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Serum Zinc Deficiency Test, Its Importance and Prevention during Pregnancy

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Abstract: Because zinc is so important across numerous functions, a deficiency of it can cause a host of problems. Zinc deficiency during pregnancy can negatively affect both the mother and fetus. A healthy, balanced diet can help provide necessary minerals and vitamins. Zinc deficiency is caused by inadequate levels of zinc in the diet. It also plays a role in carbohydrate breakdown (which supplies energy), as well as in cell growth, division and reproduction. Medical tests can determine whether our body fluids contain high levels of zinc. Samples of blood or feces can be collected in a doctor's office and sent to a laboratory that can measure zinc levels. 500 samples for this study were taken from May 2011 until December 2012, at the University Hospital of Obstetrics and Gynecology "Queen Geraldine" in Tirana, Albania. These was a random selection of these samples and groups obtained from this study resulted in normal pregnant women (control group) and high risk pregnant women, from first to third trimester of pregnancy. During this period we studied the clinical cartels of each pregnant woman, in the premises of the hospital archives, to differentiate cases according to hospitalization diagnoses, maternal age, phetus age etc. Laboratory work for this study was done at the "Public Health Institution" in Tirana, using Atomic Absorption Spectroscopy (AAS VARIAN-200); Clinical-Biochemical Laboratory "PhD. Stelijan Buzo "in Tirana, using Photometry (End-Point); "The Nuclear Physics Institution" in Tirana, using Total X-ray Fluorescence. Data taken from the corresponding laboratories, were divided into different groups, to differentiate pregnant women and make the comparison to the control group (normal pregnant women). Pregnant women were divided according to: age, number of deliveries, fetus age, education, residence and also hospitality diagnosis. The most frequent diagnosis and their prevalence of deficiency was as follows: Cephalic: 131 cases (26.2%), from which 90 cases (18%) resulted in zinc deficiency; Partus premature: 71 cases (14.2%), from which 41 cases (8.2%) resulted in zinc deficiency; Hypertension: 63 cases (12.6%), from which 44 cases (8.8%) resulted in zinc deficiency; Anemia: 45 cases (9%), from which 37 cases (7.4%) resulted in zinc deficiency. There were no significant changes ($F_{experimental} < F_{critical}$) between three laboratories using different methods (Photometry, Total X-Ray Fluorescence and Atomic Absorption).

Keywords: AAS Method, Total X-ray Fluorescence, Photometry (End-Point), Zinc Determination, Serum Zinc Test, Zinc in Pregnancy

1. Aim of the Study

This study was aimed at:

- Monitoring of serum zinc concentrations in pregnant women, from first to third trimester of pregnancy,
- Comparison of the zinc concentration in women with abnormal pregnancy, to normal pregnant women who served as a control group,
- The discovery of cases of pregnant women with zinc mild and severe deficiency,
- Identification of serious problems and reasons of results, different from normal laboratory values.

2. Introduction

Zinc is an important mineral required for a number of bodily functions involving energy and metabolism. One of its most important roles is in supporting our immune system, which protects us from pathogens, infections, and disease. Zinc also plays a role in carbohydrate breakdown (which

supplies energy), as well as growth, division, and reproduction of our cells. Physiological states that require increased zinc include periods of growth in infants and children as well as in mothers during pregnancy.

2.1. Introducing Zinc Deficiency in Humans and Pregnant Women

Zinc deficiencies were first realized in the middle-east in the 1960's. Children and adolescents (who require more zinc because they are growing rapidly) were experiencing poor growth, slow sexual maturity, diarrhea and anorexia (leading to overall malnutrition), frequent infections, poor wound healing and learning difficulties. This was because their diets were low in zinc-rich foods (such as meats) and high in unleavened breads, legumes and whole grains. Because zinc is so important across numerous functions, a deficiency of it can cause a host of problems. People with zinc deficiency can experience vision and hearing loss, susceptibility to infections, delayed sexual maturation (in men), stunted growth, hair loss, appetite and weight loss, dry skin, and anemia. A healthy, balanced diet can help provide necessary minerals and vitamins. Zinc deficiency during pregnancy can negatively affect both the mother and fetus. A review of pregnancy outcomes in women with *acrodermatitis enteropathica*, reported that out of every seven pregnancies, there was one abortion and two malfunctions, suggesting the human fetus is also susceptible to the teratogenic effects of severe zinc deficiency. However, a review on zinc supplementation trials during pregnancy did not report a significant effect of zinc supplementation on neonatal survival.

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 [1], and clinical zinc deficiency causes a spectrum from mild and marginal effects up to symptoms of severe nature [2]. Human zinc deficiency was first reported in 1961, when Iranian males were diagnosed with symptoms including growth retardation, hypogonadism, skin abnormalities, and mental lethargy, attributed to nutritional zinc deficiency [3]. Severe zinc deficiency can be either inherited or acquired. The most severe of the inherited forms is *acrodermatitis enteropathica,* a rare autosomal recessive metabolic disorder resulting from a mutation in the intestinal Zip4 transporter [4]. Symptoms of this condition include skin lesions, alopecia, diarrhea, neuropsychological disturbances, weight loss, reduced immune function and can be lethal in the absence of treatment.

