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Correlation of Serum Alanine Aminotransferase, Aspartate Aminotransferase and Total Protein with Copper in Anemic Women

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Summary

Anemia remains a major public health problem, affecting one third of all adults and almost two billion people worldwide. Main aim of this study is to determine serum copper, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and total protein and explore the possible association that might present between them in Anemic Women.

Fifty subjects were included in this study, they were divided into two groups; controls group which involved 20 females, and patients group involved 30 females, the sample collection has been attended the students of the college of applied science and some private laboratories in Al-Fallujah city, for the sampling period was from January 2022 to April 2022 and the ages of all subjects was ranged from 20-45 year. Serum levels of Copper and liver enzymes mentioned in the study were estimated by color enzymatic methods .

The results showed lower serum levels of Copper (µg/dL) with significant differences in anemic women ($P < 0.0001$) than in control group. The serum levels of ALT (U/L) was significantly higher in controls ($P < 0.0001$) in comparison with patients, also the serum levels of, AST was significantly higher in controls than patients ($P < 0.001$), while the serum levels of total protein decreased slightly in the patients group in comparison to the controls group ($P < 0.001$).

The current study showed that the correlation for Copper with Age weak positive ($r = 0.383$), ($p=0.037$), and negative correlation was notice with total protein ($r = -0.505$) ($p=0.004$) .In addition to no correlation was observed between Copper with ALT and AST.

1.Introduction

1.1 Anemia

Anemia remains a major public health problem, affecting one third of all adults and almost two billion people worldwide. (1) Anemia in women of reproductive age (WRA) (age range: 15–49 y) remains a public health problem globally. (2) Anemia is a global public health concern, provoking severe health problems and lower quality of life, the main contributory factor of anemia is the nutritional causes such as trace elements deficiency. (3)

The low hemoglobin (Hb) concentration in blood that defines anemia occurs long after tissue iron stores have been depleted to levels associated with suboptimal function, a major cause of anemia, this iron deficiency leads to diminished oxygen-carrying capacity in red blood cells, which in turn diminishes energy efficiency, work capacity and productivity. (4) According to the World Health Organization (WHO), anemia is Hb levels less than 13 g/dL in men over 15 years of age, less than 12 g/dL in women over 15 years of age, and less than

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11 g/dL in pregnant women, although the data on the prevalence of anemia vary, it was found to be 24.8% worldwide in a study, and the majority of this was iron deficiency anemia. [5]
In general, there are three major types of anemia, classified according to the size of the red blood cells. [6]

- If the red blood cells are smaller than normal, this is called microcytic anemia. The major causes of this type are iron deficiency (low level iron) anemia and thalassemia (inherited disorders of Hb).
- If the red blood cells size are normal in size (but low in number), this is called normocytic anemia, such as anemia that accompanies chronic disease or anemia related to kidney disease.
- If red blood cells are larger than normal, then it is called macrocytic anemia. Major causes of this type are pernicious anemia and anemia related to alcoholism. [7]

1.1.2 Anemia Causes

Many medical conditions cause anemia, common causes of anemia include the following. [8]

- Anemia from active bleeding: Loss of blood through heavy menstrual bleeding or wounds can cause anemia. Gastrointestinal ulcers or cancers such as cancer of the colon may slowly ooze blood and can also cause anemia.
- Iron deficiency anemia: is characterized by decreased hemoglobin synthesis leading to hypochromic and microcytic RBC production. Causes of IDA include reduced iron intake or absorption, increased iron demand during adolescence and pregnancy, bariatric surgery, heavy blood loss during menstruation, chronic gastrointestinal blood loss, polyps, or carcinoma. (9)
- Anemia of chronic disease: Any long-term medical condition can lead to anemia. The exact mechanism of this process in unknown, but any long-standing and ongoing medical condition such as a chronic infection or a cancer may cause this type of anemia.
- Anemia related to kidney disease: The kidneys release a hormone called the erythropoietin that helps the bone marrow make red blood cells. In people with chronic (long-standing) kidney disease (CKD) or end stage renal disease (ESRD), the production of this hormone is diminished, and this, in turn, diminishes the production of red blood cells, causing anemia. This is called anemia related to or anemia of chronic kidney disease. [10]

1.2 Liver Enzyme Function

Enzymes are proteins found in your body that speed up certain chemical reactions. Liver enzymes perform these jobs within the liver. Two of the common ones are known as alanine aminotransferase (ALT) and aspartate aminotransferase (AST). [11] Serum levels of liver enzymes, such as ALT, AST are highly sensitive to liver dysfunction and damage, because assays for these liver enzymes are cost-effective, they are widely used during general health check-ups worldwide. [12]

1.2.1 Alanine Aminotransferase

Alanine aminotransferase, (EC: 2.6.1.2). [13] It is the most commonly used enzyme for detecting hepatocellular damage, it is found in many tissues, but it is most active in the liver. [14] ALT concentrations are found to be lower than AST concentrations in all cells except

