numerous human neurological disorders supports the critical importance of this trace metal for proper neurodevelopment and neurological function. Copper is involved in the functions of several cupproenzymes that are essential for life (Goel and Misra, 1982). Copper plays a role in the mobilization of iron to plasma from the tissue stores (Raman and Leela, 1992) and copper deficiency during embryonic and fetal development has been found to cause numerous gross structural and biochemical abnormalities. It has been reported that more than 50% of human conception fail to implant and of those implanted, approximately 30% fail to reach term due to copper deficiency (Ebbs *et al.,* 1984).

Lithium

Lithium is used as a psychiatric medication. A number of lithium salts are used as mood stabilizing drugs, primarily in the treatment of bipolar disorder, where they have a role in the treatment of depression and particularly of mania, both acutely and in the long term. As a mood stabilizer, lithium is probably more effective in preventing mania than in preventing depression, and reduces the risk of suicide in people with bipolar disorder (Baldessarini, Ross J *et al.,* 2006). In depression alone (unipolar disorder), lithium can be used to augment other antidepressants. Upon ingestion, lithium becomes widely distributed in the central nervous system and interacts with a number of neurotransmitters and receptors, decreasing norepinephrine (noradrenaline) release and increasing serotonin synthesis (Brunton *et al.,* 2010). The specific biochemical mechanism of lithium action in mania is unknown. Lithium is used primarily for bipolar disorder. It is sometimes used when other treatments are not effective in a number of conditions, including the major depression, schizophrenia, and some psychiatric disorders in children (The American Society of Health-System Pharmacists, 2013).

In mood disorders, of which bipolar is one, it decreases the risk of suicide (Cipriani *et al.,* 2013). This benefit is not seen with other medications (Müller-Oerlinghausen *et al.,* 2003; Kovacsics *et al.,* 2009). High levels of naturally occurring lithium in drinking water have been associated with lower rates

of suicide (Ohgami H *et al.,* 2009; Gonzalez R *et al.,*2008; Kapusta ND *et al.,* 2011).

MATERIALS AND METHODS

Blood Sampling

Blood samples were collected from February to March 2013 in line with the World Health Organization protocol: i) extending the patient's arm and inspect the antecubital fossa or forearm, ii) locating a vein of a good size that is visible, straight and clear, iii) applying the tourniquet about 4–5 finger widths above the venepuncture site and re-examine the vein, iv) disinfecting the site using 70% isopropyl alcohol for 30 seconds and allow to dry completely (30 seconds), v) anchoring the vein by holding the patient's arm and placing a thumb below the venepuncture site, vi) entering the vein swiftly at a 30 degree angle, vii) once blood is sufficiently collected, tourniquet could be released before withdrawing the needle and, vii) withdrawing the needle gently and then give the patient a clean gauze or dry cotton-wool ball to apply to the site with gentle pressure (WHO, 2010).

Blood Serum Preparation

Blood serum is the present investigation used to the concentration of trace elements in women. Procedures followed to prepare blood serum are: i) 5 ml of blood obtained by venous puncture were added to the tube with gel, ii) blood was allowed to clot by leaving it undisturbed at room temperature for 5 min. The tube was put in thermostat with temperature 37© to accelerate the degradation of fibrinogen for 4 min and, iii) Once the tube was put in the thermostat and the degradation of fibrinogen was accelerated, the clot was removed via centrifugation at 3600 rpm/min using a refrigerated centrifuge.

At the end of this process were obtained the blood serum in light yellow color (WHO, 2010). The serum samples were diluted to 1:10 ratio with de-ionized water comprising 0.25% Triton X-100.

Methods

The trace metal content in serum samples was carried using:

1. AAS method involving acetylene-air flame for Mg, Ca , Zn;
2. AAS method with electrothermic atomiser equipped with graphite furnace for Pb, Fe, Cu, Cr, Ni, Mn;
3. AES method involving acetylene-air flame for Na, K and Li.

