

The Investigation of Zinc-Dependent Enzymes in Pregnant Women and their Correlation to Zinc Deficiency

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Abstract: 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 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. Enzymes are large biological molecules responsible for the thousands of chemical inter-conversions that sustain life. Enzymes are usually very specific as to which reactions they catalyze and the substrates that are involved in these reactions. Complementary shape, charge and hydrophilic/ hydrophobic characteristics of enzymes and substrates are responsible for this specificity. Specimens were collected at the University Hospital of Obstetrics and Gynecology “Queen Geraldine” in Tirana, Albania during a period of time from year 2011 to 2013. We took into consideration 500 cases of pregnant women from first to third trimester of pregnancy. Serum zinc was measured by using Photometry (End-Point) method, whereas zinc enzymes such as Alkaline Phosphatase, Amylase, Gamma-glutamyl Transpeptidase, Lactate Dehydrogenase, Creatine Kinase, Aspartate Amino-Transferase, Glutamic Pyruvic Transaminase, Lipase measured with the corresponding methods: Beckman Synchron LX20, Colorimetric ab102523 kit, GenWays GGT kit, nonradioactive colorimetric LDH kit, Max Discovery, Colorimetric kit, Abcam kit and Biovision kit. From 500 cases taken into consideration, 190 pregnant women (38%) had normal zinc values (70 – 120 mcg/dl) 58 pregnant women (11.6%) had zinc levels between 60 – 69.9 mcg/dl, patients which were identified as pregnant women with zinc deficiency, as a consequence of oral contraceptive use; 252 pregnant women (50.4%) with zinc values < 60 mcg/dl, identified as patients with zinc deficiency, a result of malnutrition and also urinary elimination of digestive liquids; 56 pregnant women (11.2%) with zinc values < 30 mcg/dl, identified as patient with definite deficiency as a result of different diseases like *acrodermatitis enteropathica* ect. According to these results, there was a positive correlation between zinc and enzymes such as ALP, CK and LDH in pregnant women suffering from hypertension, whereas a negative correlation of zinc to enzymes such GGT, AST and GPT in cases with anemia. Zinc prophylactic treatment is important before and during pregnancy. Without a proper nutritional requirement the person falls in the state of zinc deficiency.

Keywords: Enzyme Assay Kit, Zinc Dependent Enzymes, Zinc and Enzymes Correlation, Zinc Deficiency, Enzyme Test Interpretation

1. Aim of the Study

- Evaluation of serum zinc concentration in pregnant women, from first to third trimester of pregnancy.
- The comparison of zinc concentration in high risk pregnant women to a control group (normal pregnant women).
- The expression of the correlation between zinc and zinc enzyme levels in pregnant women.
- Cases detection with zinc deficiency.

- Identification of serious problems and reasons of concentrations different from normal reference values.

2. Introduction

2.1. Introduction to Zinc

Zinc is the second most abundant trace mineral in the body, after iron. It is an essential nutrient that must be ingested dietary. Not only an antioxidant, but it also helps our digestive system as well as producing certain hormones such

as testosterone and growth hormones. In plain language, zinc plays a role in immune function, protein synthesis, wound healing, DNA synthesis, and cell division. A daily intake of zinc is required to maintain a steady state because the body has no specialized zinc storage system [1].

2.2. The Concept of Zinc Deficiency

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 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 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, skin abnormalities, and mental lethargy, attributed to nutritional zinc deficiency [3]. Later studies with some Egyptian patients showed remarkably similar clinical features [4]. Additional studies in the ongoing years manifested zinc deficiency as a potentially widespread problem in developing as well as in industrialized nations [5].

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. Symptoms of this condition include skin lesions, alopecia, neuropsychological disturbances, weight loss, reduced immune function and can be lethal in the absence of treatment.

Acquired severe zinc deficiency has been observed in patients receiving total parental nutrition without supplementation of zinc, following excessive alcohol ingestion, severe mal-absorption, and iatrogenic causes such as treatment with histidine or penicillamine. The symptoms are mostly similar to those arising during *acrodermatitis enteropathica*. In mild cases of zinc deficiency, slight weight loss and hyperammonemia were observed. 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 in the United 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.

2.3. Introduction to Enzymes and Their Specificity

Enzymes are large biological molecules responsible for the thousands of chemical inter-conversions that sustain life. They are highly selective catalysts, greatly accelerating both the rate and specificity of metabolic reactions, from the digestion of food to the synthesis of DNA. Most enzymes are proteins, although some catalytic RNA molecules have been identified. Enzymes adopt a specific three-dimensional structure, and may employ organic (e.g. biotin) and inorganic (e.g. magnesium ion) cofactors to assist in catalysis. Like all catalysts, enzymes work by lowering the activation energy (E_a) for a reaction, thus dramatically increasing the rate of the reaction. As a result, products are formed faster and reactions reach their equilibrium state more rapidly. Most enzyme reaction rates are millions of times faster than those of comparable un-catalyzed reactions. As with all catalysts, enzymes are not consumed by the reactions they catalyze, nor do they alter the equilibrium of these reactions. However, enzymes do differ from most other catalysts in that they are highly specific for their substrates.

Enzyme activity can be affected by other molecules. Inhibitors are molecules that decrease enzyme activity; activators are molecules that increase activity. Many drugs and poisons are enzyme inhibitors. Activity is also affected by temperature, pressure, chemical environment (e.g., pH), and the concentration of substrate. Some enzymes are used commercially, for example, in the synthesis of antibiotics. In addition, some household products use enzymes to speed up biochemical reactions (e.g., enzymes in biological washing powders break down protein or fat stains on clothes; enzymes in meat tenderizers break down proteins into smaller molecules, making the meat easier to chew).

Enzymes are usually very specific as to which reactions they catalyze and the substrates that are involved in these reactions. Complementary shape, charge and hydrophilic/hydrophobic characteristics of enzymes and substrates are responsible for this specificity. Enzymes can also show impressive levels of stereospecificity, regioselectivity and chemoselectivity. Some of the enzymes showing the highest specificity and accuracy are involved in the copying and expression of the genome. These enzymes have "proof-reading" mechanisms. Here, an enzyme such as DNA polymerase catalyzes a reaction in a first step and then checks that the product is correct in a second step. This two-step process results in average error rates of less than 1 error in 100 million reactions in high-fidelity mammalian polymerases.