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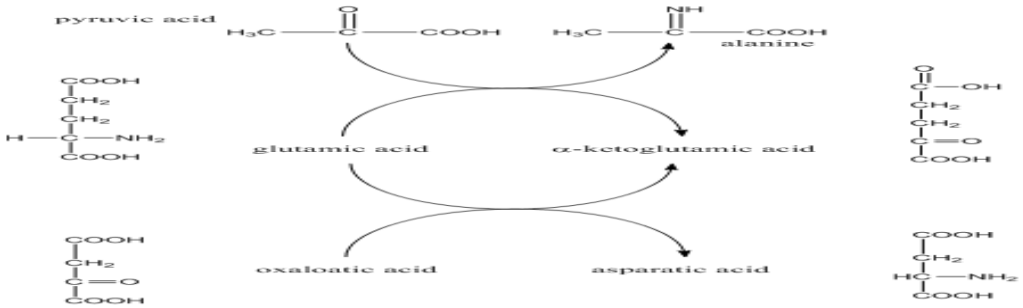
hepatic cells; thus, its elevation is particularly related to liver disease. [15] ALT facilitates the formation of glutamate and pyruvate in the hepatocyte which is important for energy production. [16] Elevations in ALT are traditionally linked to hepatocellular injury; however, there have also been reports of severe muscle damage linked to an increase in ALT activit. [17]

1.2.2 Aspartate Aminotransferase

Aspartate aminotransferase (EC 2.6.1.1). [13] AST is less specific for liver damage. [15] AST is found throughout tissues, with high concentrations in the heart, liver, kidney, and skeletal muscle as a result, it should not be used as a sole indicator of liver damage unless other supporting enzymes are measured, elevations in AST may be less pronounced in most species than increases in ALT with minimal to mild liver injury. [14] When it comes to AST, caution must be practiced when evaluating abnormal levels due to its presence in other tissues . [18]Increased AST activities are commonly associated with effects on the liver and skeletal muscle, but they can also be seen in hemolytic conditions. [17]

1.2.3 Reaction Catalyzed by The Aminotransferase

Both ALT and AST catalyze the transfer of an amino group from an amino acid to α-ketoglutarate. The amino acids are L-alanine and
L-aspartate and the reaction products are L-glutamate and either pyruvate or oxaloacetate, respectively (Figure 1-1A)The overall effect is exchange of an amino group and a keto group. Pyridoxal 5'-phosphate(PLP) vitamin B6 derivative serves as a coenzyme. [19]



The most important role of ALT is in the alanine-glucos (Figure1-1B) in muscle ALT converts pyruvate to the amino acid alanine using an amino group from glutamate.Alanine enters circulation and is taken up by the liver, where ALT in hepatocytes can convert it back to pyruvate which can be used to make glucose, this system is especially important for glucose regulation during stressful conditions such as fasting or vigorous exercise. It has also been suggested that the mitochondrial isoform of ALT is particularly important in gluconeogenesis in some cases. [20]
The most important physiological function of AST may be maintenance of the NAD+/NADH ratio within cells. AST is a critical partner in the malate-aspartate shuttle, which oxidizes NADH in the cytosol and reduces NAD+ in the mitochondrial matrix to facilitate glycolysis and electron transport, respectively (Figure1-1C). [19]

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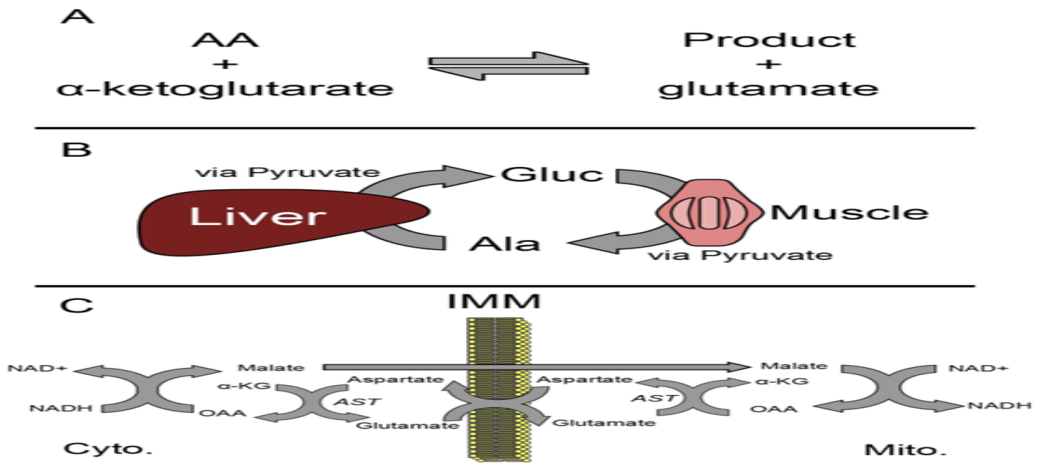

Fig. (1-1) Reaction Catalyzed by The Aminotransferase. [19]

1.3 Biochemical Markers Related to Liver Diseases

Routine liver biochemical tests includes tests of hepatic function (e.g., serum albumin, serum bilirubin), abnormal liver biochemical test are often the first clues to liver disease. [21]

1.3.1 Total protein

Total protein is one of the elements used to assess the system's protein balance. Its concentration in serum provides general information on the nutritional status of the patient, about 50–60% of total protein is albumin, the remaining percentage is globulin, fibrinogen, lipoprotein, and glycoprotein. [22] The estimation of total proteins in the body is useful in distinguishing between normal and damaged liver function because the important role of liver produces the majority of proteins such as albumin and globulin. [23] Total protein represents the sum of albumin and globulin, it may be elevations duo to chronic infection, collagen vascular disease, liver dysfunction wile it could be decreased due to malnutrition, liver diseases etc. [24]

A- Albumin

Albumin is the most abundant circulating protein found in plasma. It represents half of the total protein content(3.5 g/dL to 5 g/dL)of plasma in healthy human patients. Albumin is synthesized by liver hepatocytes and rapidly excreted into the bloodstream at the rate of about(10-15) gm per day .[25]