Under the condition of the absence of blood serum certified samples, CRM, the calibration method followed with parallel run of the blank sample and the control of one of the standard solution after each 10 measurements, and the analysis of several parallel samples (5% of the total number of samples).

RESULTS AND DISCUSSION

The concentration data for each element have been subject to the descriptive statistics and the results (Table 1) are depicted in the following figures.

Fig. 1 shows that the difference between the values of the mean and the median is very small (close to zero) by indicating that the data are normally distributed. The Na data are characterized by low value of the coefficient of the variance (CV% = 9) by indicating that the data vary in a small range (confidence interval for mean = 2.04 to 2.13, and for median = 2.05 to 2.09 mMol/L) and are more or less stable in most of people under investigation. The Na content in blood samples was characterized by high positive values of skewness (1.45) and kurtosis (6.55). The high positive values of skewness (>0) and kurtosis (>3) indicate that the data are positively skewed and are effected by different factors.

Table 1. Descriptive Statistics of measurements obtained by this study for all elements

Variable	Mean	StDev	Variance	Coef Var	Minimum	Median	Maximum	Skewness	Kurtosis
Ca	14.0	4.01	16.0	28.6	4.25	14.7	27.0	-0.41	1.18
Mg	15.7	3.82	14.6	24.2	1.79	16.2	23.4	-1.77	4.25
Zn	12.9	13.9	195	108	5.61	9.29	64.5	3.46	10.4
K	4.13	0.65	0.42	15.8	2.54	4.09	6.97	1.06	5.03
Na	2.08	0.18	0.03	9.03	1.59	2.07	2.80	1.45	6.55
Li	1.84	3.30	10.9	178	0.09	1.12	21.4	5.37	29.4
Pb	24.4	16.4	269	67.2	0.21	24.0	79.4	0.98	1.28
Fe	101	75.2	5657	74.4	25.5	81.2	400	1.67	3.37
Cu	178	92.4	8531	51.6	58.6	153	710	2.82	13.7
Mn	28.6	26.3	693	92.0	2.56	20.3	135	2.24	5.72

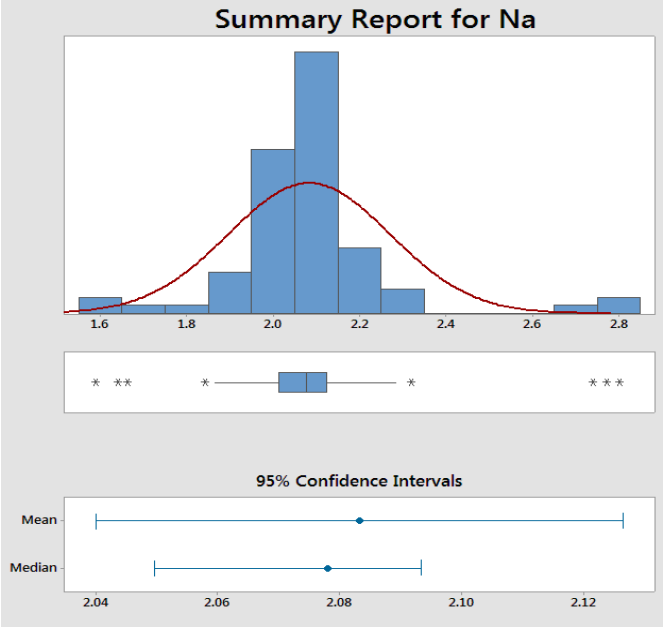

Fig. 1. Descriptive Statistics of measurements obtained by this study for Na

Fig 2 shows that the difference between the values of the mean and the median of K is small by indicating that the data are more or less normally distributed. The K data are characterized by low value of the coefficient of the variance (CV% = 16) by indicating that the data vary in a small range (confidence interval for mean = 3.98 to 4.28 and for median = 4.0 to 4.2 mMol/L) and are more or less stable in most of people under investigation. The K content in blood samples was characterized by positive values of skewness (1.06) and kurtosis (5.03). The high positive values of skewness (>0) and kurtosis (>3) indicate that the data are positively skewed and are effected by different factors.