3. Enzymes Taken into Consideration for the Study

3.1. Cardiac Enzymes

3.1.1. Lactate Dehydrogenase (LDH)

A lactate dehydrogenase is an enzyme present in a wide variety of organisms, including plants and animals. It exists in four distinct enzyme classes. Two of them are cytochrome c-dependent enzymes, each acting on either D-lactate or L-

lactate The other two are NAD(P)-dependent enzymes, each acting on either D-lactate or L-lactate. Lactate dehydrogenase catalyzes the inter-conversion of pyruvate and lactate with concomitant inter-conversion of NADH and NAD⁺. It converts pyruvate, the final product of glycolysis, to lactate when oxygen is absent or in short supply, and it performs the reverse reaction during the Cori cycle in the liver. At high concentrations of lactate, the enzyme exhibits feedback inhibition, and the rate of conversion of pyruvate to lactate is decreased. It also catalyzes the dehydrogenation of 2-Hydroxybutyrate, but it is a much poorer substrate than lactate.

3.1.2. Creatine Kinase (CK)

Creatine kinase (CK), also known as creatine phosphokinase (CPK) is an enzyme expressed by various tissues and cell types. CK catalyses the conversion of creatine and consumes adenosine triphosphate (ATP) to create phosphocreatine (PCr) and adenosine diphosphate (ADP). This enzyme reaction is reversible and thus ATP can be generated from PCr and ADP [12]. In tissues and cells that consume ATP rapidly, especially skeletal muscle, but also brain, hair cells of the inner ear, spermatozoa and smooth muscle, PCr serves as an energy reservoir for the rapid buffering and regeneration of ATP *in situ*, as well as for intracellular energy transport by the PCr shuttle or circuit. Thus creatine kinase is an important enzyme in such tissues. Clinically, creatine kinase is assayed in blood tests as a marker of myocardial infarction (heart attack), muscular dystrophy and in acute renal failure [11].

3.2. Digestive Enzymes

3.2.1. Amylase

Amylase is an enzyme that catalyses the breakdown of starch into sugars. It is produced in the pancreas and the glands that make saliva. When the pancreas is diseased or inflamed, amylase releases into the blood. It is present in the saliva of humans and some other mammals, where the chemical process of digestion begins. Foods that contain much starch but little sugar, such as rice and potato, taste slightly sweet as they are chewed because amylase turns some of their starch into sugar in the mouth. The pancreas also makes amylase (alpha amylase) to hydrolyse dietary starch into disaccharides and trisaccharides which are converted by other enzymes to glucose to supply the body with energy. Plants and some bacteria can also produce amylase

3.2.2. Lipase

Lipase is an enzyme that catalyzes the breakdown or hydrolysis of fats (lipids). Lipases are a subclass of the esterases. They perform essential roles in the digestion, transport and processing of dietary lipids (e.g. triglycerides, fats, oils) in most, if not all, living organisms.

Most lipases act at a specific position on the glycerol backbone of lipid substrate (A1, A2 or A3) (small intestine). For example, human pancreatic lipase (HPL) which is the

main enzyme that breaks down dietary fats in the human digestive system, converts triglyceride substrates found in ingested oils to monoglycerides and two fatty acids. Several other types of lipase activities exist in nature, such as phospholipases and sphingomyelinases, however these are usually treated separately from "conventional" lipases. Some lipases are expressed secreted by pathogenic organisms during the infection. In particular, *Candida albicans* has a large number of different lipases, possibly reflecting broad lipolytic activity, which may contribute to the persistence and virulence of *C. albicans* in human tissue.

3.3. Cholelstatic Enzymes

3.3.1. Alkaline Phosphatase (ALP)

Alkaline phosphatase (ALP, ALKP) is a hydrolase enzyme responsible for removing phosphate groups from many types of molecules, including nucleotides, proteins, and alkaloids. The process of removing the phosphate group is called *dephosphorylation*. As the name suggests, alkaline phosphatases are most effective in an alkaline environment. It is sometimes used synonymously as basic phosphatase [8]. ALP is present in a number of tissues including liver, bone, intestine, and placenta. Serum ALP is of interest in the diagnosis of 2 main groups of conditions-hepatobiliary disease and bone disease. Each tissue—liver, bone, placenta, and intestine—produces a slightly different alkaline phosphatase. These variations are called isoenzymes. In the laboratory, alkaline phosphatase is measured as the total amount or the amount of each of the four isoenzymes. During pregnancy, alkaline phosphatase is made by the placenta and leaks into the mother's bloodstream. Some tumors, however, start production of the same kind of alkaline phosphatase produced by the placenta. These tumors are called germ cell tumors and include testicular cancer and certain brain tumors [6,7].

3.3.2. Gamma-Glutamyl Transpeptidase (GGT)

Gamma-glutamyltransferase is an enzyme that transfers gamma-glutamyl functional groups. It is found in many tissues, the most notable one being the liver, and has significance in medicine as a diagnostic marker. GGT catalyzes the transfer of the gamma-glutamyl moiety of glutathione to an acceptor that may be an amino acid, a peptide or water (forming glutamate). GGT plays a key role in the gamma-glutamyl cycle, a pathway for the synthesis and degradation of glutathione and drug and xenobiotic detoxification. This is a test which helps in determining the level of GGT in the bloodstream. The abnormal high or low level shows alcohol abuse, bone disease & bile duct obstructions are the few names. As a thumb rule, the higher the GGT level the higher would be the chances of liver damage. There may be other situations which might lead to this & hence, other tests will be recommended along with the GGT test. As GGT is particularly found in the areas near liver & other organs a raised level of GGT in the blood indicates the flowing of the enzymes out of the liver into the bloodstream & can be identified by its raised level. Many

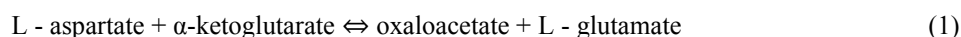
other blood tests are also performed with GGT test to figure out the causes of damaged liver [8,9].

3.4. Liver Enzymes

3.4.1. Aspartate Aminotransferase (AST)

Aspartate aminotransferase is an enzyme that catalyzes the conversion of α -ketoglutarate in the amino acid glutamic acid catalyze. Without this step, the reaction would malate-aspartate shuttle and thus the utilization of carbohydrates in the metabolism of eukaryotes impossible.