Very little albumin is stored in the liver, and most of it gets rapidly excreted into the bloodstream. In humans, serum albumin functions as a significant modulator of plasma oncotic pressure and transporter of endogenous and exogenous i.e. drugs ligands. In clinical medicine, serum albumin can be measured via standard serum laboratory testing, and this measure has been advocated as a highly sensitive marker for an individual patient's nutritional status. [26]

Globulins

Globulins are a family of globular proteins that have higher molecular weights than albumins and are insoluble in pure water but dissolve in dilute salt solutions. Some globulins are produced in the liver, while others are made by the immune system. The normal concentration of globulins in human blood is about(g/dL 4.6-2.6).[27]

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Globulins play an important role in liver function, blood clotting, and fighting infection, Globulin is one type of protective antibody produced by immune system that helps identify and fight infections, increased globulin levels may be due to chronic inflammation, kidney infection, stress, liver disease and parasite infestation, while decreased globulin levels may be due to anemia, depressed immune systems.[28]

B-1 Type of globulin

All globulins fall into one of the following four categories: [29]Alpha 1 globulins

- Alpha 2 globulins
- Beta globulins
- Gamma globulins (one group of gamma globulins is the immunoglobulins, which are also "known as "antibodies"

Shown the type in (Figure 1-2). [27]

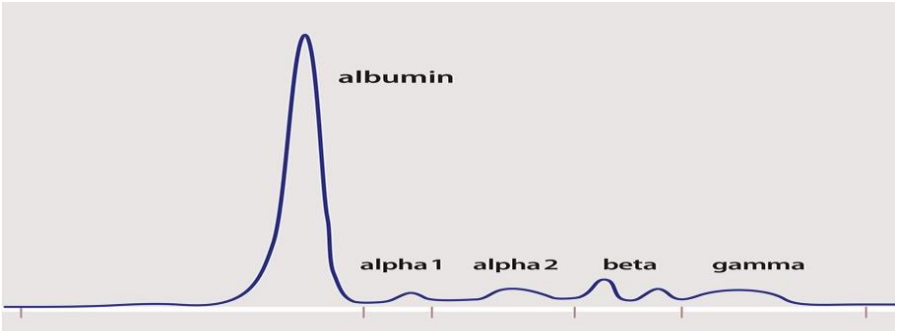

Fig. (1-2)Schematic representation of a protein electrophoresis gel. [27]

1.4 Clinical Significance of Copper in Anemia

Copper a trace element, is heavily involved in cell oxidation and signaling systems. [30] Trace elements play an important role as essential components or cofactors of enzymes throughout hemopoiesis. Most of the trace elements are critically involved during hemopoiesis via the metabolically important enzymatic pathway .[31] In addition, when the trace elements enter into the body, they may bind to red blood cells to be transported to target organs.Thus, trace elements not only can alter the synthesis of red blood cells but also can influence the distribution and the storage of blood cells in the target organs, thereby altering the status of blood parameters in the body, in case of Cu acts as an essential component of the functioning enzymes such as ferroxidase, hephestin, and ceruloplasmin; it is related to the etiology of anemia due to defect in iron immobilization. [32]

Copper deficiency reduces hemoglobin synthesis and leads to anemia. [3] Copper is a crucial micronutrient needed by animals and humans for proper organ function and metabolic processes such as hemoglobin synthesis, as a neurotransmitter, for iron oxidation, cellular respiration, and antioxidant defense peptide amidation, and in the formation of pigments and connective tissue. [33]

Trace elements, such as copper and zinc, are found in the structures of enzymes that act on iron metabolism (e.g., copper and ceruloplasmin). [34] Copper may also contribute to anemia development through reductions in erythropoietin (EPO) and antioxidant enzymes that require copper, thus increasing oxidative stress and reducing red blood cell (RBC) life span. [35]

A hereditary or acquired copper unbalance, including deficiency, overload, or misdistribution, may cause or aggravate certain diseases such as Menkes disease, Wilson

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disease, neurodegenerative diseases, anemia, metabolic syndrome, cardiovascular diseases, and cancer. A full understanding of copper metabolism and its roles in diseases underlies the identification of novel effective therapies for such diseases. [36] More than 95% of the copper found in plasma is present in a serum ferroxidase called as Ceruloplasmin.This sialo glycosylated oxidase protein contains 07 atoms of copper. Ceruloplasmin activates mobilizable stores of iron present in the specific cells and bring about the release of iron. [37]

Etiology of anemia in copper deficiency is complex and multifactorial. Ceruloplasmin, a major copper carrying protein in the blood, oxidizes ferrous iron to ferric form which allows iron to be transported in the circulation and bind to transferrin. Another important element in copper–iron interaction is Hephaestine, which is a copper-dependent ferroxidase. This is a transport protein involved in iron absorption from enterocytes which can explain the development of microcytic hypochromic anemia in some patients. [30] Mechanism of copper deficiency anemia shown in figure (1-3). [33]