Clinical manifestations of moderate zinc deficiency are mainly found in patients with low dietary zinc intake, alcohol abuse, mal-absorption, chronic renal disease, and chronic debilitation. Symptoms include growth retardation (in growing children and adolescents), skin changes, poor appetite, mental lethargy, delayed wound healing, taste abnormalities etc.

One population in which mild zinc deficiency occurs with high prevalence, even in industrialized countries, are the elderly. Here, a significant proportion has reduced serum zinc levels, and zinc supplementation studies indicate that this deficiency contributes significantly to increased susceptibility to infectious diseases. The overall frequency of zinc deficiency worldwide is expected to be higher than 20%. In developing countries, it may affect more than 2 billion people. Furthermore, it has been estimated that only 42.5% of the elderly (=71 years) in the Unites States have adequate zinc intake. This widespread occurrence combined with the variety of clinical manifestations makes zinc deficiency a serious nutritional problem, which has a far greater impact on human health than the relatively infrequent intoxication with zinc.

2.2. Causes of Zinc Deficiency and Risk Factors

Zinc deficiency is caused by inadequate levels of zinc in the diet. It also plays a role in carbohydrate breakdown (which supplies energy), as well as in cell growth, division and reproduction. It is harder for our body to obtain zinc from vegetable sources than from meat sources; therefore, some people with vegetarian diets may be deficient in zinc. Eating a balanced, healthy diet that incorporates foods high in zinc, including protein-rich foods, such as beans, red meat (beef and lamb), and peanuts, can help reduce your risk of zinc deficiency. If our diet is largely vegetarian, we may need to take zinc supplements.

We may be at risk for zinc deficiency because of a number of factors. Not all people with risk factors will get zinc deficiency. Risk factors for zinc deficiency include:

- Limited or no intake of animal protein
- Living in a region without access to proper nutrition
- Malnourishment

2.3. Reducing the Risk of Zinc Deficiency

Fortunately, zinc deficiency is preventable. Our health care provider can advise us about steps we can take to reduce your risk, including providing guidelines for required daily zinc intake.

We may be able to lower our risk of zinc deficiency by:

- Following the guidelines established by the Institute of Medicine's Food and Nutrition Board,
- Obtaining zinc from dietary sources, such as peanuts and beef or lamb,
- Taking zinc supplements if your diet does not provide sufficient zinc.

3. Zinc Medical Tests

Before we start popping zinc at random, take note that there is an upper limit to dietary zinc. Zinc toxicity has produced poor immune health and infertility, just as low zinc compromises the immune system. Scientists suggest we perform a zinc test to measure our level and then supplement accordingly. Once we start taking zinc, our levels will rise and we should do another test six to eight weeks later for best

results.

Medical tests can determine whether our body fluids contain high levels of zinc. Samples of blood or feces can be collected in a doctor's office and sent to a laboratory that can measure zinc levels. It is easier for most high levels of zinc in the feces can mean recent high zinc exposure. High levels of zinc in the blood can mean high zinc consumption and/or high exposure. High zinc levels in blood or feces reflect the level of exposure to zinc. Measuring zinc levels in urine and saliva also may provide information about zinc exposure. Tests to measure zinc in hair may provide information on long-term zinc exposure; however, no useful correlation has been found between hair zinc levels and zinc exposure and these tests are not routinely used. Since zinc levels can be affected by dietary deficiency and cell stress, these results may not be directly related to current zinc exposure [5].

3.1. Serum Zinc

This is the simplest way of assessing zinc status but the factors that can be inaccuracies are high. They include stress, pregnancy, certain malignancies, renal failure, low albumin concentrations etc. Analysis of the 250µL blood sample is done usually by atomic absorption spectroscopy. The reference range in our laboratory was 70-120 µg/dl. A concentration below 40 µg/dl is indicative of a decided deficiency [6].

3.2. Plasma zinc

This is the main lab test done to establish zinc deficiency. Although it is a very good at picking up major deficiencies, it is quite insensitive to marginal deficiency because a change in plasma zinc does not occur until zinc intake is extremely low. Plasma levels of zinc can be influenced by hypo or hyper-proteinemia, stress, pregnancy, liver disease and anemia. Clinical signs of zinc deficiency may occur when plasma zinc concentration drops below 65µg/dl [7].