Further, the reaction is part of the degradation of several



ASAT is required for their function of pyridoxal phosphate, which serves as a prosthetic group is bound to the enzyme.

3.4.2. Glutamic-Pyruvic Transaminase (GPT)

Alanine transaminase is a transaminase enzyme. It is also called serum glutamic-pyruvic transaminase (SGPT) or alanine aminotransferase (ALAT). ALT is found in plasma and in various bodily tissues, but is most commonly associated with the liver. It catalyzes the two parts of the alanine cycle. It catalyzes the transfer of an amino group from L-alanine to α -ketoglutarate, the products of this reversible transamination reaction being pyruvate and L-glutamate. ALT (and all transaminases) require the coenzyme pyridoxal phosphate, which is converted into pyridoxamine in the first phase of the reaction, when an amino acid is converted into a keto acid.

Aspartate transaminase, as with all transaminases, operates via dual substrate recognition; that is, it is able to recognize and selectively bind two amino acids (Asp and Glu) with different side-chains. In either case, the transaminase reaction consists of two similar half-reactions that constitute what is referred to as a ping-pong mechanism. In the first half-reaction, amino acid 1 (e.g., L-Asp) reacts with the enzyme-PLP complex to generate ketoacid 1 (oxaloacetate) and the modified enzyme-PMP. In the second half-reaction, ketoacid 2 (α -ketoglutarate) reacts with enzyme-PMP to produce amino acid 2 (L-Glu), regenerating the original enzyme-PLP in the process. Formation of a racemic product (D-Glu) is very rare.

4. Material and Methods

4.1. Method for Zinc Measurement

Serum zinc was measured by using Photometry (End-Point) method. Specimens were collected at the University Hospital of Obstetrics and Gynecology "Queen Geraldine" in Tirana, Albania during a period of time from year 2011 to 2013. We took into consideration 500 cases of pregnant women from first to third trimester of pregnancy.

4.1.1. Principle of the Method

Zinc dissociated from proteins, in particular conditions of

amino acids, because the AST is found in all living things. We distinguish cytoplasmic and mitochondrial AST (c-AST, m-AST), with the corresponding genes *GOT1* and *GOT2*. Plants have another form in the chloroplasts. Humans produce ASAT especially in skeletal muscle, heart muscle and liver. The ratio of c-ASAT/m-ASAT in blood serum suggests the condition of the heart and liver. AST catalyzes the transfer of the common L-amino group of an amino acid to an α -keto acid.

This occurs for example for the reaction:

ionic strength gives with chromogen Nitro-PAPS a stable colored complex, where the intensity of color is proportional to the concentration of Zinc in the sample [17].

4.1.2. Reagents Used for the Study

REAGENT 1 (liquid) 2 x 16 mL
Buffer pH 8.2 >0.1 mol/L
Masking agents: Stabilizers and detergents
REAGENT 2 (liquid) 1 x 8 mL
Nitro-PAPS >0.1 mmol/L
REAGENT 3 Standard (liquid) 1 x 5 mL
Zinc 200 $\mu\text{g/dL}$

4.1.3. Performance Characteristics

- Stability: The reagents, at 2-8°C, are stable up to the expiry date shown on the package if not contaminated during handling [18].
- Linearity: The Zinc concentration is determined between 10-1000 $\mu\text{g/dL}$. For concentrations ≥ 1000 $\mu\text{g/dL}$ dilute the sample 1:5 with saline sol., repeat the determination and multiply the result x 5.
- Wavelength: 580 nm (570-600 nm)
- Pathlength: 1 cm
- Temperature: 37°C
- Method: end point
- Reaction: 5 minutes
- Sample/Reagent: 1/20
- Sensitivity: The minimum detectable is 10 $\mu\text{g/dL}$ [19].

4.2. Methods for Enzyme Measurements

We took into consideration 8 zinc-dependent enzymes such as: Alkaline Phosphatase (ALP), Amylase, Gamma-glutamyl Transpeptidase (GGT), Lactate Dehydrogenase (LDH), Creatine Kinase (CK), Aspartate Aminotransferase (AST), Glutamic Pyruvic Transaminase (GPT) and Lipase.

4.2.1. Method Used for ALP Determination

(i) Principle of the method

The Beckman Synchron LX20 method uses an enzymatic rate by using 2-amino-2-methyl-1-propanol (AMP) buffer to measure ALP activity in serum or plasma. In the reaction, the ALP catalyzes the hydrolysis of the colorless organic phosphate ester substrate, p-Nitrophenylphosphate, to the yellow colored product p-Nitrophenol and phosphate. This

reaction occurs at an alkaline pH of 10.3. The system monitors the rate of change in absorbance at 410 nm over a fixed-time interval. This rate of change in absorbance is directly proportional to the ALP activity in the serum. Alkaline phosphatase measurements are used in the diagnosis and treatment of liver, bone, and parathyroid disease [20,21].

(ii) Equipments used and reagent preparation

- Beckman Synchron CX Micro Sample Tube (Part #448774)
- S/P Plastic Transfer Pipet (Cat. #P5214-10)
- S/P Brand Accutube Flange Caps (Cat. #T1226-37)
- Reagent Preparation: Beckman Synchron System ALP Reagent (Part #442670, 200 tests/cartridge or Part #476821, 400 tests/cartridge).
- No preparation required.
- When stored unopened at 2-8°C, the reagent is stable until the expiration printed on the label.
- When first opened or installed on the instrument, the reagent is stable for ten days unless the expiration date is exceeded.
- Caustic reagent. Avoid skin contact with reagent. Use water to wash reagent from skin.
- Standards Preparation: None required.
- Control Material

4.2.2. Method Used for Amylase Determination

(i) Principle of the method

Amylase Assay kit (Colorimetric ab102523) detects activity of α -amylase through a two-step reaction. α -Amylase will cleave the substrate ethylenedip NP-G7 to produce smaller fragments that are eventually modified by α -glucosidase, causing the release of a chromophore that can then be measured at OD = 405 nm. The assay can detect α -amylase content as low as 0.2 mU.