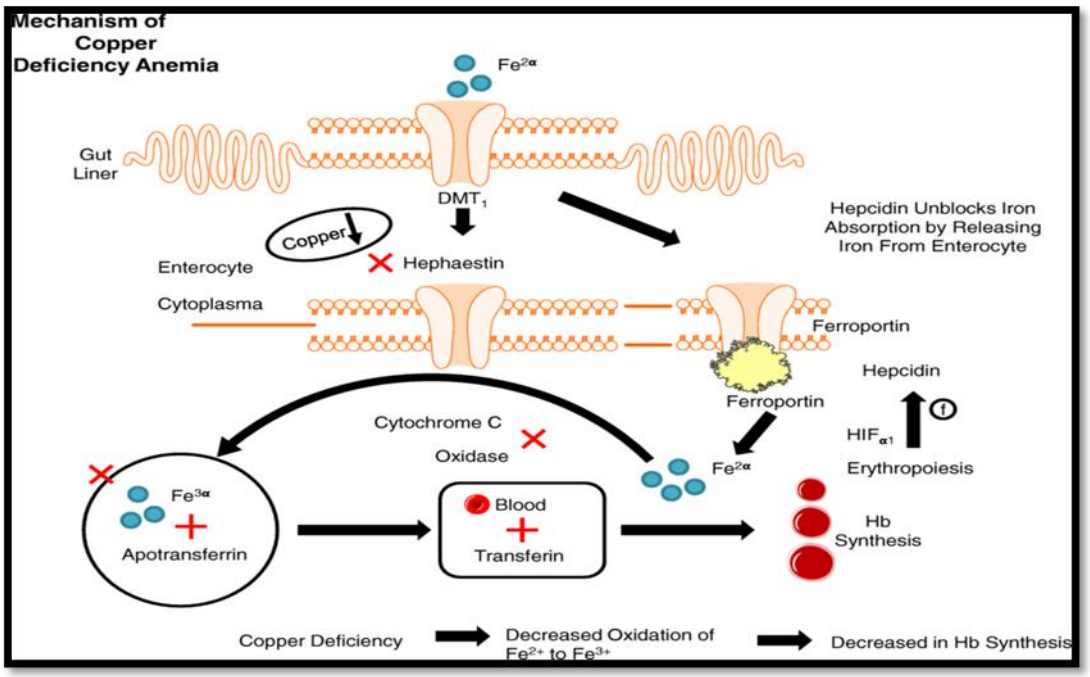

Fig (1-3) Mechanism of copper deficiency anemia. [33]

1-5 Aims of the Study

The main aim of this study was to evaluate serum levels of copper in anemia woman and explore the correlation and strength of associations between the variables with others studied parameters ALT, AST, Total protein .

2. Materials and Methods

2.1 Subjects

Fifty subjects females in the study. The age range was within (20 to 40 years), the study was carried out in those attending the Fallujah teaching hospital and some private laboratories between October to December 2021.Questionnaire sheet was completed for each subjects (**Appendix I**).

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2.2 Materials

2.2.1. Instruments that are used:

The sources of devise in this study are shown in table (2-1).

N	The Device Name	Company	Origin
1	Spectrophotometry	Emclab	Germany
2	Spectrophotometry	Mindray BA-88A	Chine
3	Centrifuge	Hettich Universal	Germany
4	Water bath	Memmert	Germany
5	Deep freeze	Beko	Turkey
6	Normal refrigerator	LG	South Korea
7	Timer with alarm	junghans	Germany

The tools that are were used in the studay are shown in table (2-2*)*

N	Tools	Company	Origin
1	Pipette tips	Gilson	France
2	Eppendroff tubes	Eppendroff	Germany
3	Micropipette	Gilson	France
4	Plastic disposable syrings 3ml	Meheco	China
5	Blood collection gel tubes	Afmh	England

2.1.2 Materials

The material that were used in this study are shown in Table (3-2)

N	Materials	Company	Origin
1	ALT Kit	Agappe	Switzerland
2	AST Kit	Agappe	Switzerland
3	TP Kit	Bio system	
4	Copper	Rame	Italy

