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THE EVALUATION OF MICRO-NUTRIENTS ESPECIALLY ZINC, AS DETERMINANTS FOR A HEALTHY DIET DURING PREGNANCY

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Abstract

Zinc is an essential nutrient that must be ingested dietary and can also be a danger to unborn and newborn children. When their mothers have absorbed large concentrations of zinc, the children may be exposed to it, through blood or milk of their mothers.

We took into consideration 500 cases of pregnant women, from first to third trimester of pregnancy. We collected 2mL blood from each of them, where serum zinc levels were measured by using two different methods: Atomic Absorption Spectroscopy (AAS) and Total Reflection X-ray Fluorescence (TXRF).

According to a statistical data processing, using SPSS Statistics, Descriptive Statistics and ANOVA: Single Factor, there was no significant change ($P > 0.05$) between zinc levels measured by two methods of analyzing. The study showed that the prevalence deficiency of zinc in pregnant women was 62% (310 cases), this because of an eating disorder or when they are breastfeeding. The identification of pregnant women with zinc deficiency was a problem to be considered, because the continuation of the pregnancy until the end was not enough, foetus complete development was also necessary.

Zinc deficiency can be prevented, by ensuring intake of the recommended amounts of zinc, through obtaining zinc from dietary sources (such as peanuts and beef or lamb) and by using zinc supplements if their diet does not provide adequate levels of zinc.

It is advised for pregnant women to pay special attention to food hygiene during pregnancy, in addition to avoid certain foods, in order to reduce the risk of exposure to substances that may be harmful to the developing foetus. Modification of lifestyle is an effective intervention strategy, for improvement of maternal metabolism and the prevention of adverse outcomes.

Key words: *Zinc and pregnancy, Healthy diet, Micronutrients, Serum zinc, Zinc determination.*

1. Introduction

The purpose of this study was the investigation of maternal zinc status during pregnancy, its measurement with two different methods (Atomic Absorption Spectroscopy and Total Reflection X-Ray Fluorescence), and its role as an important micronutrient in human health.

Micronutrients are nutrients required by humans and other organisms throughout life, in small quantities to orchestrate a range of physiological functions [1]. For people, they include dietary trace minerals in amounts generally less than 100 milligrams/day - as opposed to macronutrients, which are required in larger quantities. The microelements include at least iron, cobalt, chromium, copper, iodine, manganese, selenium, zinc and molybdenum.

Micronutrients also include vitamins, which are organic compounds required as nutrients in tiny amounts by an organism [2]. Micronutrients are different from macronutrients because they are necessary only in small amounts. Micronutrients are essential for good health, and their deficiencies can cause serious health problems. When our body is being supplied with the micronutrients it needs, it functions the way it is designed to, and helps us metabolize the proteins, carbohydrates, and fats better.

Nutrition and pregnancy refers to the nutrient intake, and dietary planning that is undertaken before, during and after pregnancy [3]. It is very important that expecting mothers should change their personal habits like smoking, alcohol, caffeine, using certain medications and street drugs as soon as they know they are pregnant or even when they are planning to conceive. All these can affect the development of the organs like brain, which happen in early stages of pregnancy. They can cause irreparable damage to the growing foetus [4]. Folic acid is the only supplement, which is recommended to be used by every pregnant woman. It is essential for the formation of the neural tube. Using folic acid increases the probability for the foetus to have healthy brain. In the other hand, lack of calcium may cause preeclampsia and postnatal depression. If a pregnant woman feels good and eats plenty and various foods, there is no need to use food supplements. Even supplements for pregnant women may be harmful (they are known to cause allergies for the baby). The positive effect of magnesium for pregnant women and foetus is not proven yet. Still, magnesium is recommended when muscle spasms, convulsions and weakness manifest**.** In some cases, iron might be even harmful and must be taken only when the pregnant woman is anaemic.

Zinc is an essential nutrient that must be ingested dietary and can also be a danger to unborn and newborn children. When their mothers have absorbed large concentrations of zinc, the children may be exposed to it, through blood or milk of their mothers. It is the second must abundant trace mineral in the body, after iron. Zinc is present throughout the body in low concentration, but in most tissues. It performs multiple critical functions and must be supplied at adequate levels consistently or deficiency states will result, from mild to severe. Zinc is a necessary element, when it comes to human health. It can plays an important role in keeping the body healthy and It's quite interesting to know that zinc helps improve the natural texture of the skin. A deficiency of zinc can result in skin issues, such as over dryness. Zinc is a trace element that is essential for human health. When people absorb too little zinc they can experience a loss of appetite, decreased sense of taste and smell, slow wound healing and skin sores. Although humans can handle proportionally large concentrations of zinc, too much zinc can still cause eminent health problems, such as stomach cramps, skin irritations, vomiting, nausea and anemia. Very high levels of zinc can damage the pancreas and disturb the protein metabolism, and cause arteriosclerosis. Zinc can be a danger to unborn and newborn children. When their mothers have absorbed large concentrations of zinc the children may be exposed to it through blood or milk of their mothers.