(ii) Equipments used and materials required

These materials are not included in the kit, but will be required to successfully utilize this assay:

- MilliQwater or other type of double distilled water (ddH₂O)
- PBS
- Micro centrifuge
- Pipettes and pipette tips
- Colorimetric microplate reader equipped with filter for OD 405nm
- 96 well plate: clear plate for colorimetric assay
- Orbital shaker
- Vortex

4.2.3. Method Used for GGT Determination

(i) Principle of the method

GenWay's Gamma-Glutamyl Transferase Assay Kit provides a convenient tool for sensitive detection of the GGT in a variety of samples. The GGT in sample will recognize L- γ -Glutamyl-pNA as a specific substrate leading to proportional color development. The activity of GGT can be easily quantified colorimetrically (λ = 418 nm). This assay detects GGT activity as low as 0.5 mIU [8,9].

(ii) Kit content and reagents used

- GGT Assay Buffer WM GWB-AXR359-1
- GGT Substrate NM GWB-AXR359-2
- GGT Positive Control GWB-AXR359-3
- pNA Standard (2mM)
- Reaction mix

4.2.4. Method Used for LDH Determination

(i) Principle of the method

The non-radioactive colorimetric LDH assay is based on the reduction of the tetrazolium salt MTT in a NADH-coupled enzymatic reaction to a reduced form of MTT which exhibits an absorption maximum at 565 nm. The intensity of the purple color formed is directly proportional to the enzyme activity [22].

(ii) Kit content and materials required

- Pipeting devices and accessories (e.g. multi-channel pipettor).
- Procedure using 96-well plate: Clear bottom 96-well plates (e.g. Corning Costar) and plate reader.
- Procedure using cuvette: Spectrophotometer and cuvetts for measuring OD 565nm.
- Substrate buffer
- NAD solution
- PMS solution
- MTT solution
- Calibrator [23,24].

4.2.5. Method Used for CK Determination

(i) Principle of the method

The MaxDiscovery™ Creatine Kinase (CK) Enzymatic Assay Kit is a colorimetric, plate-based assay to determine the amount of creatine kinase in serum. The kit enables biomedical researchers to detect heart damage in mice and rats. The test is based on a proven method for creatine kinase determination which utilizes a coupled enzymatic assay to specifically detect creatine kinase enzyme in fluids. It provides accurate, proven results even in complex samples [10,11].

(ii) Kit contents and Materials required

- Microtiter Plate 1 x 96-well Plate
- Reagent Mix bottle
- Standard vial - 20°C
- Standard Dilution Buffer
- Microtiter plate reader (with 340 nm absorbance filter)
- Microcentrifuge
- De-ionized or distilled water
- 1.5 mL microcentrifuge tubes
- Multichannel pipet (recommended)

4.2.6. Method Used for AST Determination

(i) Principle of the method

Alanine Transaminase Activity Assay Kit (colorimetric) is a rapid and simple assay where ALT catalysis the transfer of an amino group from alanine to α -ketoglutarate, the products of this reversible transamination reaction being pyruvate and glutamate [12,13]. The kit provides a rapid, simple, sensitive, and reliable test suitable for high throughput activity assay of

ALT with a detection limit of 10mU per well [14].

(ii) Kit components and materials required

- ALT positive control (lyophilized)
- ALT substrate (lyophilized)
- Pyruvate standard
- ALT assay buffer
- ALT enzyme mix
- OxiRed in DMSO

4.2.7. Method Used for GPT Determination

(i) Principle of the method

In Abcam's Alanine Transaminase Activity Assay Kit, ALT catalyzes the transfer of an amino group from alanine to α -ketoglutarate, the products of this reversible transamination reaction being pyruvate and glutamate. The pyruvate is detected in a reaction that concomitantly converts a nearly colorless probe to both color ($\lambda_{\max} = 570 \text{ nm}$) and fluorescence ($\text{Ex/Em} = 535/587 \text{ nm}$). The kit provides a rapid, simple, sensitive, and reliable test suitable for high throughput activity assay of ALT with a detection limit of 10 mU per well.

(ii) Kit component and materials required

- ALT Assay Buffer
- OxiRed Probe (in DMSO)
- ALT Enzyme Mix (Lyophilized)
- ALT Substrate (Lyophilized)
- Pyruvate Standard
- ALT positive control (Lyophilized)
- Microcentrifuge
- Pipettes and pipette tips

- Fluorescent or colorimetric microplate reader
- 96 well plate
- Orbital shaker

4.2.8. Method Used for Lipase Determination

(i) Principle of the method

In BioVision's Lipase Assay Kit, lipase hydrolyzes a triglyceride substrate to form glycerol which is quantified enzymatically by via monitoring a linked change in the OxiRed probe absorbance ($\lambda=570\text{nm}$). This assay is rapid, simple, sensitive, and reliable, as well as, suitable for high throughput activity screening of lipase. This kit detects lipase activity as low as 10mU per well [15,16].

(ii) Kit component and materials required

- Lipase Assay Buffer
- OxiRed Probe (in DMSO)
- Enzyme Mix (lyophilized)
- Lipase Substrate
- Glycerol Standard (100mM)
- Lipase Positive Control

5. Results and Discussion

5.1. The Results of Serum Zinc Measurements in Pregnant Women

Serum zinc in pregnant women was measured using Photometry (End-Point), at the Medical Laboratory "PhD Stiljan Buzo", in Tirana, Albania. All the laboratory values were expressed in table 1.

Table 1. Serum zinc levels in pregnant women.

Serum zinc values ($\mu\text{g/dl}$)	Possible condition	Causes	Number of cases	%
300 – 700	Acute Intoxication		0	0
160 – 299	Intoxication or increase of the level due to high consumption of zinc	High consumption of sea production	0	0
70 – 120	Normal values	-	190	38
50 – 69.9	Zinc deficiency causes by drug uses	Loss of digestive fluids	94	19
30-49.9	Deficiency	Oral Contraceptive uses	160	32
< 30	Definite deficiency	-	56	11

If these values were expressed in percentage, we would have the chart below (figure).

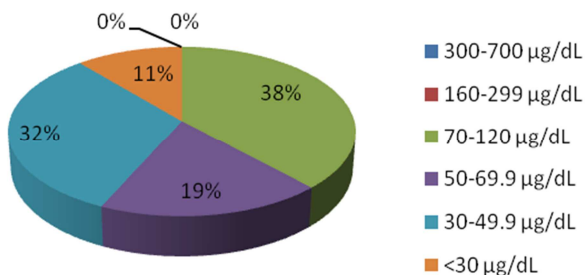


Figure 1. Zinc values expressed in percentage.

