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Estimation of the Ratio between Copper & Zinc in Pregnant Women Suffering from Iron Deficiency Anemia

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Copper & Zinc, Pregnant Women, Iron Deficiency Anemia.

Abstract

Anemia is one of the pathological conditions that have multiple and varied causes, including what is known as iron deficiency, especially in women and during pregnancy, where the condition is known as “iron deficiency anemia”. One of the objectives of this research is to investigate and study the clinical significance of iron and other elements known as “minor elements” in the blood such as copper, zinc, and the possible relationship of these elements to this type of anemia.

The results of this research showed an increase in the level of copper in the blood serum of pregnant women with iron deficiency anemia in all stages of pregnancy, especially in the third stage, compared to the corresponding stages in normal pregnancy, as well as an increase in the level of copper in the blood of healthy pregnant women compared to the healthy group of non-pregnant women. The study also showed a decrease in the level of zinc in the serum of the pregnant mother with iron deficiency anemia in all stages of pregnancy, especially in the third stage, where it was a significant decrease (probability <0.001) compared with the corresponding stages in normal pregnancy. A significant decrease in the level of zinc was also observed in the serum of healthy pregnant women (probability <0.001) compared with that of non-pregnant healthy women.

Low levels of both hemoglobin and the volume of pressurized blood cells were recorded, and this decrease increases with the aging of pregnancy in pregnant women with iron deficiency anemia, especially in the second stage, where it becomes a significant decrease (probability <0.001) compared to healthy non-pregnant women. This significant decrease was also recorded in healthy pregnant women (P<0.001) compared to non-pregnant healthy women.

The ratio of copper to zinc increased in its results in the healthy pregnant mother and in all stages of pregnancy compared to the non-pregnant woman.

This percentage also increased in the pregnant mother suffering from iron deficiency anemia in the three stages of pregnancy compared to the corresponding stages in the normal pregnancy. In the second stage of normal pregnancy, the results of the study showed a positive significant relationship between the level of hemoglobin and the level of zinc, probability (0.001), while there was a negative significant relationship with the level of copper (probability <0.001).

In general, the results of this research show that the level of copper and zinc and the ratio between them that has been studied is affected (increase or decrease) by

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the condition of iron deficiency anemia in pregnant women.
The results of measuring the levels of these elements in addition to ratio between the copper and zinc can be used as additional laboratory tests to assess the anemia caused by iron deficiency in women during the period of time Pregnancy. The ratio of both elements should be optimized in pregnancy, to improve reproduction results and reduce the chance of spontaneous abortion.

1. Introduction

The prevalence of anemia in pregnant women ranges from 17% to 31% in Europe and North America, 53–61% in Africa, and 44–53% in Southeast Asia (1). Milman et al. (2017),(2) found that the prevalence of iron deficiency (ID) and iron deficiency anemia (IDA) in pregnant women from Europe is 10–32% and 2–5%, respectively, and the lack of Fe supplementation during pregnancy increased the prevalence. Only 20–35% of women of childbearing age did not require additional Fe supplementation. Insufficient supply of Fe can cause disorders in oxygen transport and consequently lead to anemia (3).The concept of molecular disease, now an integral part of medicine (4). The capability of reliably quantitating trace element levels in biological Specimens have led to discoveries of the vital roles that trace elements play in human and animal metabolism. Basically, an essential element is one that is uniquely required for growth and for the maintenance of life or health (5). Macro elements and micro elements are essential for the proper functioning of living organisms. They participate in many processes, including cellular metabolism and antioxidant and anti-inflammatory defenses, and also influence enzyme activity, regulate gene expression, and take part in protein synthesis (6). A deficiency of the element produces a functional impairment, which is alleviated by physiological supplementation of only that element. A biochemical basis for the element's functions must be demonstrated. For trace metals this is often the identification of a unique metallo enzyme, which contains the metal as an integral part or as an enzyme activator.

At low nutrient levels, dependent biological functions are impaired, thus defining a deficiency state. Very high nutrient levels may result in another disorder consequences and toxicity states. Each nutrient has its own characteristic, since pathology due to deficiency is different from that due to toxicity [7]. During pregnancy, the diet should meet the needs of the baby, as well as that of the mother, whose health is closely related to the provision of adequate amounts of essential elements including Fe, Cu, and Zn. Their levels before pregnancy can also be of significance [8].The study by Caan *et al* 1987. [9], showed that the Special Supplemental Nutrition Program for Women, Infants, and Children (WIC) initiated 5–7 months before pregnancy resulted in an increase in birth weight by 131 g and length by 0.3 cm. Providing the right amount of essential elements with a diet or by supplementation can reduce the risk of fetal malformation and preterm birth [10], including multiple pregnancies—associated with a higher risk of perinatal complications [11]. These complications are likely to cause premature birth, miscarriage, hypotrophy of one or both fetuses, preeclampsia, fetal death, or fetal atrophy syndrome [12]. Figueiredo *et al* 2018. [13] Proved that maternal anaemia was associated with a higher risk of low birth weight. Pregnant women, especially those with multiple pregnancies, should monitor Fe levels from the beginning of the pregnancy. Low Fe concentration at the beginning of pregnancy significantly correlates with the occurrence of anemia in the last trimester of pregnancy [14]. Therefore, it is recommended to supplement pregnant women's daily diet with 27 mg of Fe a day,and with as much as60mg if they are diagnosed with anemia[15, 16].