1.1 The effect of zinc Deficiency in human health (the lack of micronutrients)

Zinc deficiency is insufficient zinc to meet the needs of biological organisms. Due to its essentiality, a lack of this trace element leads to far more severe and widespread problems. Both, nutritional and inherited zinc deficiency, generate similar symptoms [5], and clinical zinc deficiency causes a spectrum from mild and marginal effects up to symptoms of severe nature [6].

The following are some risk factors for zinc deficiency.

1. *Inadequate calorie intake* - Zinc deficiency is widespread in parts of the world where famine and inadequate nutrition are endemic. Anorexics generally have chronically low zinc levels - a chicken and egg situation because one sign of low zinc stores is loss of appetite.
2. *Over-reliance on Refined and Processed Foods* - Refining of whole grains routinely removes most zinc. It follows that highly processed diets are often zinc deficient
3. *Alcohol* - Alcohol is a double whammy when it comes to zinc. Ethanol decreases zinc absorption and increases urinary zinc excretion. This may explain why 30% - 50% of alcoholics are estimated to be zinc deficient.
4. *Mal-absorption conditions* - A host of medical conditions can lead to zinc deficiency including: Mal-absorption syndrome, Liver disease, Kidney disease, Diabetes, Cancer, Sickle cell disease.
5. *Vegetarian or Vegan Diet* - Some of the most bio-available dietary sources of zinc are animal products, especially oysters and red meat. Plant based sources of zinc are sometimes poorly absorbed because of the presence of phytates that diminish zinc absorption.
6. *Pregnancy and lactation* - Both conditions increase substantially a woman's need for zinc.
7. *Older infants* who are exclusively breastfed, unless the mother consumes adequate zinc.

According to all these effects of micronutrients, especially zinc, we came to a conclusion that the investigation of maternal zinc status was very useful to pregnant women, for their health and pregnancy outcome. We built a study, based on two main methods of serum zinc analysis such as, Atomic Absorption Spectroscopy and Total Reflection X-Ray Fluorescence, to see which of them, was the most appropriate method for maternal zinc measurement.

The identification of pregnant women with zinc deficiency was a problem to be considered, because the continuation of the pregnancy until the end was not enough, fetus complete development was also necessary.

2. Materials and Methods

2.1 Experimental part

Laboratory work for this study was done at the Public Health Institution, by using Atomic Absorption

Spectroscopy (AAS), and at the Nuclear Physics Institution by using Total Reflection X-Ray Fluorescence (TXRF).

According to a randomized selection, we collected 500 blood samples from pregnant women, at different stages of pregnancy (from first to third trimester of pregnancy) in which we measured serum zinc levels by using the two different methods mentioned above (AAS and TXRF). We collected 2 m L from every pregnant woman, in appropriate tubes necessary for zinc analysis, which did not contain anticoagulants such as Heparin or EDTA. A specimen collected in a blood activation tube with clot activator, was inverted 5 times in order to facilitate the clotting process, and then we allowed the specimens to sit at ambient temperature until a clot has formed, and after this the specimen was ready for centrifugation using Centrifuge Biofuge Pico, at 1100 - 1300 rpm for 15 minutes. Once the spin time was completed, using a transfer pipette (graded GILSON pipette), we removed the serum from the red top tube (being careful not to disturb the clot), and dispersed it in the appropriate transport tubes (Eppendorf test tubes 1.5 mL). Finally we placed the labelled transport tubes in a cooler or cold storage device until ready for shipping at the corresponding Institutions (Public Health Institution and Nuclear Physics Institution, using the two methods AAS and TXRF for serum zinc analysis.

2.1.1 Atomic Absorption Spectroscopy (AAS)

Atomic absorption spectroscopy (AAS) is a spectro-analytical procedure for the quantitative determination of chemical elements (Figure 1), employing the absorption of optical radiation (light) by free atoms in the gaseous state [7]. AAS has been the method of choice for the elemental analysis of soils extracts and aqueous samples because of their utility, sensitivity and reliability.