5.2. Zinc Correlation to Enzymes

Pregnant women were divided according to maternal diagnosis. We took into consideration only diagnosis that had many cases, to have a better idea about the correlation between zinc and enzyme levels.

5.2.1. Zinc in Correlation to Alkaline Phosphatase (ALP)

Values taken from the corresponding laboratory were expressed in figure 2. As we can see, the correlation coefficient is respectively $R^2 = 0.7656$ in pregnancies with anemia and $R^2 = 0.7476$ in pregnancies with hypertension.

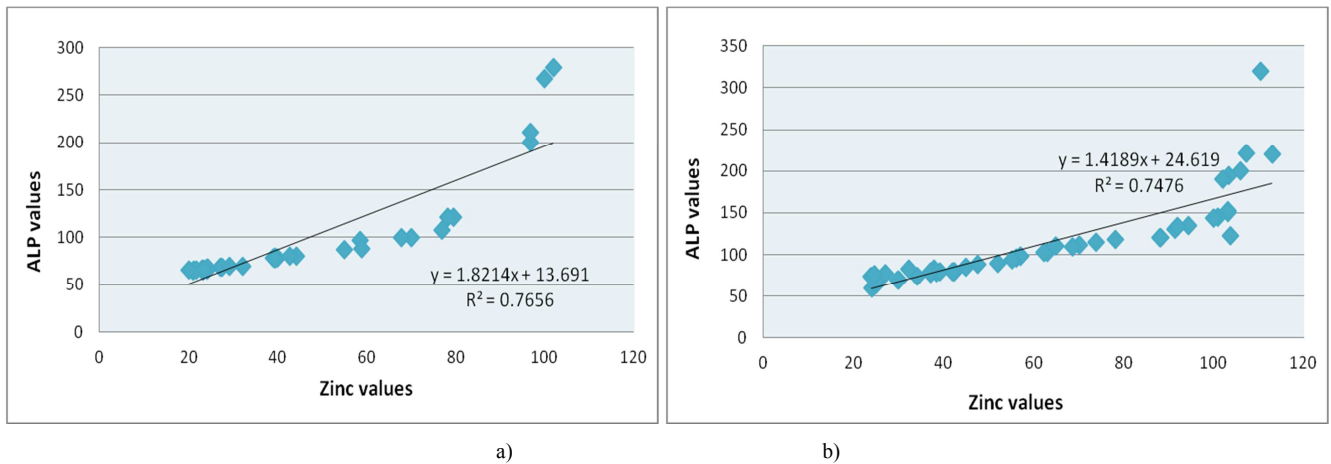


Figure 2. Correlation of zinc to ALP in different maternal diagnosis, a) anemia, b) hypertension.

5.2.2. Zinc in Correlation to Amylase

According to the figure 3, the correlation coefficient between zinc and amylase for pregnancies with abortion was $R^2 = 0.7033$, whereas for pregnancies with hypertension $R^2 = 0.7472$.

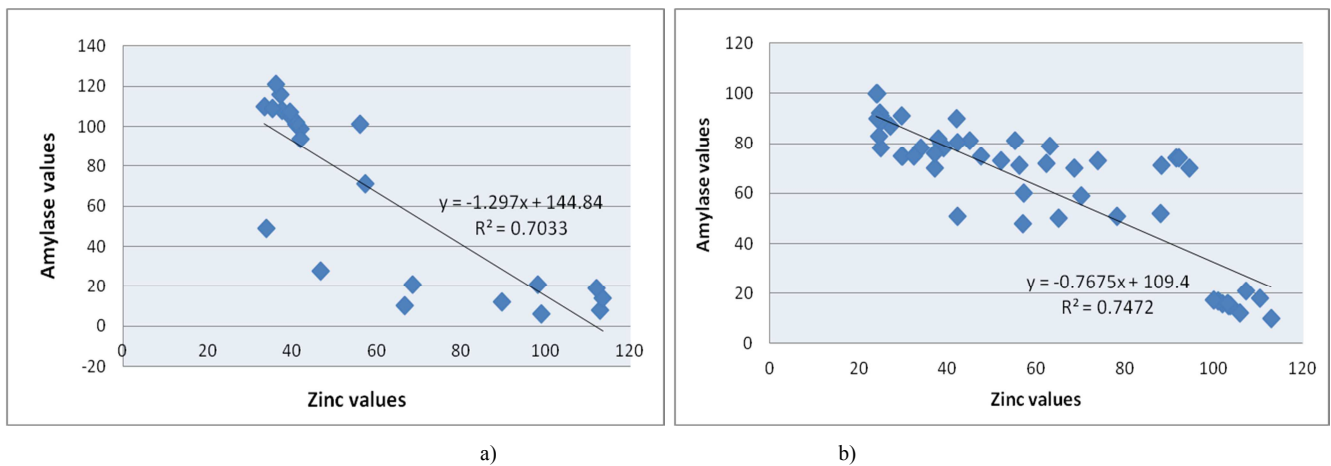


Figure 3. Correlation of zinc to Amylase in different maternal diagnosis, a) abortion, b) hypertension.

5.2.3. Zinc in Correlation to Gamma-glutamyl Transpeptidase (GGT)

According to the figure 4, the correlation coefficient between zinc and GGT in pregnancies with anaemia was $R^2 = 0.7591$, whereas in pregnancies with hypertension $R^2 = 0.8385$.