Conditions associated with iron, zinc, and copper deficiencies may be of potential clinical importance for ultra-trace metal nutrition as well. Such conditions include:

1- Inborn errors of metabolism, which affect absorption, retention or excretion of the element.

2- Disruptions to element metabolism, as a secondary consequence to malnutrition, disease, injury, or stress.

3- The absence of an element in total parenteral or synthetic formula Solutions fed over long periods of time.

4- Marginal deficiencies induced by various dietary manipulations or by antagonistic interactions with other nutrients or drugs [17].

An overabundance of one trace element can interfere with the metabolic utilization of another element present in – normal or marginal concentrations[18, 19].Alternatively, the effect of a toxic trace element may be ameliorated by another "protective" trace element [7, 20]. The addition of large amounts of zinc to a diet interferes with the intestinal copper absorption system, resulting in copper deficiency in spite of otherwise adequate copper intake. Hence, zinc is said to antagonize copper absorption. Copper deficiency, in trace, is known to provoke iron deficiency and anemia [19]. During pregnancy, plasma volume increases by 40–60% and red cell mass by 18–25% [21, 22, 23]. Hemoglobin concentration decreases to 10.5–11 g/dL and the hematocrit value to 30–32% [22,24]. This process, known as hemodilution, usually intensifies between the 17th and 36th weeks of pregnancy [23]. Additionally, it may result in a slightly decreased vitamin B_{12} concentration (in about 10–28% of women), [25] and a slight decrease in platelet count [26]. It is also worth noting in that during this time (i.e., 17–36 weeks) the number of white blood cells increases by about 20% due to increased hormonal activity (e.g., glucocorticosteroids) and cytokine synthesis (an increase in granulocyte-macrophage colony-stimulating factor) [27].

Human food most often contains Fe^{3+}, reduced by ferrireductase to Fe^{2+} in the intestinal lumen [28]. Fe^{2+} absorption is based on the principle of active transport with a divalent metal transporter (DMT-1) in the apical membrane of the duodenum and the upper part of the small intestine [29, 30]. The divalent metal transporter 1 (DMT-1) is a non-selective transporter of bivalent metal ions, including iron, zinc, copper, manganese, cobalt, and cadmium, whose transport through the membrane occurs via proton-coupled divalent metal ion transporters [31,32]. Fe levels may also be influenced by the expression of intestinal copper transporting P-type ATPases copper-transporting ATPase α (ATP7A), which indirectly impairs Fe absorption by affecting the expression of Fe transporters [33].

2. Clinical Manifestations of Zinc Deficiency

Zinc is necessary for growth and development. Immunologic impairment, hypogonadism growth retardation and delayed wound healing are reported in zinc deficiency **[34, 35]**. Other manifestations of zinc deficiency are skin lesions, poor appetite, anorexia, iron deficiency anemia, abnormal leukocyte function, abnormal dark adaptation and hyperpigmentation **[36, 37].** General appearance of apathy; muscle or tissue wasting; excessive irritability; underweight, undersized, underdeveloped for age; paleness and loss of color of the skin, nail beds, lips, or hair. Hair that is dries, wiry, stiffs, brittle, loss

of color, or easily pulled out [38]. .Zinc depletion can cause tremors, cerebellar ataxia, mental lethargy, confusion, and atrophic changes in cranial and peripheral nerves; hyperlipidaemia and glucose intolerance [39].

3. Zinc Toxicity

Zinc is considered non-toxic particularly if taken orally. Most causes of acute zinc toxicity have been reported in response to food poisoning resulting from storage of food or drink in galvanized containers. Acute effect of zinc intoxication appears when the amount of zinc exceeds 15 mg/day in normal adult and it involves nausea, vomiting, epigastria pain, fever, fatigue, abdominal pain and diarrhea [40, 41]. Large quantities of zinc ingestion during a prolonged period of time can lead to copper deficiency with secondary anemia and neutropenia [42, 43, 44]. Obeck (1978) . [45], found a disease referred to as "white monkey" or "fading infant syndrome" caused by zinc toxicity, and characterized by gradual whitening of the hair (achromotrichia) accompanied by alopecia.

4. Copper

Copper is an essential micronutrient. It has been estimated that the amount of this element in an adult human body ranges from 50 to 120 mg [46]. The highest concentrations of Cu were found in the brain and the liver [47]. Cu is involved in the formation and metabolism of bone tissue and participation in oxidation–reduction reactions as a coenzyme, a regulator of Fe metabolism and transport, as well as collagen metabolism [16,48]. Cu participates in the metabolism of fatty acids, in RNA synthesis, supports the absorption of Fe in the gastrointestinal tract, and participates in the synthesis of myelin [49, 50]. Cu also participates in the synthesis of melanin and—as a component of tyrosinase—is involved in the conversion of tyrosine to melanin [51].