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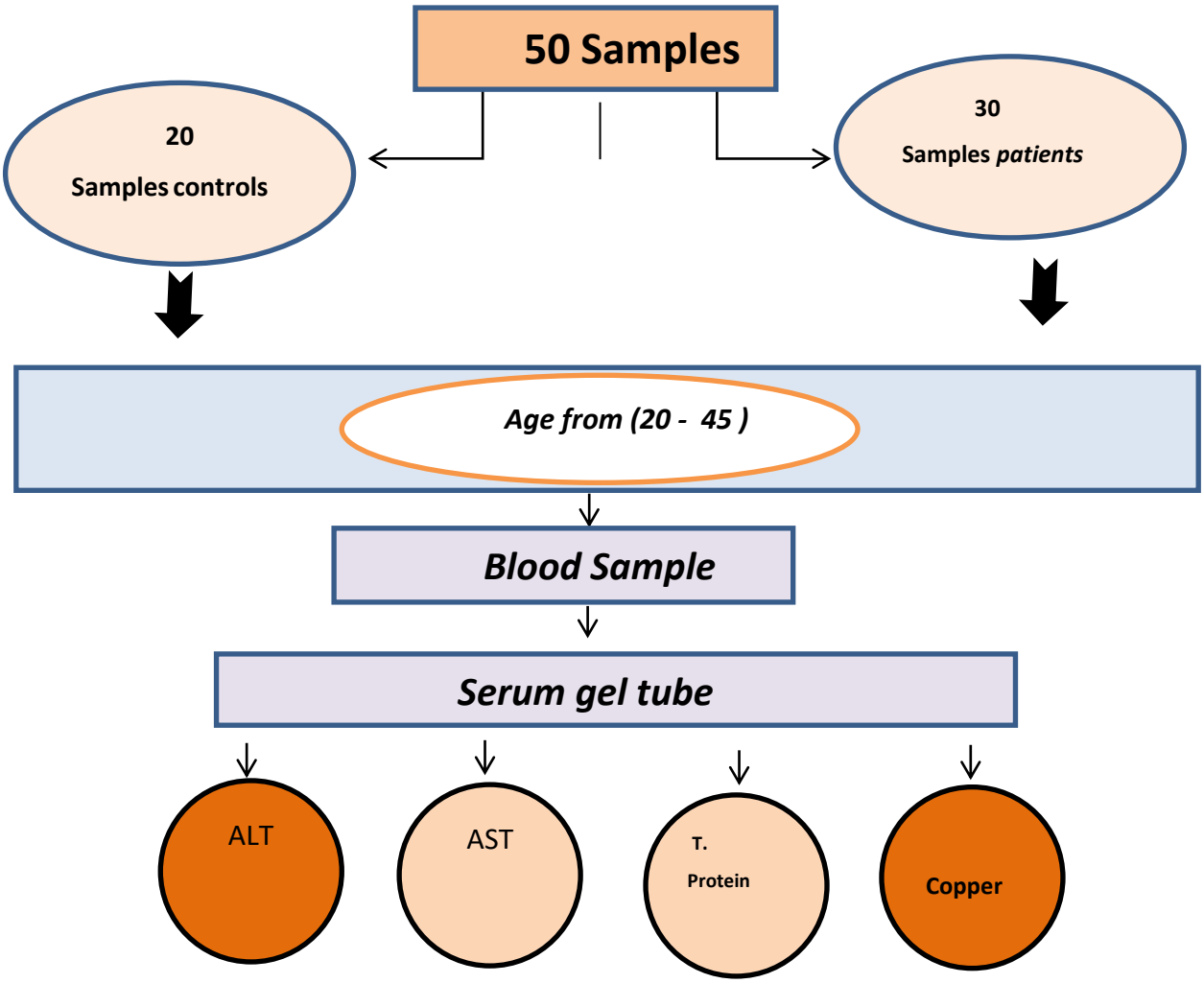
Diagram of the project



2.3 Blood Sampling

About 3 mL of blood were taken from all subjects participate in this study by using disposable syringe, blood was left for 15 minutes for clotting at room temperature , the blood was centrifuged at 4000 xg for 15 minutes. The serum was divided into two parts, the first one was used for estimating liver function test and the second part was stored in two eppendorff tubes (250µL) and froze at (-20 °C) until use to estimate Copper.

2.4 Laboratory Methods

2.4.1 Determination Serum Activity of Alanine Amino transferase.

Principle

Alanine aminotransferase stimulates the reverse transfer of an amino group of alanine to α-ketoglutarate forming pyruvate and glutamate and the pyruvate produced is reduced to lactate by lactate dehydrogenase(LDH) and NADH.

Kinetic determination of ALT is according to the following reaction

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L-Alanine + alpha –ketoglutarate $\xrightarrow{ALT}$ Pyruvate +L-Glutamate

Pyruvate + NADH + H^+ $\xrightarrow{LDH}$ L-Lactate + NAD^+

- **Kit Components**

Reagents Types	Materials	Concentration
SALT (S.L) R1	Tris buffer (PH 7.5) L-Alanine LDH	110 mmol /L 600 mmol/L > 1500 U/L
SALT (S.L) R2	Alpha- Ketoglutarate NADH	16 mmol /L 0.24 mmol /L

Assay Procedure

1- Reagents and samples were left at temperature between 20-25 ˚C

2-Reagent 2(R2) was added to Reagent 1(R1) and mixed gently to prepare working reagent

3-The spectrophotometer was set to zero with distilled water (D.W)

4-This tube was prepared (4 volumes was mixed for R1 with 1 volume of R2 + sample)

Table (2 - 4): Procedure for ALT Determination

Laboratory Procedure for Semi Auto Analyzer	
Working reagent	1000 µL (800 µL R1+200 µL R2)
Sample	100 µL

5- The tube was incubated for 1 minute in a water bath at 37 ˚C.

6- The absorbance was read at 340 nm during 3 minute.

7- The serum activity of ALT was calculated according to equation:

ALT activity (U/L) = (ΔOD/min) 1745

- 1745 proved factor for estimation of this assay on semi auto

Normal value for ALT	
ALT	Serum up to 49 U/L

2.4.2 Determination Serum Activity of Aspartate Amino transferase

Principle

Aspartate aminotransferase catalyzes the reversible transfer of an amino group from aspartate to α-ketoglutarate forming oxaloacetate and glutamate. The oxaloacetate produced is reduced to malate by NADH and malate dehydrogenase (MDH).

Kinetic determination of AST is based upon the following reaction

L-Aspartate +alpha –ketoglutarate $\xrightarrow{AST}$Oxaloacetate+L-Glutamate

Oxaloacetate +NADH+H^+ $\xrightarrow{MDH}$L-Malate +NAD

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- **Kit Components**

Reagents Types	Materials	Concentration
AST (S.L)R1	Tris buffer (PH 7.8) L-Aspartate LDH MDH	88 mmol /L 260 mmol /L >1500 U/L >900 U/L
AST (S.L)R1	Alpha- Ketoglutarate NADH	12 mmoL/L -0.24 mmol/L

- **Assay Procedure**

1- Reagents and samples were left at temperature between 20-25 ˚C.
2- R2 was added to R1 and mixed gently to prepare working reagent.
3- The spectrophotometer was set to zero with D.W.
4-This tube was prepared (4 volumes was mixed for R1 with 1 volume of R2 + sample).