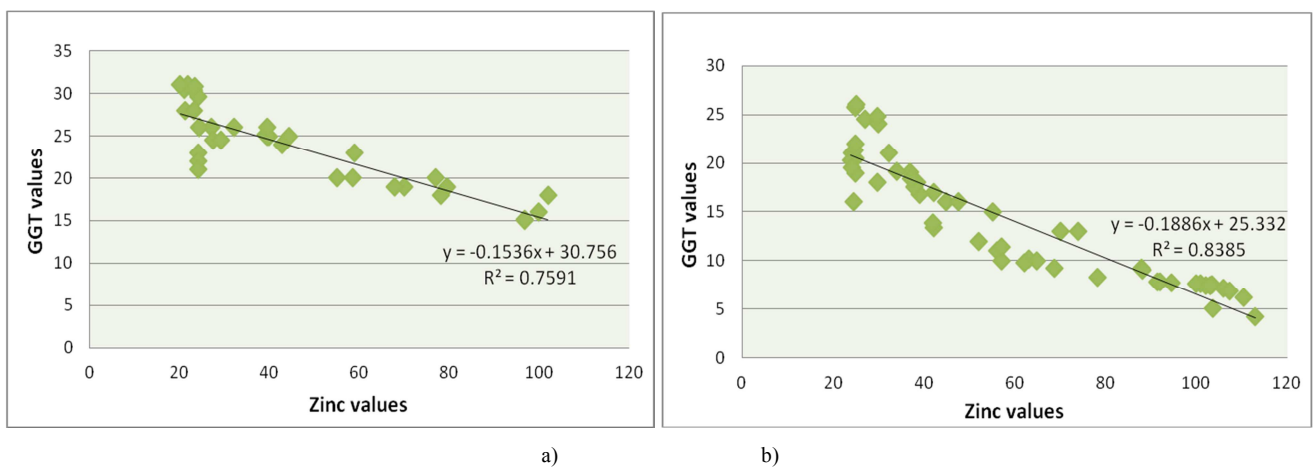


Figure 4. Correlation of zinc to GGT in different maternal diagnosis, a) anemia, b) hypertension.

5.2.4. Zinc in Correlation to Creatine Kinase (CK)

According to figure 5, the correlation coefficient between zinc and CK in pregnancies with abortion was $R^2 = 0.8859$, in cases with anaemia $R^2 = 0.8697$, whereas in pregnancies with hypertension $R^2 = 0.8465$.

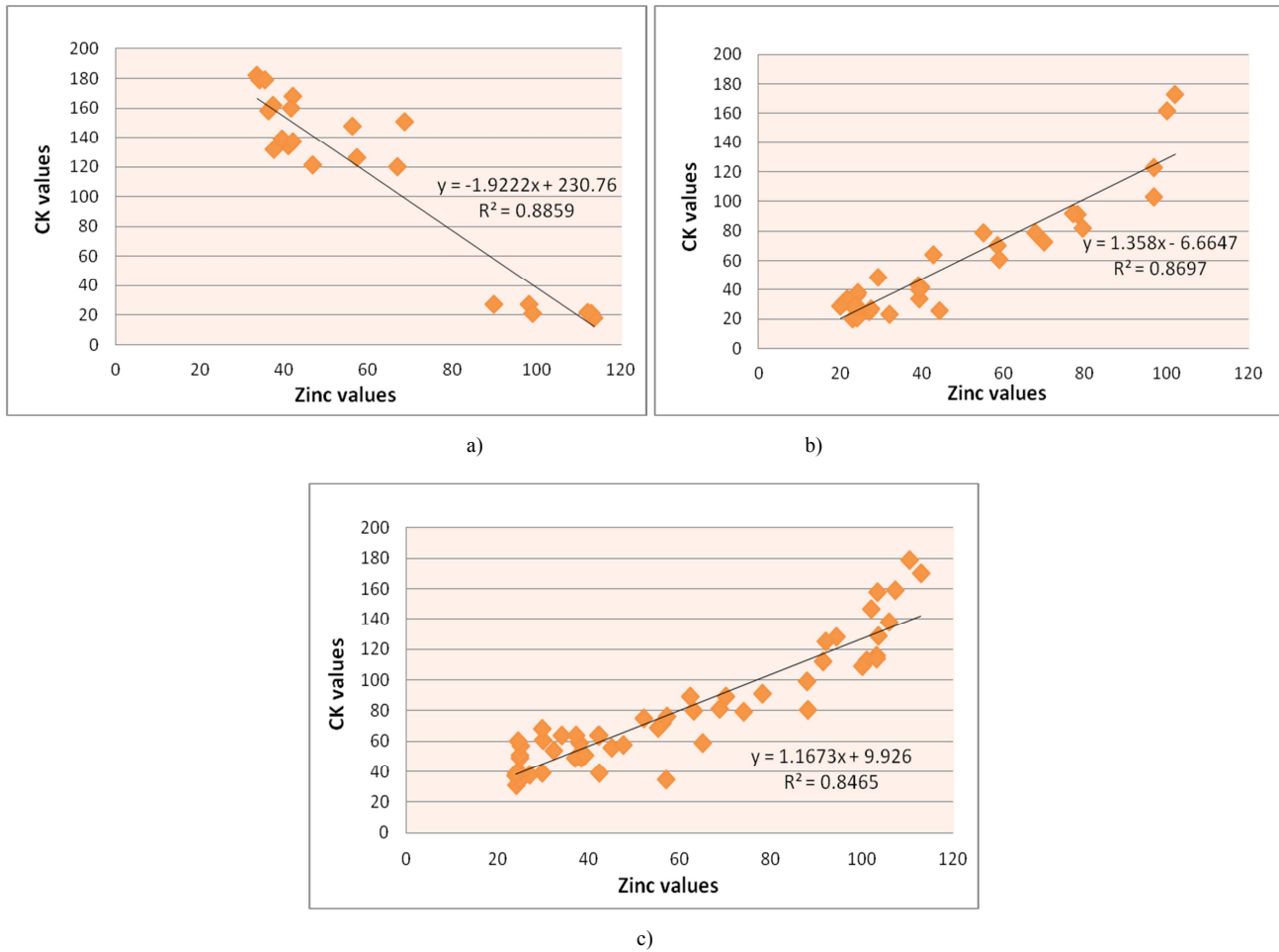
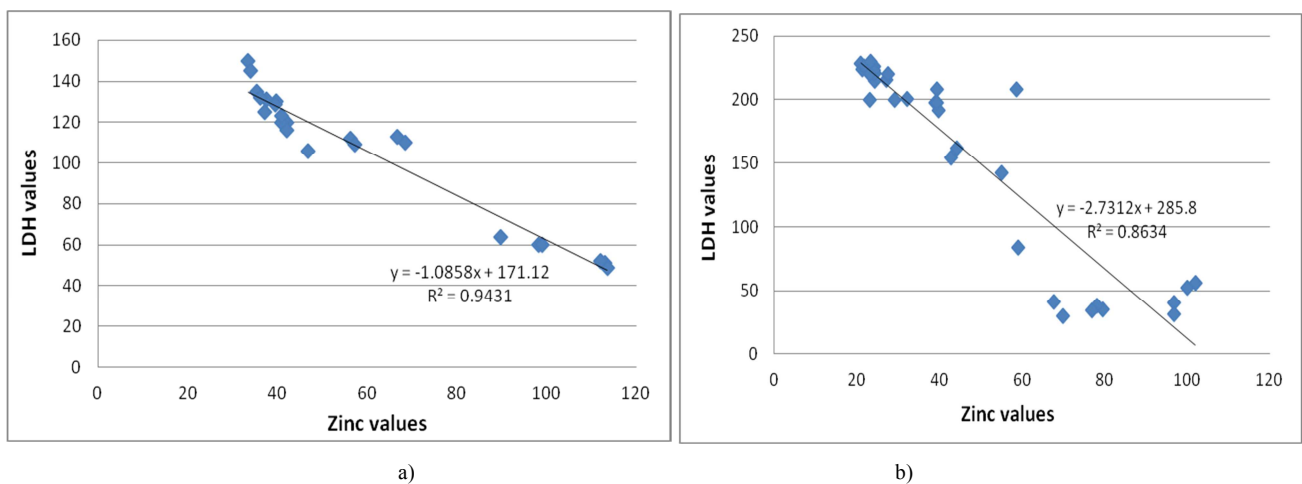
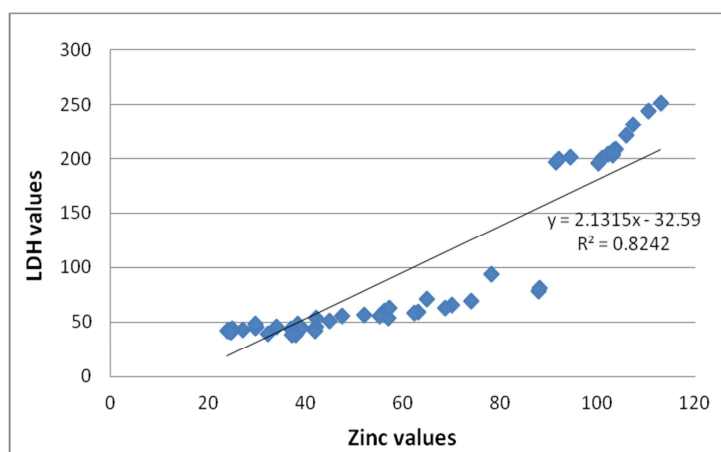