This method can rapidly determine metals in trace amounts in many types of matrices. This method is well-characterized and widely used, often for establishing analytical reference values for site samples. Atomic absorption spectroscopy is based on absorption by ground state atoms of an element present in the sample which is atomized in the flame or graphite furnace [8]. Depending on absorption of selected wavelength of the element the concentration is estimated. The technique provides valuable information on concentration of required elements present in the sample. Concentrations are possible in parts per million, or parts per billion levels, depending on source of sample excitation. It uses the absorption of light to measure the concentration of gas-phase atoms. Since samples are usually liquids or solids, the analyte atoms or ions must be vaporized in a flame or graphite furnace [9].

II. Material basis used for zinc analysis:

Reagents used:

- Hydrochloric Acid 1:1
- Metallic zinc 99.99%
- Commercial standard of zinc 1000 parts per million
- Glycerol 5%
- Distilled water

Materials used:

- 2 L flask
- 1 L container
- 100 mL volumetric container

In Tables 1 and 2 are presented all the fixed and changeable conditions necessary for work with Atomic Absorption Spectrometer.

Figure 1. Different views from an Atomic Absorption Spectrometer used for analysis (VARIAN AAS-200)

AAS characteristics and parameters:

Table 1. Fixed conditions for Atomic Absorption (VARIAN AAS-200)

Current of lamp	5 mA
Fuel	Acetylene
Support	Air

Table 2. Changeable conditions for Atomic Absorption

Wavelength (nm)	Sled length (nm)	Optimal range (μg/dL)
213.9	1.0	1 - 200
307.6	1.0	10.000 - 1.400.000

2.1.2 Total Reflection X-Ray Fluorescence (TXRF)

An X-ray Fluorescence Spectrometer, as expressed in Figure 2, is an X-ray instrument used for routine, relatively non-destructive chemical analysis of rock, minerals, sediments and fluids [10].

The XRF method depends on fundamental principles that are common to several other instrumental methods involving interactions between electron beams and X-rays with samples [11], including X-ray Spectroscopy, X-ray diffraction, and wavelength dispersive spectroscopy. System scheme of X-ray Fluorescence, used for zinc analysis, is submitted below:

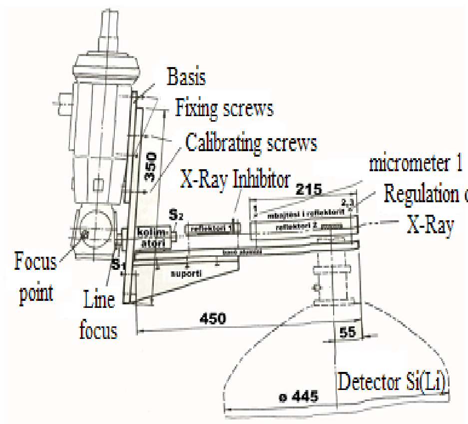

Figure 2. X-ray Fluorescence Instrument used for zinc analysis

II. Material basis used for zinc analysis:

Reagents used:

- Zinc standard 1000 parts per million
- Hydrochloric Acid (10%)
- Distilled water
- Nitric Acid (10%)

Materials used:

- Teflon cups
- Si-PIN detector
- X radiation tube, with Ag anode and power of 3 Watt

III. X-ray characteristics and parameters:

- Negligible effect of the matrix
- Quantification is possible
- It detects elements from Na (11) to U (92),
- Non-destructive method
- Minimal training
- Fast method
- Easy to use method
- Detection limit: 50 - 500 parts per million
- Low cost per analysis.

3. Results and Discussion

Our study consisted of normal pregnant women (who served as control group) and high risk pregnant women, whose maternal age was 17- 44 years old.

Serum zinc values of pregnant women, taken from the corresponding laboratories, were expressed in percentages as seen in Figure 3.

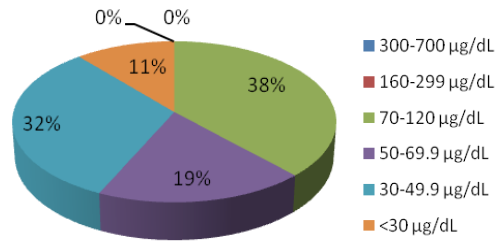

Figure 3. Percentage of serum zinc level in pregnant women

As seen from the chart:

- 38% of pregnant women resulted nomal (190 cases) with zinc levels 70 - 140 μg/dL.
- 62% resultet (310 cases) zinc deficient patient, with zinc levels < 70 μg/dL *(of which 32% with zinc deficiency as a result of digestive fluids loss, 19% with zinc deficiency as a result of oral contraceptive using, and 11% with definite zinc deficiency).*

According to a statistical data processing, using SPSS Statistics, Descriptive Statistics (Table 3) and ANOVA: Single Factor (Table 4)**,** there was no significant change (P > 0.05) between zinc levels measured by two methods of analyzing, $F_{experimental}$ (0.04) < $F_{critical}$ (2.60).