4. Material and Methods

4.1. Steps Taken during the Relevant Work

Steps taken during the laboratory work were as follows:

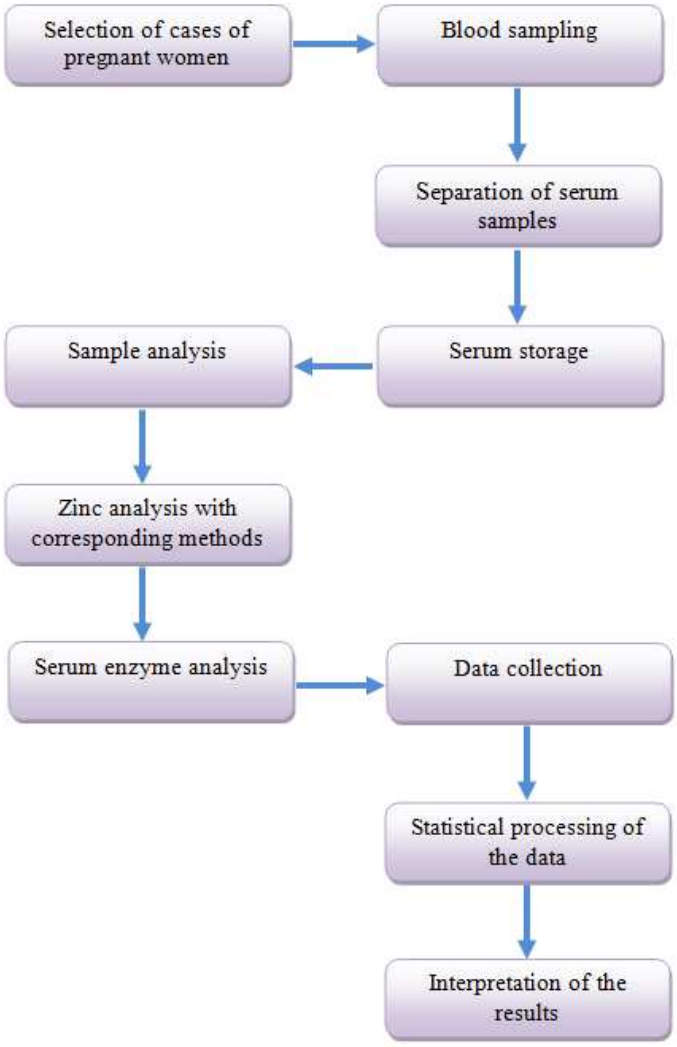

Figure 1. *Steps during relevant work.*

4.2. Steps Followed for Zinc Measurements

- Samples must be collected and processed using zinc-free needles, syringes, centrifuge tubes, storage vials, and transfer pipettes, while avoiding the destruction of red blood cells, hemolysis, and contamination of specimens with ambient zinc in air or water, or by contact with the analyst.
- Ideally, specimens should be collected according to a strict protocol that controls the time of day and fasting status of the specimen donor.
- Because it may not always be possible to collect specimens at the same time of day from all subjects, the time of the blood drawing should be recorded, so the resulting values can be adjusted statistically as necessary.
- Because it is not always possible to ensure that all subjects have either fasted or eaten within a defined time period (for children), the time of the previous meal also should be noted.
- It should be stored in a cool box or in a refrigerator until centrifuged to separate the serum or plasma from the blood cells. This will reduce the introduction of possible artifact into the final results due to transfer of zinc from the blood cells to the serum or plasma.
- Ideally, the serum or plasma should be separated from the cells within 20 to 30 minutes.
- Following centrifugation, the serum or plasma should then be transferred to a screw-top vial for storage, under refrigeration (for up to several days) or frozen, until analysis.
- Zinc concentration can be measured by a number of different analytic instruments, such as atomic absorption spectrometry, photometry and also total X-ray fluorescence [8].
- The measurement method depends on the local availability of these instruments and the desired level of precision.

4.3. Laboratory Methods Used for the Study

500 samples for this study were taken from May 2011 until December 2012, at the University Hospital of Obstetrics and Gynecology "Queen Geraldine" in Tirana, Albania. These was a random selection of these samples and groups obtained from this study resulted in normal pregnant women (control group) and high risk pregnant women, from first to third trimester of pregnancy. During this period we studied the clinical cartels of each pregnant women, in the premises of the hospital archives, to differentiate cases according to hospitalization diagnoses, maternal age, phetus age etc.

Laboratory work for this study was done at the:

- "Public Health Institution" in Tirana, using Atomic Absorption Spectroscopy (AAS VARIAN-200),
- Clinical-Biochemical Laboratory "PhD. Stelijan Buzo "in Tirana, using Photometry (End-Point),
- "The Nuclear Physics Institution" in Tirana, using Total X-ray Fluorescence,
- "Center of Molecular Diagnostics and Genetic Researches" at the University Hospital of Obstetrics-Gynecology "Queen Geraldine" in Tirana, using the necessary equipments for serum preparation, specimen storage etc.