Figure 5. Correlation of zinc to CK in different maternal diagnosis, a) abortion, b) anemia, c) hypertension.

5.2.5. Zinc in Correlation to Lactat Dehydrogenase (LDH)

According to figure 6, the correlation coefficient between zinc values and LDH, in pregnancies with abortion was $R^2 = 0.9431$, in pregnancies with anemia $R^2 = 0.8634$, whereas in cases with hyperemesis $R^2 = 0.8242$.



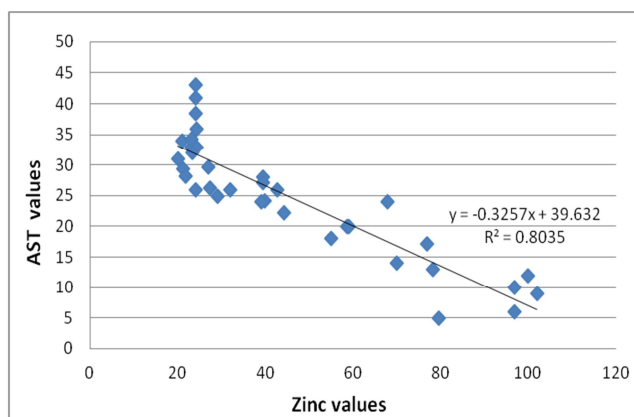


c)

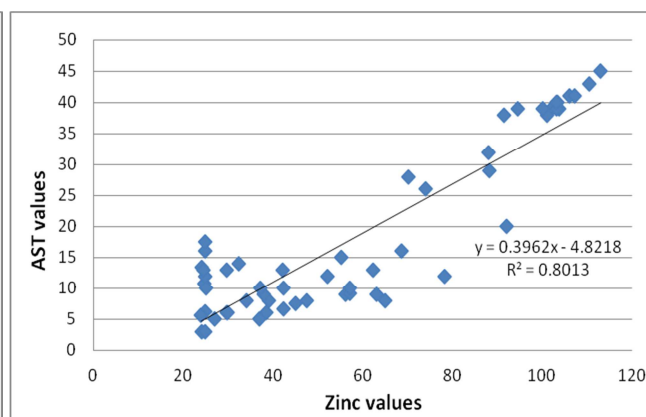
Figure 6. Correlation of zinc to LDH in different maternal diagnosis, a) abortion, b) anemia, c) hypertension.

5.2.6. Zinc in Correlation to Aspartate Aminotransferase (AST)

As we can see in figure 7, the correlation coefficient between zinc values and AST, in pregnancies with anaemia was $R^2 = 0.8035$, whereas in pregnancies with hypertension $R^2 = 0.8013$.



a)

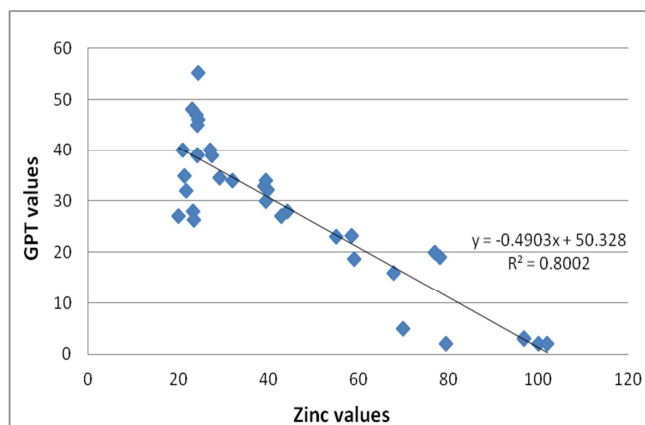


b)