It was shown that only 4.3 % of the cases show K content lower than the confidence interval of the mean, while 7.2 % of the cases under investigation show K content higher than the confidence interval of the mean. It is a significant argument confirming that the female people under investigation have normal concentration of K.

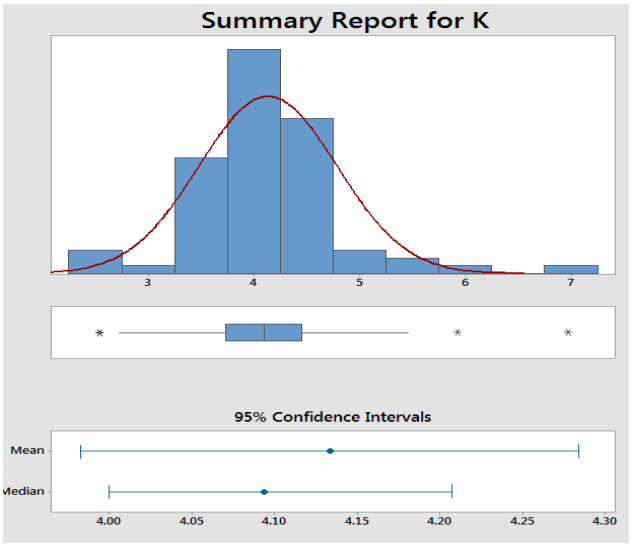

Fig. 2. Descriptive Statistics of measurements obtained by this study for K

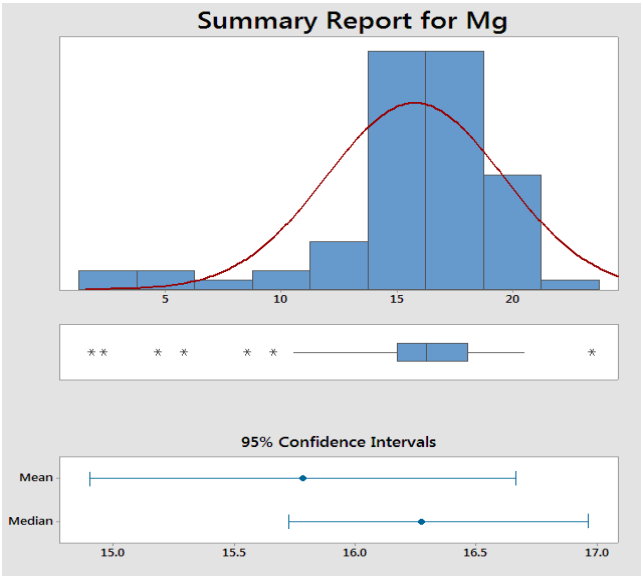

Fig. 3. Descriptive Statistics of measurements obtained by this study for Mg

Descriptive Statistics of measurements for iron illustrated at the Fig. 9 indicates that 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. 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 nr 1 indicates that the values are repeated and systematic errors are negligible.