4.3.1. Atomic Absorption Spectroscopy (Varian AAS-200)

(i). Principle of the Method

In general atomic absorption spectroscopy (AAS) is a spectro-analytical procedure for the quantitative determination of chemical elements (figure 2). Atomic absorption techniques are preferred due to their specificity and simplicity [9]. This technique is also used for the measurement of specific elements in the sample to be analyzed [10]. AAS was originally used as an analytical technique, and principles were highlighted in the second half of the nineteenth century by Robert Wilhelm Bunsen and Gustav Robert Kirchhoff, both professors at the University of Heidelberg in Germany [11].

(ii). The Base Material Used for Analysis

- Hydrochloric acid (HCl) 1: 1
- Zinc metal particles 99.99%
- Commercial Standard 1000 ppm zinc
- Glycerin 5%
- Distilled water
- Flask with volume 2 liters
- Container with volume 1 liter
- Containers of 100 mL

(iii). Instrumental Parameters and Performance Characteristics

The performance characteristics are as follows:

- Analysis in less than 2 minutes,
- Defines an element at a time,
- The more determined element, the greater is the time saved and productivity for laboratory.

Table 1. *Fixed condition of AAS method (VARIAN AAS-200).*

Lamp intensity	5 mA
Fuel	Acetylene
Support	Air
Flame stoichiometry	Oxidising

Table 2. *Changeable conditions of AAS method.*

Wavelength (nm)	Element concentration (µg/dl)
213.9	1-200
307.6	10.000 – 1.400.000

Figure 2. *Atomic Absorption Instrument (VARIAN AAS-200).*

4.3.2. Photometry (End-Point)

(i). Principle of the Method

Zinc dissociated from proteins, in particular conditions of ionic strength, gives with chromogen Nitro-PAPS (figure 3), a stable colored complex which intensity of color is proportional at the concentration of Zinc in the sample [12].

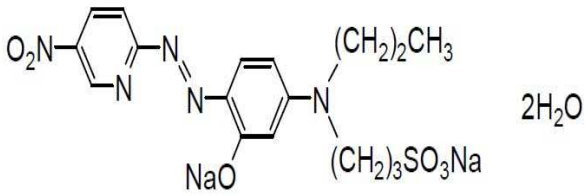

Figure 3. *Chemical structure of Nitro PAPS.*

(ii). Instrumental Parameters and Performance Characteristics

- Wavelength: 580 nm (570-600 nm)
- Path length: 1 cm
- Temperature: 37°C
- Method: End point
- Reaction: 5 minutes
- Linearity: up to 1000 µg/dL
- Sample/Reagent: 1/20
- Working reagent: 1000 µl
- Distilled water: 50 µl
- Standard: 50 µl
- Specimen: 50 µl
- Sensitivity: The minimum detectable is 10 µg/dL.

4.3.3. Total X-ray Fluorescence (TXRF)

(i). Principle of the Method

A very important method of fluorescence Energy Dispersion [14] and fluorescence radiation is Total X Ray Fluorescence. There are two features that distinguish this method:

- Radiation downward in the sample, it forms an angle of less than or very close to the total reflection angle X-rays,
- The angle formed between the incident radiation and a plan that serves as the sample holder (thin film analysis) or is itself the object of analysis (surface analysis).

Total X-Ray Fluorescence is a method used to analyze the samples in liquid, solutions with a particular concentration in the samples that we want to study. A scheme of TXRF apparatus is provided below (Figure 4).

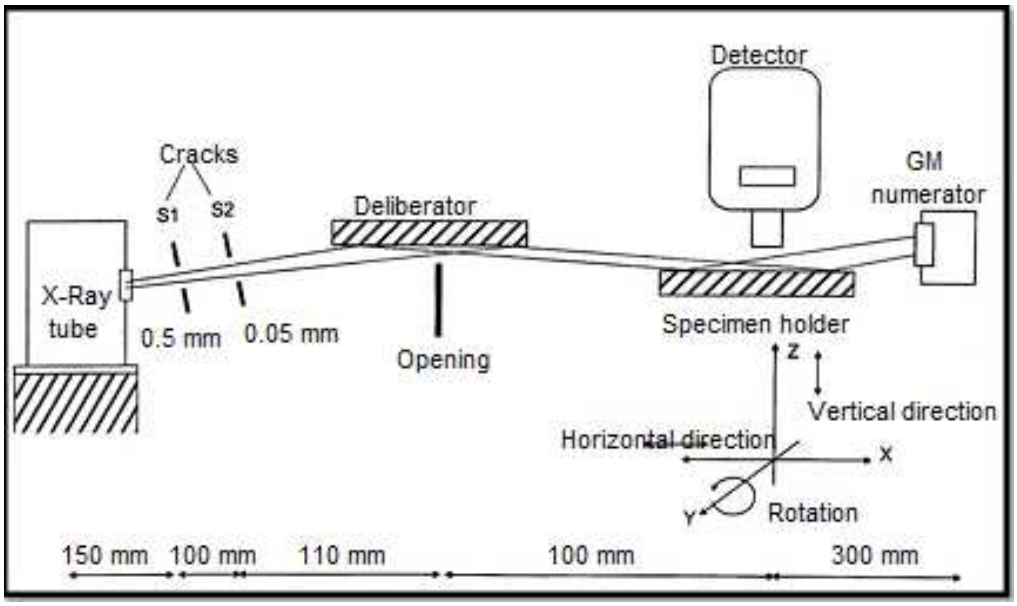

Figure 4. *TXRF instrument.*

TXRF instrument that was used for examinations in this study, is presented below in figure 5.