Table (2-5): Procedure for AST Determination

Laboratory Procedure for Semi Auto Analyzer	
Working reagent	1000 µL (800 µL R1+200 µL R2)
Sample	100 µL

5- The tube was incubated for 1 minute in a water bath at 37 ˚C.
6- The absorbance was read at 340 nm during 3 minute.
7- The serum activity of AST was calculated according to equation:
AST activity (U/L) = (ΔOD/min) × 1745
• 1745 proved factor for estimation of this assay on semi auto analyzer.

Normal value for AST	
AST	Serum up to 46 U/L

2.4.4 Determination Level of Total Protein

Principle

Total protein colorimetric determination is based on the Biuret reaction principle (copper salt in an alkaline medium). When protein in plasma or serum samples is treated with cupric ions in alkaline solution, it forms a blue complex. The intensity of the blue color is directly proportional to the protein concentration**.**

- **Kit Components**

Reagents Types	Materials	Concentration
Total Protein Reagent	Potassium Iodide Copper (11) acetate Sodium Hydroxide	12 mmol /L 8 mmol /L 1.15 mol /L
Total Protein Standard	Total Protein Standard	6 g /dL

- **Assay Procedure**

1- Reagents and samples were left at temperature between 20-25 ˚C.

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2- Standard of total protein was added to total protein reagent and mixed gently to prepare working reagent.

3- The spectrophotometer was set to zero with blank..

4- Three tubes were prepared as shown in table (2-7)

Table (2-7): Procedure for Total Protein Determination

Laboratory Procedure			
	Blank	**Standard**	**Sample**
Reagent	1000 µL	1000 µL	1000 µL
Standard	----------	20 µL	---------
Sample	----------	----------	20 µL

5- The tube was incubated for 10 minutes in a water bath at37 ˚C.

6- The absorbance was read at 545 nm.

7- Serum level of total protein was calculated according to equation:

$$\text{TSP Concentration (g/L)} = \frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}} \times 69.6$$

- 69.6 g /L Standard Concentration.

Normal value:	
Total protein	(64–83) g /L

2.4.5 Determination of copper in serum

Principle

The chromogen 3,5-Di-Br-PAESA react with cupric ions and forming a blue –violet compound , which intensity is prpportional to the copper concentration in the sample

- **it Components**

Reagents Types	Materials	Concentration
Reagent A	Acetate buffer	0.1M PH 4.9
Reagent B	3.5-Di-Br-PAESA	
Standard	Ion copper	200 µg/dl

1- Reagents and samples were left at temperature between 20-25 ˚C

2-Equal amounts of reagent (R2) was added to Reagent (R1) and mixed gently to prepare working reagent

3-The spectrophotometer was set to zero with distilled water (D.W)

4- Three tubes were prepared as shown in table (2-8)

Table (2-8): Procedure for Copper Determination

Laboratory Procedure			
reagent	**Blank**	**Standard**	**Sample**
Work Reagent	1ml	1ml	1ml
Standard	----------	66 µL	---------

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Distilled water	66 µL	-------	------
Sample	--------	--------	66 µL

5- The absorbance was read at 580 nm during 3 minute.

6- The serum level of Copper was calculated according to equation:

Copper µg/dl = $\frac{A(sample)}{A(standard0}$x200

Normal value:	
Copper	85-180µg /dL

2.5 Statistical Analysis

• A statistical analysis of data was carried out using SPSS version 25.

• The statistical significance level was set to less than 0.05.

• Descriptive statistics consist of mean, standard deviation (SD), and standard error of mean (SEM) was calculated separately for each parameter

• The associations between ALT, AST, Total protein with characteristics of Copper was studied courtesy of Pearson's correlation (r = −1 to 1).

3. Results and Discussion

3.1 Anthropometric Analysis

This study shows a statistical analysis of 50 subjects, which included two groups consist of 30 anemic women (patients) and women healthy (controls) , their ages ranged from 20 to 45 years and the sample in our study were selected from non –pregnant women. The obtained results of this study were presented in **appendix II.**

3.1.1 Age

There was significant statistical difference in mean age (years) between controls and patient (P<0.0001). The results showed that the mean age of patients (28.2333±7.894499) was lower than control

(30.35±7.942), as shown in table **(3-1)** and **figure (1) in appendix III**

Table (3-1): Mean ± S.D of age in anemia Patients and Controls

Parameter	**controls**	**anemia patients**	**p-value**
	Mean ± S.D	**Mean ± S.D**	
Age (Years)	30.35±7.942	28.2333±7.894499	<0.0001

Anemia has been considered as a leading public health problem mainly among women of reproductive age group.[38] This problem can be attributed to multiple factors such as consuming inappropriate diet, certain socio-demographic factors, susceptibility to develop some infections and hemoglobinopathies anemia during the reproductive cycle. [39] there are several factors including age affects hemoglobin concentration. [40] Heavy menstrual bleeding (menorrhagia) and pregnancy and delivery can cause significant iron loss leading to severe anemia. [41]

3.2 Biochemical Investigations (Liver function test)

The statistical analysis in **table (3-2)** shows the examined of liver function, data of this study detect high significant statistical difference in liver enzymes , the serum level of ALT