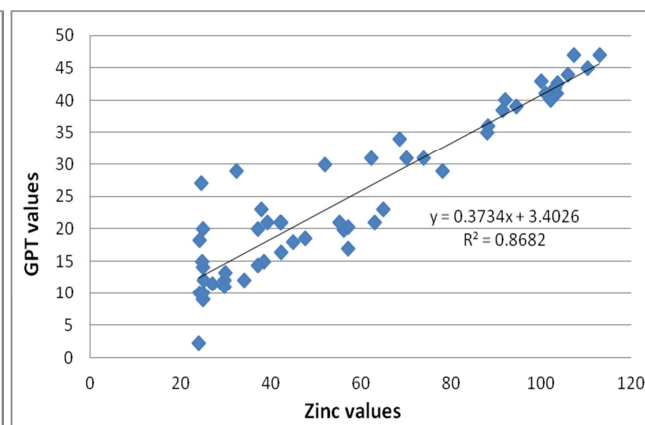
Figure 7. Correlation of zinc to AST in different maternal diagnosis, a) anemia, b) hypertension

5.2.7. Zinc in Correlation to Glutamic Piruvic Transaminase (GPT)

According to the figure 8, the correlation coefficient between zinc and GPT in pregnancies with anemia is $R^2 = 0.8002$, whereas in pregnancies with hypertension is $R^2 = 0.8682$.



a)



b)

Figure 8. Correlation of zinc to GPT in different maternal diagnosis, a) anaemia, b) hypertension.

5.2.8. Zinc in Correlation to Lipase

According to figure 9, the correlation coefficient between zinc and Lipase in pregnancies with *hyperemesis* is $R^2 = 0.9502$, whereas in *partus premature* pregnancies $R^2 = 0.8734$.

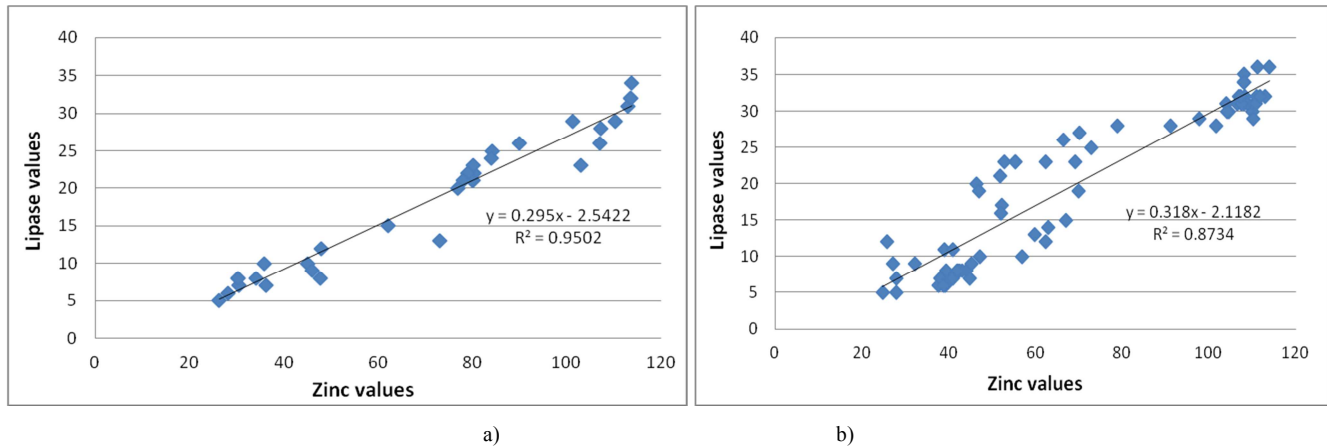


Figure 9. Correlation of zinc to Lipase in different maternal diagnosis, a) *hyperemesis* b) *partus premature*.

6. Conclusions

6.1. Conclusions about Zinc Measurements

Our study consisted of normal pregnant women, who served as control group and of zinc deficiency pregnant women. The maternal age was 17- 44 years old.

We analyzed 500 cases of pregnant women, from which:

- 190 pregnant women (38%) had normal zinc values (according to lab values 70 – 120 mcg/dl)
- 58 pregnant women (11.6%) had zinc levels between 60 – 69.9 mcg/dl, these patients were identified as pregnant women with zinc deficiency, as a consequence of oral contraceptive use.
- 252 pregnant women (50.4%) with zinc values < 60 mcg/dl, identified as patients with zinc deficiency, a result of which may be malnutrition and also urinary elimination of digestive liquids.
- 56 pregnant women (11.2%) with zinc values < 30 mcg/dl, were identified as patient with definite deficiency as a result of different diseases.

The prevalence of zinc deficiency in pregnant women was 62% (310 cases).

6.2. Conclusions about Enzyme Measurements

From the measurements done at the corresponding laboratory, it resulted:

- There was a strong positive linear correlation ($R^2 > 0.7$) between zinc and ALP in both diagnosis anemia and hypertension,
- There was strong negative linear correlation between zinc and Amylase in both diagnosis abortion and hypertension,
- There was a strong negative linear correlation between zinc and GGT in both diagnosis anemia and hypertension,
- There was a strong positive linear correlation between

zinc and CK in both in both diagnosis anemia and hypertension, whereas a strong negative linear correlation in pregnancies with abortion,

- There was a strong negative linear correlation between zinc and LDH in both diagnosis abortion and anemia, whereas a strong negative linear correlation in pregnancies with hyperemesis,
- There was a strong negative linear correlation between zinc and AST in pregnancies with anemia, whereas a strong positive linear correlation in pregnancies with hypertension,
- There was a strong negative linear correlation between zinc and GGT in pregnancies with anemia, whereas a strong positive linear correlation in pregnancies with hypertension,
- There was a strong positive linear correlation between zinc and Lipase in both diagnosis hypertension and *partus premature*.

Recommendations

- An average adult woman should consume about 7 milligrams of zinc daily, while an average male should be consuming 9.5 milligrams daily. The risk for women to have a zinc deficiency is much greater than a man, especially if they are malnourished because of an eating disorder or when they are breastfeeding.
- If you are concerned about your zinc intake, taking a good multivitamin should be sufficient. Be sure to only take the recommended daily dose, as zinc overdoses can also occur, which can be toxic to the body. Consuming too much zinc can cause nausea, vomiting and fever because too much of the mineral can interfere with how the body processes other minerals.
- Without a proper nutritional requirement the person falls in the state of zinc deficiency
- Zinc prophylactic treatment is important before and

during pregnancy.

- Everyone needs zinc. Children need zinc to grow, adults need zinc for health. Growing infants, children and adolescents, pregnant women and lactating mothers, athletes, vegetarians and the elderly often require more zinc.

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