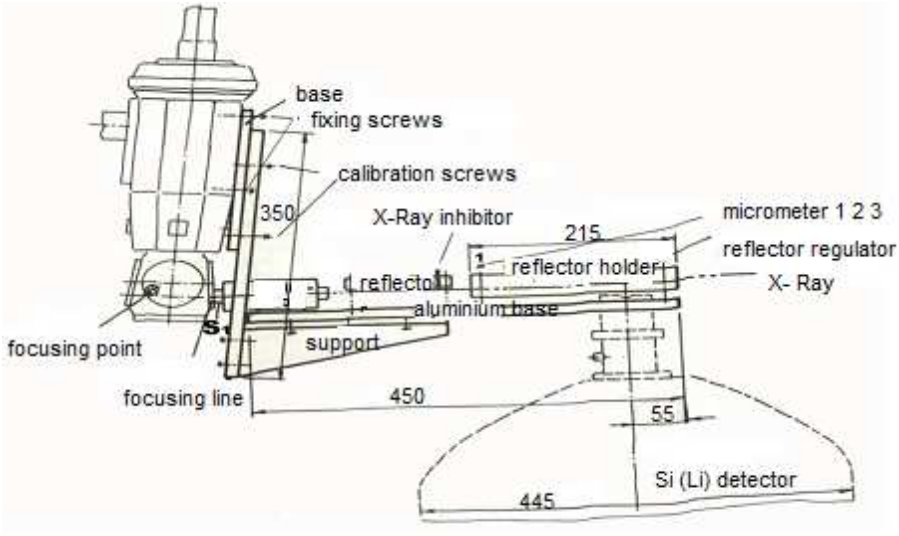

Figure 5. *The TXRF instrument used during the examination.*

TXRF apparatus is an instrument used for routine chemical analysis, relatively non-destructive, minerals, sediments and biological fluids. Low cost analysis and stability X-ray spectrometer, make this method accessible to footprint elements analysis, mineral and sediments [15, 16].

(ii). The Base Material Used for Analysis

- Zinc standard 100 ppm
- HCl (10%)
- Distilled water
- HNO_3 (10%)
- Teflon cups
- Si-PIN detector
- X radiation tube with Ag anode and force 3 Wat

(iii). Instrumental Parameters and Performance Characteristics

- The effect of the matrix is negligible,
- Determinates elements from Na (11) up to U (92),
- Sensitivity of the elements depends on their atomic number,
- Quantification requires the addition of a standard element,
- Non-destructive method
- Requires minimal preparation,
- Quick method (about 6-8 minutes per sample, depending on the measure),
- Easy to use method (under computer control),
- Detection limit: 50-500 ppm,

5. Results and Discussion

5.1. Division of Cases According to Different Groups

Data taken from the corresponding laboratories, were divided into different groups, to differentiate pregnant women and make the comparison to the control group (normal pregnant women). Pregnant women were divided according to: age, number of deliveries, fetus age, education, residence and also hospitality diagnosis.

Table 3. *Cases division according to age.*

	Values of Zn<70		Values of Zn>70		
Age	**No:**	**%**	**No:**	**%**	**Total**
< 20 year	21	56.7	16	43.3	37
20-30 years	206	63.2	120	36.8	326
> 30 years	83	60.6	54	39.4	137
Totali	310	62	190	38	500

Zinc concentration resulted related to maternal age. Women at the age >30 years, had zinc deficiency lower than women at the age 20-30 years. This was due to the increasing of zinc requirements at young women, in comparison to older ones [12, 13].

Table 4. *Cases division according to number of deliveries.*

	Values of Zn<70		Values of Zn>70		
Deliveries	**No:**	**%**	**No:**	**%**	**Total**
1 delivery	153	60	102	40	255
2 deliveries	115	64.2	64	35.8	179
3 deliveries	22	57.9	16	42.1	38
>3 deliveries	20	71.4	8	28.6	28
Total	310	62	190	38	500

Table 5. *Cases division according to residence.*

	Values of Zn<70		Values of Zn>70			
Residence	**No:**	**%**	**No:**	**%**	**Total**	**%**
Village	196	81.6	44	18.4	240	48
Town	56	36.1	99	63.9	155	31
District	58	55.3	47	44.7	105	21
Total	310	62	190	38	500	100

From 500 cases taken into consideration, 240 pregnant women (48%) resulted village residents; 155 (31%) town residents whereas 105 (21%) district residents. In this study

resulted that zinc deficiency was also related to residence.