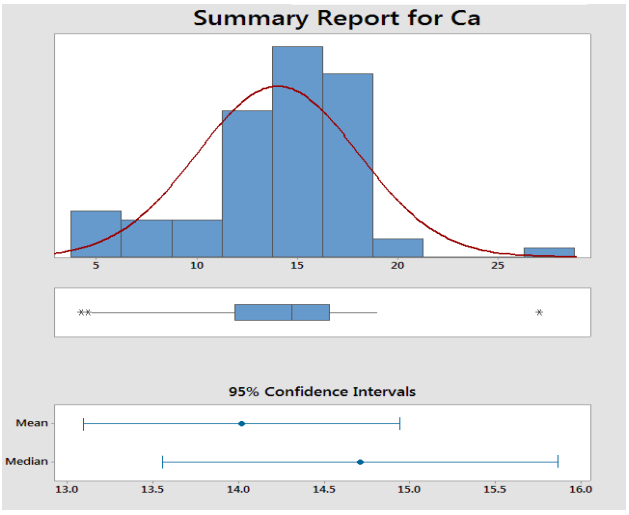

Fig. 4. Descriptive Statistics of measurements obtained by this study for Ca

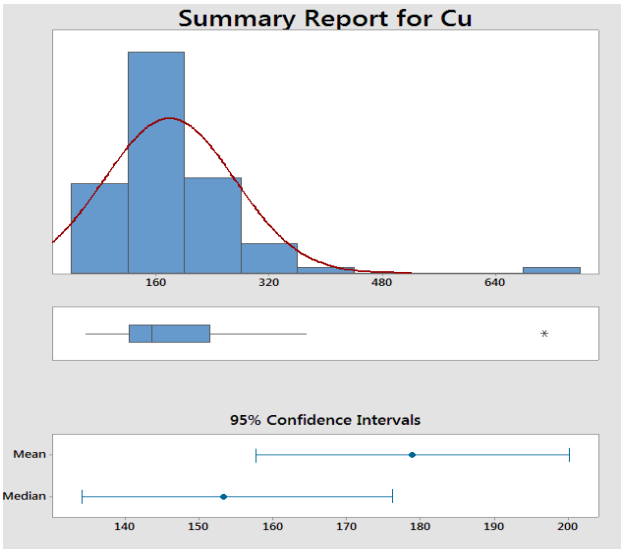

Fig. 5. Descriptive Statistics of measurements obtained by this study for Cu

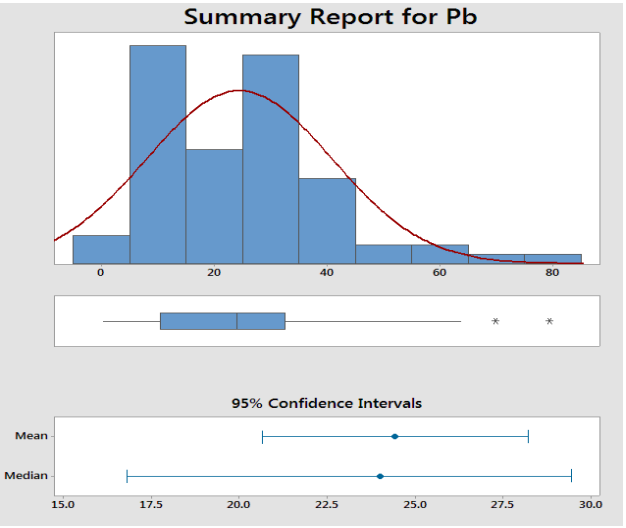

Fig .7. Descriptive Statistics of measurements obtained by this study for Pb

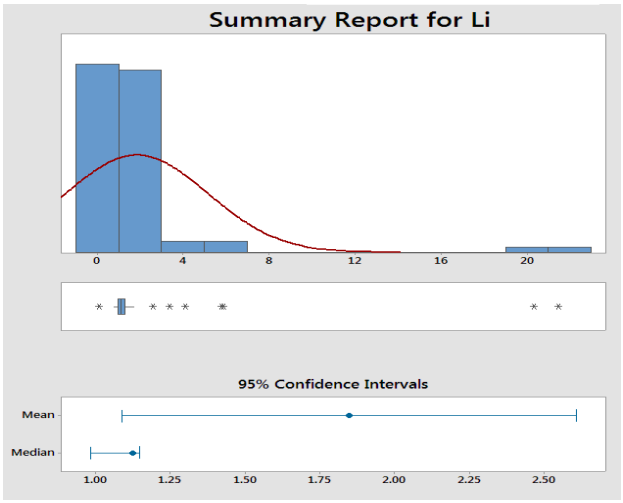

Fig. 7. Descriptive Statistics of measurements obtained by this study for Pb

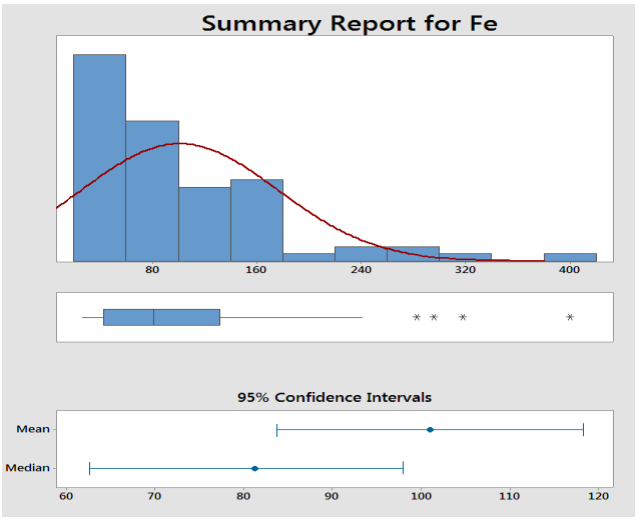

Fig. 9. Descriptive Statistics of measurements obtained by this study for Fe

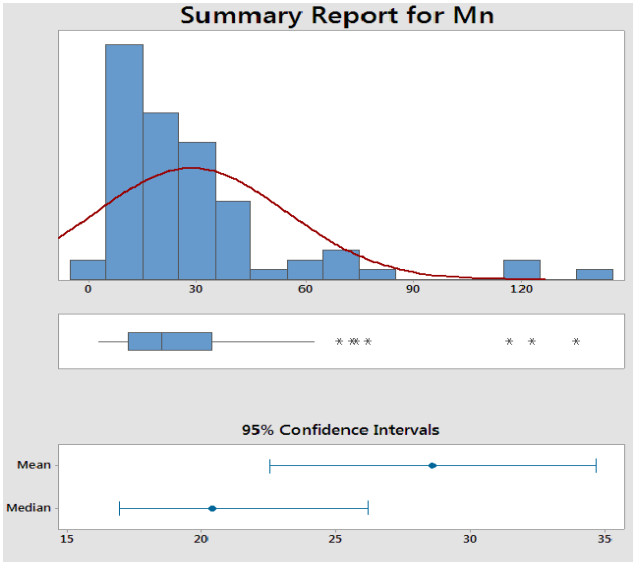

Fig.10. Descriptive Statistics of measurements obtained by this study for Mn

Fig. 10 shows that the Mg content in blood samples was characterized by high positive values of skewness (-1.8) and negative value of kurtosis (4.25). The high negative values of the skweness indicate that the data are negatively skewed and are affected by complicated factors (kurtosis value>3). The mean and the median values of both parameters