The study showed that the prevalence deficiency of zinc in pregnant women was 62 % (310 cases), this because of an eating disorder or when they are breastfeeding.

Table 3. Data processing using descriptive statistics

Fluorescence	Parameters analyzed	atomic absorption	Parameters analyzed
Mean	64.0784	Mean	64.1196
Standard Error	1.297783229	Standard Error	1.303148135
Median	59.55	Median	60.45
Mode	25	Mode	47
Standard Deviation	29.0193152	Standard Deviation	29.13927815
Relative Standard Deviation	0.4528 (45.28%)	Relative Standard Deviation	0.4543 (45.43%)
Variance	842.1206547	Variance	849.0975309
Minimum	19	Minimum	21
Maximum	116	Maximum	115
Sum	32039.2	Sum	32059.8
Count	500	Count	500
Confidence level (95.0%)	2.549792745	Confidence level (95.0%)	2.560333333

Table 4. Data processing using ANOVA: Single factor

Methods	Count	Sum	Mean	Variance	P-value	F critical	F experimental
TXRF	500	32039.2	64.0784	842.1206547	0.999616858	2.609360773	0.004261628
atomic absorption	500	32059.8	64.1196	849.0975309			

4. Conclusions

As a conclusion of this study we can say that:

- Both methods AAS and TXRF were successful in serum zinc analyzing in high risk and normal pregnant women.

- According to the data taken from this study, in comparison to the two methods, (AAS and TXRF), TXRF is considered as the fastest method and with lowest cost per analysis.

- The identification of pregnant women with zinc deficiency was a problem to be considered, because the continuation of the pregnancy until the end was not enough, fetus complete development was also necessary.

- It is advised for pregnant women to pay special attention to food hygiene during pregnancy, in addition to avoid certain foods, in order to reduce the risk of exposure to substances that may be harmful to the developing fetus. Modification of life-style is an effective intervention strategy, for improvement of maternal metabolism and the prevention of adverse outcomes.

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5. References

[1] Canadian UNICEF Committee. (2006). *Global Child Survival and Health*. <URL:http://en.wikipedia.org/wiki/Micronutrient. Accessed 4 April 2014.

[2] Lieberman S., Bruning N. (1990). *The Real Vitamin & Mineral Book*. Avery Group, New York, USA.

[3] Rasmussen K. M. (1992). *The influence of maternal nutrition on lactation*. Annual Review of Nutrition, 12, pp. 103-117. doi:10.1146/ annurev.nu. 12.070192.000535. PMID 1503799.

[4] Barasi E. M. (2003). *Human Nutrition - A Health Perspective*. Arnold. London, GB.

[5] Prasad A. S. (1985). *Clinical manifestations of zinc deficiency.* Annu. Rev. Nutr., 5, pp. 341-363.

[6] Prasad, A. S., Halsted A. J., and Nadimi M. (1961), *Syndrome of iron deficiency anemia, hepatosplenomegaly, hypogonadism, dwarfism and geophagia*. Am. J. Med., 31, pp. 532-546.

[7] Butrimovitz G. P, and Purdy C. W. (1977). *The Determination of Zinc in Blood Plasma By Atomic Absorption Spectrometry*. Anal. Chim. Acta, 94, pp. 63.

[8] Fernandez F. J, and Kahn L. H. (1971). *Clinical Methods for Atomic Absorption Spectroscopy.* Clin. Chem. Newsl., 3, pp. 24.

[9] Parker M. M., Humoller L. F., and Mahler J. D. (1967). *Determination of Copper and Zinc in Biological Material.* Clin. Chem., 13, pp. 40.

[10] Fitton G. (1997). *X-Ray fluorescence spectrometry.* In: Modern Analytical Geochemistry: An Introduction to Quantitative Chemical Analysis for Earth, Environmental and Material Scientists, Gill, R. (ed.), Addison Wesley Longman, UK.

[11] Potts P. J. (1987). *A Handbook of Silicate Rock Analysis.* Chapman and Hall, UK.