- From 240 village residents pregnant women, 196 (81.6%) were zinc deficient patients,
- From 105 district resident pregnant women, 58 (55.3%) were zinc deficient patients,
- From 155 town resident pregnant women, 56 (36.1%) resulted zinc deficient patients

According to table 3, zinc deficiency was higher at village residents pregnant women than in other cases, which may be due to mal-nutrition, nutrition absence (especially proteins).

Table 6. *Cases division according to fetus age.*

	Values of Zn<70		Values of Zn>70			
Fetus age	**No:**	**%**	**No:**	**%**	**Total**	**%**
First trimester	21	51.2	20	49	41	8
Second trimester	72	65.5	38	34.5	110	22
Third trimester	217	62.1	132	38	349	70
Total	310	62	190	38	500	100

Zinc concentration resulted related to fetus age, which was increased with fetus age, due to increasing zinc requirements.

- 21 cases (4% of total cases) resulted in zinc deficiency in the first trimester of pregnancy;
- 72 cases (15%) with zinc deficiency in the second trimester of pregnancy;
- 217 cases (43%) resulted with zinc deficiency in the third trimester of pregnancy.

Table 7. *Cases division according to education.*

	Values of Zn<70		Values of Zn>70		
Education	**No:**	**%**	**No:**	**%**	**Total**
8-year education	50	55.6	40	44.4	90
Secondary education	159	64.6	87	35.4	246
Higher education	101	61.6	63	38.4	164
Total	310	62	190	38	500

Table 8. *Cases division according to education and residence.*

		8-year education	%	Secondary education	%	Higher education	%	Total	%
Values of Zn<70	Village	120	61.2	30	15.3	46	23.5	196	63.2
	Town	12	21.4	12	21.4	32	57.2	56	18
	District	10	17.2	32	55.2	16	27.6	58	18.8
	Total	142	45.8	74	23.8	94	30.4	310	62
Values of Zn>70	Village	21	47.8	13	29.5	10	22.7	44	23.1
	Town	50	50.5	29	29.3	20	20.2	99	52.3
	District	11	23.4	29	61.7	7	14.9	47	24.6
	Total	82	43.2	71	37.4	37	19.4	190	38
Total		224	44.8	145	29	131	26.2	500	

Table 9. *Cases division according to hospitality diagnosis.*

	Cases with Zn<70		Cases with Zn>70		Total	%
Hospitality diagnosis	**Nr**	**%**	**Nr**	**%**		
Abortion	22	4.4	4	0.8	26	5.2
Amniorrhea	0	0	3	0.6	3	0.6
Anemia	37	7.4	8	1.6	45	9
Anomalies	23	4.6	9	1.8	32	6.4
Prolonged labour	0	0	1	0.2	1	0.2
Cephalic	90	18	41	8.2	131	26.2
Diabetes	3	0.6	1	0.2	4	0.8
Placental abruption	4	0.8	3	0.6	7	1.4
Feto morto in utero	6	1.2	4	0.8	10	2
Twin pregnancy	6	1.2	3	0.6	9	1.8
Hyperemesis	13	2.6	20	4	33	6.6
Hypertension	44	8.8	19	3.8	63	12.6
Phetal Hypotrophy	3	0.6	0	0	3	0.6
Urinary infection	6	1.2	4	0.8	10	2
Cardiopathy	1	0.2	1	0.2	2	0.4
Membrane ruptures	6	1.2	9	1.8	15	3
Obesity	1	0.2	0	0	1	0.2
Partus premature	41	8.2	30	6	71	14.2
Placenta Previa	3	0.6	1	0.2	4	0.8
Breech delivery	10	2	10	2	20	4
Hemorrhagic shock	1	0.2	1	0.2	2	0.4
Transversal	2	0.4	0	0	2	0.4
Obstetrical trauma	1	0.2	0	0	1	0.2
Baby's death	1	0.2	4	0.8	5	1
Total	324	64.8	176	35.2	500	100

5.2. Data Processing by Using Statistical Methods

Zinc measurements from the corresponding laboratories were processed using different methods such as SPSS, Descriptive Statistics (table 8 and 9) and Anova Single Factor (table 10) to interpret clearly the results.