are positioned left (close to minimum values). Pearson correlation analysis and multivariate analysis were carried out for an accurate interpretation of the data.

Pearson correlation analysis was carried out for an accurate interpretation of the data.

Table 2. The results of the correlation analysis between the elements

	Ca	Mg	Zn	K	Na	Li	Pb	Fe	Cu
Mg	0.446[1]								
	0.000								
Zn	0.007	0.131							
	0.949	0.261							
K	0.098	-0.121[2]	-0.080						
	0.403	0.302	0.496						
Na	0.042	0.034	-0.108[2]	0.127[2]					
	0.723	0.773	0.356	0.276					
Li	-0.481[1]	-0.561[1]	-0.081	0.007	-0.002				
	0.000	0.000	0.490	0.951	0.989				
Pb	0.028	0.234[2]	0.060	0.021	0.027	-0.174			
	0.811	0.043	0.609	0.858	0.821	0.135			
Fe	-0.009	0.106	0.192	-0.084	0.213[2]	-0.006	-0.059		
	0.940	0.365	0.099	0.472	0.067	0.961	0.615		
Cu	-0.136[2]	-0.027	-0.032	-0.020	-0.038	0.162[2]	0.302	-0.105	
	0.246	0.818	0.785	0.867	0.749	0.165	0.008	0.369	
Mn	-0.238[1]	-0.212[2]	-0.159[2]	-0.025	0.048	0.596[1]	-0.094	-0.088	0.139[2]
	0.040	0.068	0.172	0.829	0.684	0.000	0.425	0.452	0.234