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(U/L) in control was higher than controls with mean for patients (19.5517 ± 5.49065) and controls (21.8440 ± 7.64502) with (P <0.0001) for all variables. The serum levels of AST (U/L), controls were higher than patients with mean for patients (23.9423 ± 6.32165) and controls (27.7310 ± 5.94088), with (P < 0.0001). The serum levels of Total protein in controls were lower than patients with mean for patients (76.6117 ± 8.03407) and control (72.2085 ± 10.4528), as showed in the table **(3-2)** with **figure (2,3,4) in appendix III.**

Table (3-2): Mean ± S.D of Biochemical Investigations in patients and Controls

Parameter	controls	patients	p-value
	Mean ± S.D	Mean ± S.D	
ALT (U/L)	21.8440 ± 7.64502	19.5517 ± 5.49065	<0.0001
AST (U/L)	27.7310 ± 5.94088	23.9423 ± 6.32165	<0.0001
Total protein (g/dL)	72.2085 ± 10.4528	76.6117 ± 8.03407	<0.0001

The liver performs a major role in iron homeostasis. It is the main organ for the production of the iron regulatory hormone hepcidin, expressed in iron excess conditions as well as in cases of inflammation, blocking the absorption of iron from the enterocytes. The role of hepcidin in liver diseases, with or without cirrhosis, is still under investigation, but is probably one of the contributing factors to the anemia of chronic disease present in a variety of liver conditions.[42]An understanding of the proportion of anemia that is attributable to iron deficiency is particularly relevant to the design and implementation of public health programs such as the fortification of staple foods with iron and the promotion and distribution of iron supplements. The fortification of cereal grains with iron is currently mandatory in 85 countries . [38]

The incidence of anemia is also found in other studies, this is supported by research from Agustina, 2020 which states that the diet of adolescent girls who attend school as a whole in the study area has poor quality and low diversity, and several dietary variables, food quality and dietary diversity was associated with anemia and overweight. [43] Factors related to the incidence of anemia among young women are breakfast habits, nutritional status, protein intake, dietary patterns of iron absorption inhibitors and length of menstruation. Lack of consumption of animal foods, dietary habits to reduce body weight, and poverty that causes insufficient consumption of nutritional foods, which can lead to anemia. [44]

The results of this study are also supported according to research from Hesty, 2018 which states that anemia in adolescent girls is deficient in protein, iron and other micronutrient deficiencies, exacerbated by a lack of knowledge related to nutrition that can affect behavior.[45]. Balci, et al. 2012 reported that 59% of the incidence of anemia was caused by iron deficiency and 41% was a combination of iron and vitamin B12 deficiency. [46] The data states that in urban areas, about 60% of adolescent girls and around 76% in rural areas lack protein intake. Statistics The results of the analysis show a correlation between protein intake and the incidence of anemia in adolescent girls. Lack of animal protein intake and combined with single consumption of staple foods results in lower iron intake. [47]

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3.3 Serum Level of Copper

The statistical analysis in **table (3-3)** shows the examined of copper, data of this study was significant statistical difference in mean the serum level of copper **(µg/dL)**in controls was higher than patients with mean for patients (77.3263 ± 32.41531) and controls (162.0365± 27.6094) with (P <0.0001) as showed in the table **(3-3)** with **figure (5) in appendix III.**

Table (3-3): Mean ± S.D of Copper in patients and Controls

Parameter	controls	patients	p-value
	Mean ± S.D	Mean ± S.D	
Copper (µg/dL)	162.0365± 27.6094	77.3263 ± 32.41531	<0.0001

Copper binds to albumin, glutathione, and amino acids in the portal blood after being released from intestinal cells into the serosal capillaries. In the liver, copper is either incorporated into copper dependent proteins or is excreted into the bile10. Liver regulates .copper release and maintains homeostatic control on the extrahepatic distribution of copper . (37)

Fortification and supplementation interventions often have the explicit aim to reduce anemia, but data on the etiology of anemia are lacking for many countries. Moreover, the use of anemia prevalence as a criterion for implementing iron interventions may not be the most effective approach particularly where the infection burden is high. (38)

Increase in the levels of copper causes oxidative damage and alterations in the function of cell membranes, peroxidation of lipids,inhibition of receptors, abnormalities in liver functions,decline in fluidity, changes in the enzymes function and ionic permeation.The dietary allowance of copper varies according to age, pregnancy, lactation, and for adults. (37)

3.5 Pearson Correlation Analysis

3.5.1 Correlation of Copper with Studied Parameters.

The correlations between Copper and variables were investigated in this study. The results are presented in a **table (3-4)** and **figure (3-1)** as showed a weak positive correlation of Copper with Age levels (r= 0.383, P =0.037), as shown in **figure (3-1 A)**, well observed with negative correlation of Copper with serum levels of Total protein (r= -0.505, P =0.004) as shown in **table (3-4)** and **figure (3-1 B)**.

While no significant correlation was observed between Copper and ALT (r= -0.265, P= 0.156), AST (r= -0.032, P=0.866).