Table 10. *Comparison of zinc measurements using different methods using Descriptive Statistics.*

Photometry (End-Point)		**Total X-ray Fluorescence**		**Atomic Absorption**	
Mean	63.9518	Mean	64.0784	Mean	64.1196
Standard Error	1.306616933	Standard Error	1.297783229	Standard Error	1.303148135
Median	59.45	Median	59.55	Median	60.45
Mode	34.1	Mode	25	Mode	47
Standard Deviation	29.21684282	Standard Deviation	29.0193152	Standard Deviation	29.13927815
Relative Standard Deviation	0.4567 (45.67%)	Relative Standard Deviation	0.4528 (45.28%)	Relative Standard Deviation	0.4543 (45.43%)
Variance	853.6239046	Variance	842.1206547	Variance	849.0975309
Minimum	20.1	Minimum	19	Minimum	21
Maximum	114	Maximum	116	Maximum	115
Sum	31975.9	Sum	32039.2	Sum	32059.8
Count	500	Count	500	Count	500
Confidence level (95.0%)	2.567148581	Confidence level (95.0%)	2.549792745	Confidence level (95.0%)	2.560333333

Table 11. *Comparison of zinc measurements within same methods using Descriptive Statistics.*

Atomic Absorption (Lab 1)		**Atomic Absorption (Lab 2)**	
Mean	64.1196	Mean	63.9572
Standard Error	1.303148135	Standard Error	1.308896128
Median	60.45	Median	60
Mode	47	Mode	24
Standard Deviation	29.13927815	Standard Deviation	29.26780718
Relative Standard Deviation	0.4543 (45.43%)	Relative Standard Deviation	0.4576 (45.76%)
Variance	849.0975309	Variance	856.6045372
Minimum	21	Minimum	21
Maximum	115	Maximum	114
Sum	32059.8	Sum	31978.6
Count	500	Count	500
Confidence level (95.0%)	2.560333333	Confidence level (95.0%)	2.571626583

Table 12. *Data comparison using Anova Single Factor.*

Groups	**Count**	**Sum**	**Mean**	**Variance**
Photometry	500	31975.9	63.9518	853.6239046
Total X-Ray Fluorescence	500	32039.2	64.0784	842.1206547
Atomic Absorption (Lab 1)	500	32059.8	64.1196	849.0975309
Atomic Absorption (Lab 2)	500	31978.6	63.9572	856.6045372

Table 13. *Data comparison using Anova Single Factor (source of variance)*

Source of variance	**SS**	**df**	**MS**	**F**	**P-value**	**Critical F**
Between groups	10.8717750024516 (RSS)	3	3.623925001	0.0042	0.9996	2.6093
Within groups	1697321.8671 (SSE)	1996	850.3616569			
Total	1697332.738875 (SST)	1999				