Cell Contents: Pearson correlation

P-Value: 1 P<0.001, 2 P<0.005, 2 P<0.05

Pearson correlation analysis reported a positive correlation between: Mg-Ca, Mn-Li and a high negative correlation between Li-Ca, Li-Mg, Mn-Ca.

Conclusion

The present investigation aims to:

- Determine trace metals content in blood serum of pregnant and non-pregnant female,
- Find a possible correlation in the measurement between the metals under this study,
- Show the effectiveness of therapy with substitute of some elements during pregnancy.

Results reported that

- The normal values were higher in pregnant women who were treated with substitute of Na, K, Ca, Mg, Zn, Cu, Mn, Fe demonstrating the effectiveness of therapy with substitute of these elements,
- Female under investigation have normal values of Li, an element related to the nervous system, and low values of Pb.
- High positive correlation between: Mg-Ca, Mn-Li.

• High negative correlation between Li-Ca, Li-Mg, Mn-Ca.

REFERENCES

"Lithium salts". The American Society of Health-System Pharmacists. Retrieved 24 Sep 2013.

Arnold, D.L., Williams, M.A., Miller, R.S., Qiu, C. and Sorensen, T.K. 2009. Maternal iron deficiency anaemia is associated with an increased risk of abruption placentae – a retrospective case control study. *Journal of Obstetrics and Gynaecology Research*, 35,446–452.

Bagrnski ES, Marie SS, Clark WL, Zak B. 1973.Direct micro determination of serum calcium. *Clinical Chimica Acta*, 46 (1):46-54.

Baldessarini, Ross J; Tondo, Leonardo; Davis, Paula; Pompili, Maurizio; Goodwin, Frederick K; Hennen, John 2006. "Decreased risk of suicides and attempts during long-term lithium treatment: A meta-analytic review". Bipolar Disorders 8(5p2): 625–39.

Barnett RN, Skodon SB, Goldberg MH. 1973. Performance of kits used for clinical chemical analysis of calcium in serum. *The American Journal of Clinical Pathology*, 59 (6): 836-845.

Bonardi R., Deambrogio V., Oliaro A. 1999. Interpretazione dei dati di Laboratorio, Torino.

Bronner F, Coburn JW.1981. Disorders of mineral metabolism. New York. Academic Press.

Brunton, L; Chabner, B; Knollman, B 2010. Goodman and Gilman's The Pharmacological Basis of Therapeutics (12th ed.). New York: McGraw-Hill Professional.

Cipriani, A.; Hawton, K.; Stockton, S.; Geddes, JR. 2013. "Lithium in the prevention of suicide in mood disorders: updated systematic review and meta-analysis". BMJ 346: f3646.

Cogswell Mary E, *et al*. 2003. Iron supplementation during pregnancy, anemia, and birth weight: a randomized controlled trial1,2,3. *The American Journal for Clinical Nutrition*, October 2003 vol. 78 no. 4 773-781.

Colomer J, *et al*. 1990. Anaemia during pregnancy as a risk factor for infant iron deficiency: report from the Valencia Infant Anaemia Cohort (VIAC) study. Paediatric and Perinatal Epidemiology Volume 4, Issue 2, pages 196–204, April 1990.

Cotzias GC, Miller ST, Edwards J, Neutron activation analysis: the stability of manganese concentrations in human blood and serum. *Journal of Laboratory and Clinical Medicine*, 1966, 67: 836-849.