Table (3-4): The Correlation of Copper with the Studied Parameters

Copper (µg/dL)	r	p-value
Age (Years)	0.383	0.037
ALT (U/L)	-0.265	0.156
AST (U/L)	-0.032	0.866

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TSP (g/dL)	**-0.505**	**0.004**

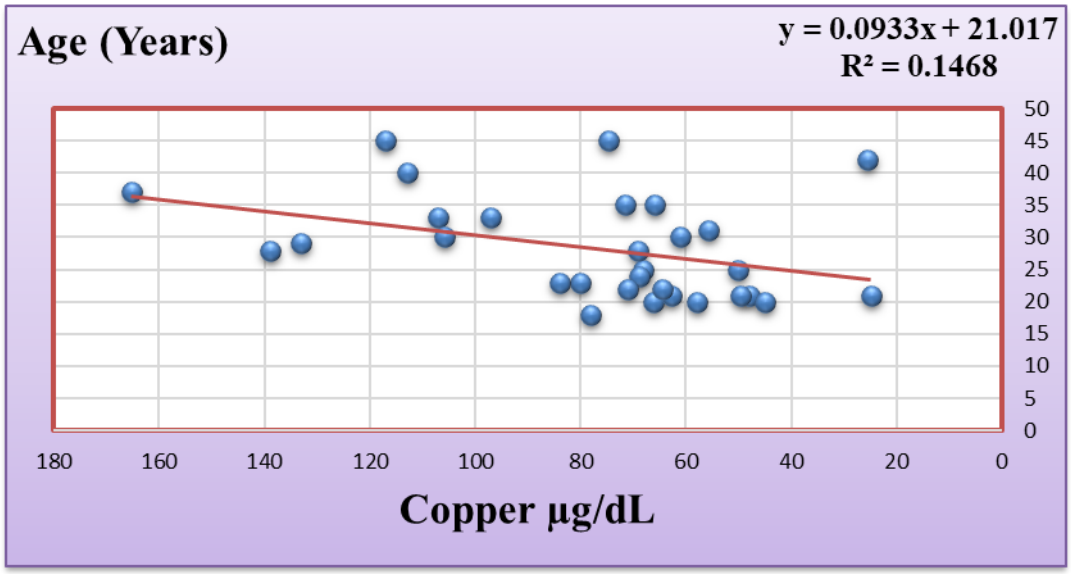

Fig. (3-1 A): Correlation of Copper with Age

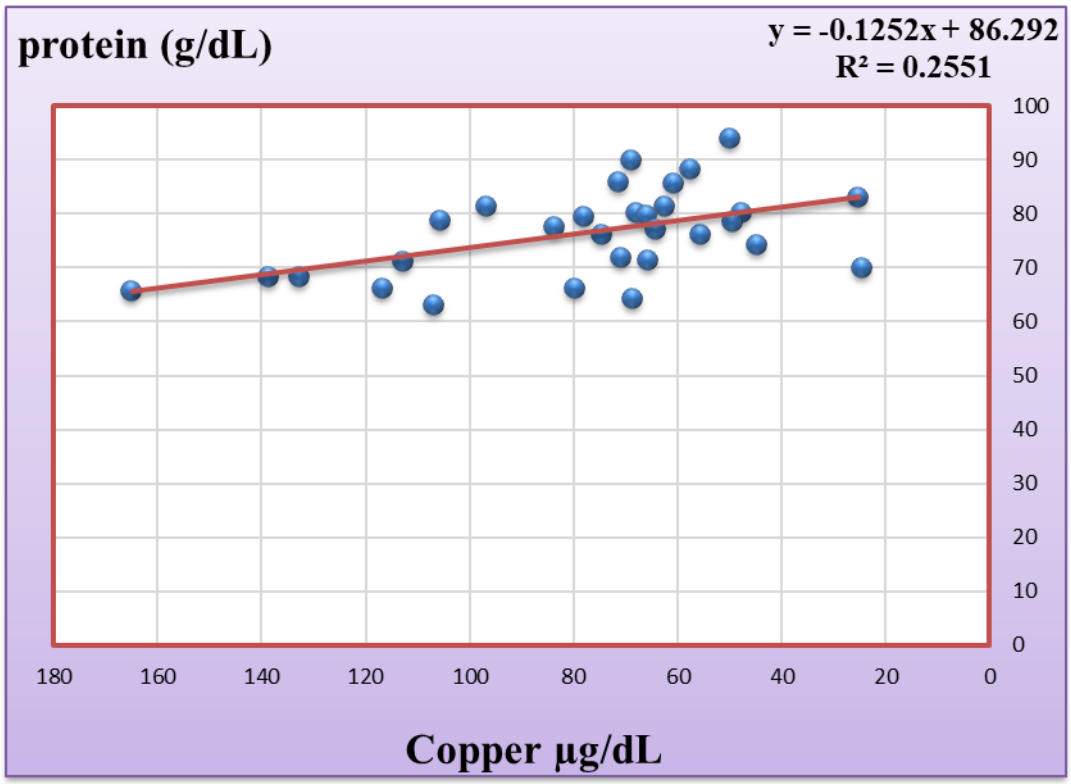

Figure (3-1 B): Correlation of Copper with total protein

Conclusions

Based on the current findings, this study concludes:

1. In our analysis,There was a weak correlation between serum levels of Copper with total protein.

2. Anemia-reduction programs for WRA can be improved by considering the underlying infection burden of the population and by assessing the overlap of micronutrient deficiencies and anemia**.**

Recommendations

This study recommends the following based on its current findings:

1- This study recommend examining anthropometric measurements which could be used as free predictive and/or diagnostic indicators in the future, especially if they are assessed and confirmed in papers with a larger sample size.

2- A small sample size of this case control study which may be not successful to explain the vital correlation between Copper levels and additional measured variables in anemic women, our data suggets studies with larger samples for additional ethnic groups that are required to test the biological roles of serum Coppe

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