5.3. Zinc Correlation within and between Methods

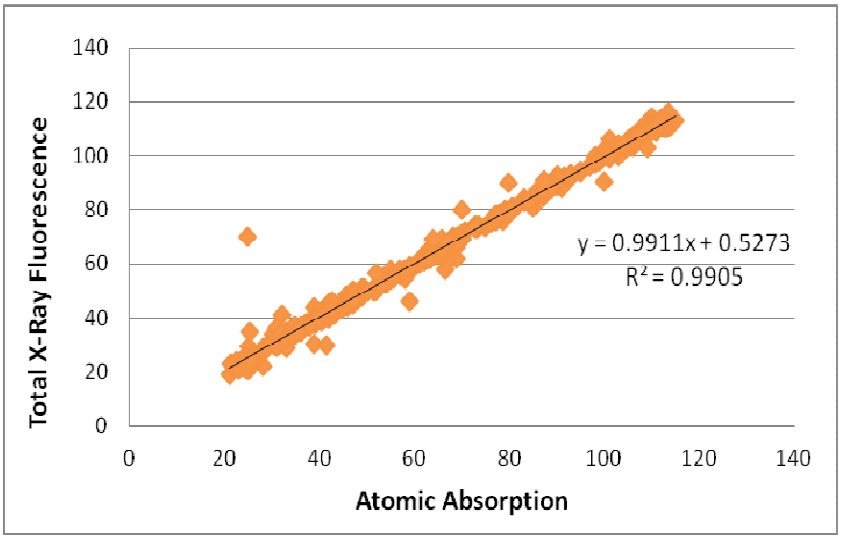

Figure 6. *Correlation of zinc between two methods TXRF and AAS.*

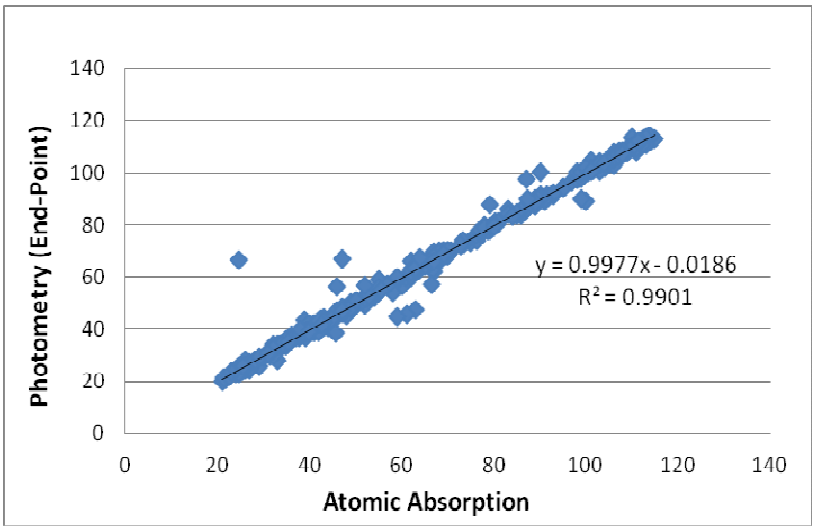

Figure 7. *Correlation of zinc between two methods Photometry and AAS.*

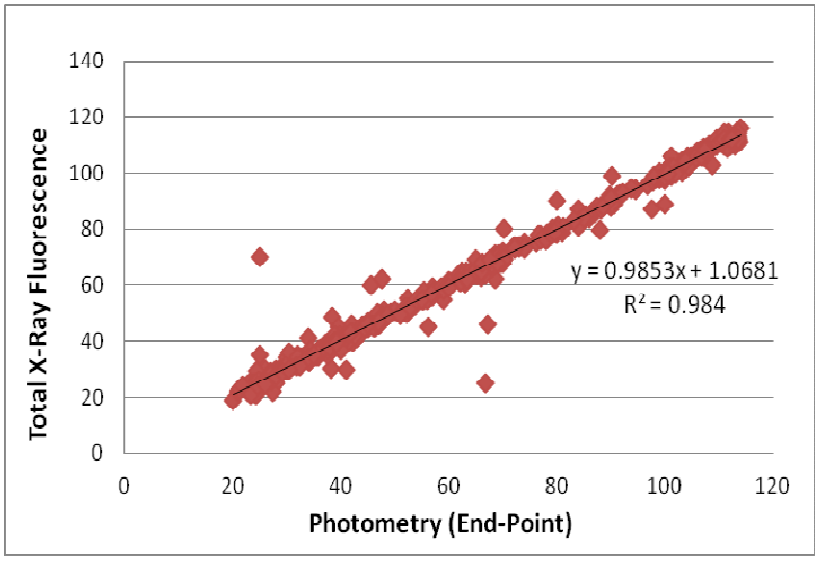

Figure 8. *Correlation of zinc between two methods TXRF and Photometry.*

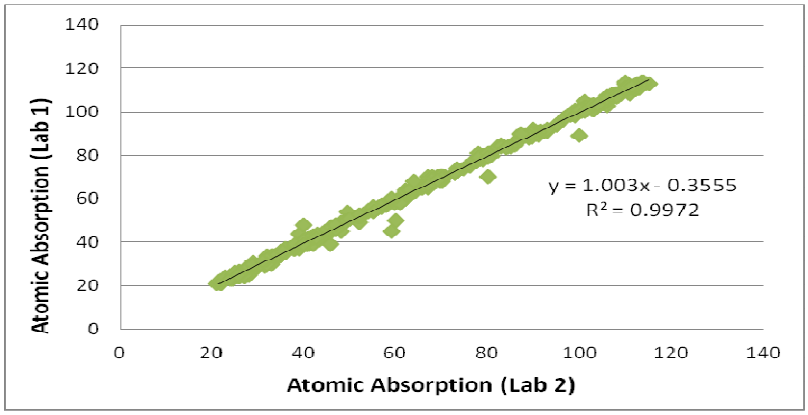

Figure 9. *Correlation of zinc between 2 laboratories using the same method (AAS).*

6. Conclusions

The most frequent diagnosis and their prevalence of deficiency was as follows:

- Cephalic: 131 cases (26.2%), from which 90 cases (18%) resulted in zinc deficiency;
- Partus premature: 71 cases (14.2%), from which 41 cases (8.2%) resulted in zinc deficiency;
- Hypertension: 63 cases (12.6%), from which 44 cases (8.8%) resulted in zinc deficiency;
- Anemia: 45 cases (9%), from which 37 cases (7.4%) resulted in zinc deficiency;

From the data processing by using the corresponding methods, resulted that:

- There were no significant changes ($F_{experimental} < F_{critical}$) between three laboratories using different methods (Photometry, Total X-Ray Fluorescence and Atomic Absorption)
- There were no significant changes between to laboratories (Lab 1 and Lab 2) that used the same method (Atomic Absorption).

As can be seen at the charts:

- There was a strong positive correlation of zinc between two methods TXRF and AAS ($R^2 = 0.99$),
- There was a strong positive correlation between two methods Photometry and AAS ($R^2 = 0.99$),
- There was a strong positive correlation between two methods TXRF and Photometry ($R^2 = 0.98$), so data taken from the corresponding laboratories are aproximitely the same in three different methods used for zinc analysis, what means that there are no significant changes.

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