Cox DW, Moore SDP. Copper transporting P-type ATPases and human disease. *J Bioenerg Biomembr*., 2002;34:333–8.

Ebbs, J. H., Tisdall, F. F. and Scott, W. A. (1984). The influence of prenatal nutrition on mother and child. *Journal of Nutrition*, 22: 515-526.

Elin RJ. 1988. Magnesium metabolism in health and disease. Disease-a-Month. 34: 161-218.

Farrell EC. 1984. Magnesium in Clinical Chemistry. Theory, Analysis and Correlation. The CV Mosby Company.Kaplan LA, Pesce AJ (Ed), Chapter 55: 1065-70.

Fawcett WJ, Haxby EJ, Male DA. 1999. Magnesium: Physiology and Pharmacology. *British Journal of Anaesthesia*, 83 (2): 302-20.

Fewtrell L, Kaufmann R, Prüss-Üstün A 2003. Lead: Assessing the environmental burden of disease at national and local levels. Geneva, World Health Organization (Environmental Burden of Disease Series, No. 2; http://www.who.int/quantifying_ehimpacts/publications/en/leadebd2.pdf).

Fraser CG., Harris EK. 1989. Generation and application of data on biological variation in clinical chemistry. *Critical Reviews in Clinical Laboratory Sciences*, 27:409-437.

Friedman BJ *et al*. Manganese balance and clinical observations in young men fed a manganese-deficient diet. Journal of nutrition, 1987, 117: 133-143.

Gambling L, *et al*. 2001. Iron deficiency during pregnancy affects postnatal blood pressure in the rat. *The Journal of Physiology*, 552, 603-610.

Gindler M, King JD. 1972. Rapid colorimetric determination of calcium in biological fluids with methylthymol blue. *American Journal of Clinical Pathology*, 58: 376-382.

Goel, R. and Misra, P. K. 1982. Study of plasma zinc in neonates and their mothers. *Indian Paediatrics*, 19: 611-614.

Gonzalez, R; Bernstein, I; Suppes, T 2008. "An investigation of water lithium concentrations and rates of violent acts in 11 Texas counties: Can an association be easily shown?". *The Journal of Clinical Psychiatry,* 69.

http://www.who.int/entity/nutrition/publications/micronutrients/guidelines/daily_ifa_supp_pregnant_women/en/

http://www.who.int/nutrition/publications/micronutrients/anaemia_iron_deficiency/WHO_NHD_01.3/en/

Human Vitamin and Mineral requirements, Chapter 16. Zinc; Agriculture and Consumer Protection Department, Food and Agriculture Organization of the United Nations, 2002.

IARC 2006. Summaries and evaluations: Inorganic and organic lead compounds. Lyon, International Agency for Research on Cancer (IARC Monographs for the Evaluation of Carcinogenic Risks to Humans, Vol. 87; http://www.inchem.org/documents/iarc/vol87/volume87.pdf).

IPCS 1995. Inorganic lead. Geneva, World Health Organization, International Programme on Chemical Safety (Environmental Health Criteria 165; http://www.inchem.org/documents/ehc/ehc/ehc165.htm).

Johnson PE, Lykken GI. Manganese and calcium balance and absorption in women fed two levels of Ca and Mn. *FASEB Journal*, 1989, 3: A760.

Kapusta, ND; Mossaheb, N; Etzersdorfer, E; Hlavin, G; Thau, K; Willeit, M; Praschak-Rieder, N; Sonneck, G; Leithner-Dziubas, K. 2011. "Lithium in drinking water and suicide mortality". *The British Journal of Psychiatry*, 198 (5): 346–350.

Keen CL *et al*. Whole blood manganese as an indicator of body manganese. *New England Journal of Medicine*, 1983, 308: 1230.

Keen CL, Lonnerdal B, Hurley LS. Manganese. In: Frieden E, ed. Biochemistry of the essential ultratrace elements. New York, Plenum Press, 1984: 89-132.

Kovacsics, Colleen E.; Gottesman, Irving I.; Gould, Todd D. 2009. "Lithium's Antisuicidal Efficacy: Elucidation of Neurobiological Targets Using Endophenotype Strategies". *Annual Review of Pharmacology and Toxicology*, 49: 175–198.

Kruse K., Kracht U., Kruse U. 1984. Reference values for urinary calcium excretion and screening for hypercalciuria in children and adolescents. *Eur J Pediatr*., 143: 25-31.

Madsen E, Gitlin JD. Copper and iron disorders of the brain. *Annu Rev Neurosci*., 2007;30:317–37.

Mena I. Manganese. In: Bronner F, Coburn JW, eds. Disorders of mineral metabolism. New York, Academic Press, 1981: 233-270.

Müller-Oerlinghausen, B; Berghöfer, A; Ahrens, B 2003."The Antisuicidal and Mortality-Reducing Effect of Lithium Prophylaxis: Consequences for Guidelines in Clinical Psychiatry". Canadian Journal of Psychiatry 48 (7): 433–9.

Ohgami, H; Terao, T; Shiotsuki, I; Ishii, N; Iwata, N 2009. "Lithium levels in drinking water and risk of suicide". *The British Journal of Psychiatry : The Journal of Mental Science*, 194 (5): 464–5; discussion 446.

Prof JH Beattie - Zinc on a plate; The Nutrition Society, Nov 2012.

Puolakka J. 1980. Serum Ferritin in the Evaluation of Iron Status in Young Healthy Women. Acta Obstetricia et Gynecologica Scandinavica, 1980, Vol. 59, No. s95 : Pages 35-41.

Raman, L. and Leela, J. 1992. Role of maternal nutrition. In: PIH Annual report, *National Institute of Nutrition*, 93: 47-50.

Robertson WC, Marshall RW. 1981. Ionized calcium in body fluids. *Critical Reviews in Clinical Laboratory Sciences*, 15(2): 85-125.

Scholl T O. and Hediger M L. 1994. Anemia and iron-deficiency anemia: compilation of data on pregnancy outcome. *The American Journal for Clinical Nutrition*, February 1994 vol. 59 no.2 492S-500S discussion 500S.

Walmsley RN., Watkinson LR., Cain HJ. 1999, Cases in Chemical Pathology A Diagnostic Approach. 4th ed. Singapore. *World Scientific*, p. 110-6.

WHO (in preparation). Safety evaluation of certain food additives and contaminants in food. Geneva, World Health Organization (WHO Food Additives Series, No. 64; (http://www.who.int/ipcs/publications/jecfa/monographs/en/index.html)

WHO 2007. Blood lead levels in children. Copenhagen, World Health Organization Regional Office for Europe, European Environment and Health Information System (Fact Sheet No. 4.5; http://www.enhis.org/object_document/o4738n27387.html).

WHO/UNICEF/UNU. Daily iron and folic acid supplementation in pregnant women. Geneva, World Health Organization, 2012. Available at

WHO/UNICEF/UNU. Iron deficiency anaemia: assessment, prevention and control, a guide for programme managers. Geneva, World Health Organization, 2001. Available at

World Health Organization (WHO). 2010.WHO guidelines on drawing blood. http://whqlibdoc.who.int/publications/2010/9789241599221_eng.pdf

World Health Organization, World Health Assembly, 2012. Resolution WHA 65.11. Nutrition. Maternal, infant and young child nutrition: draft comprehensive implementation plan. In: Sixth-fifth World Health Assembly, Geneva, 21–26 May 2012. Resolutions and decisions, and list of participants. Geneva, World Health Organization, 21–26 May 2012 (A65/11) Annex: 5–23 (http://apps.who.int/gb/ebwha/pdf_files/WHA65/A65_11- en.pdf, accessed 13 June 2013).

Zinc - Fact Sheet for Health Professionals; Office of Dietary Supplements, National Institutes of Health, June 2013.

****