5. Hypercupraemia

The Hypercupraemia is a condition that is related to changes in serum caeruloplasmin [52]. The most striking effect of copper and caeruloplasmin in the plasma is the increases produced by oestrogens, which stimulate caeruloplasmin synthesis [53].Oral contraceptives, testosterone and progesterone administrations, exposure to copper containing fungicides and ingestion of contaminated solutions cause Hypercupraemia [54].

6. Copper toxicity

Copper toxicity is relatively uncommon. Acute copper intoxication has been described following ingestion of more than 15mg of elemental copper. Acute copper intoxication in man causes nausea, vomiting, epigastric pain, dizziness, headache, acute hepatic necrosis, and striking increase in serum caeruloplasmin concentration. In more severe case tachycardia, hypertension and coma may ensure followed by jaundice, haemolytic anaemia haemoglobinuria, tubular swelling, glomerular congestion, uraemia and death [55].

7. Copper Deficiency

Is generally not considered a problem in adult humans because copper is widely distributed in food [56]. Nutritional copper deficiency has been observed in infant and in adult patients receiving Total Parenteral Nutrition (TPN) [56, 57]. A deficiency of this micronutrient during pregnancy may lead to oxidative stress, which often results in

reduced fetal growth [16]. Cu has an important role in the production of collagen and elastin, and an insufficient amount of this element can lead to a reduction in the tensile strength of the fetal membrane, resulting in its interruption and premature birth [58]. On the other hand, excessive administration of Cu causes vomiting, diarrhea, as well as liver necrosis, acute kidney damage, and ultimately death [59,60]. Numerous clinical pathological conditions have been associated with hypocupraemia such as nephrotic syndrome, cystic fibrosis, celiac disease, and kwashiorkor. Wilson's disease is a rare autosomal recessive disease, occurring as the result of a defect in copper metabolism which is characterized by hypocupraemia, decreased plasma concentration of caeruloplasmin, elevated tissue copper content, degeneration of brain tissue and cirrhosis of the liver. In contrast, patients with Menkes "kinky-hair syndrome" have hypocupraemia but in association with a low tissue copper level [61]. It is possible that a high dose of zinc therapy may result in copper deficiency [62].Clinical signs and symptoms produced by copper deficiency are anemia, vascular abnormalities and degeneration of brain and spinal cord and cardiac failure [35,64, 65].The most consistent laboratory features of human copper deficiency are hypocupraemia, hypocaeruloplasminaemia, microcytic hypochromic anemia, and neutropenia [62, 66].

8. Copper in Pregnancy

Of all the cations evaluated in the maternal serum in pregnancy, copper levels fluctuate most. Although there are variations among different individuals, each individual normally exhibits an increasing and rapidly decreasing plasma copper levels 2-3 weeks postpartum [67].

With respect to the origin of the elevated copper level in pregnancy, which remains an enigma, many researchers have reported that tissue copper levels in pregnant dogs are 25% less than in non-pregnant animals, but that the copper content in the blood flowing from the liver was 2.5 times greater in the pregnant animals. Apparently the liver is the basic storehouse that supplies the pregnant organisms with its copper [68, 69].

The plasma copper and caeruloplasmin have been found to increase during pregnancy. The plasma caeruloplasmin levels reached a peak value at 22 weeks of gestation, then its level decreased and gradually increased again to the maximum level [70].

An increase in plasma copper level was observed in normal and abnormal gestations [71]; others found that the plasma copper concentration is lower than normal in abnormal pregnancy [72].

9. Zinc Copper Interactions

For many years, it has been recognized that zinc and copper are mutually antagonistic during the intestinal absorption [73, 74].

As copper and zinc enter mucosal cells from the lumen, intracellular ligands, rather than being absorbed directly into the serum bind them. The most thoroughly studied ligand is Metallothionein (MT), a low molecular cytosolic protein found in the intestinal mucosa, liver and Kidney, which is necessary for normal heavy metal metabolism [44, 75, 76]. (MT) synthesis is influenced directly by the dietary zinc supply and serum zinc levels. Zinc absorption is inversely proportional to the (MT) content of enterocytes [75]. Similar to Cu, DMT-1 transporter may help in the absorption of Zn, by transporting Zn from the

intestinal lumen into the enterocytes [77]. (MT) play an important role in cellular transport [78]. These low-molecular proteins, rich in cysteine residues, are located in the extra- and intracellular environment [79]. Their intracellular pool acts as a reservoir of important heavy metals, including Zn, and participates in the detoxification of Reactive Oxygen Species (ROS), nitrogen, excess heavy metals, and organic compounds [80,81,82,83]. Their extracellular pool is responsible for the transport of Zn^{2+} (and other heavy metals) and organic compounds, and is also a free radical scavenger [80]. One (MT) molecule is capable of binding seven Zn^{2+} ions and up to 12 monovalent Cu ions [84,85]. However, the ratio of both elements should be optimized in pregnancy, to improve reproduction results and reduce the chance of spontaneous abortion [86].

10. Zinc Iron Interactions

Zinc, like iron, has also the peculiarity, unlike other metals, to form compounds with multiple degrees of coordination, thus giving it superior qualities in biology [87].

Previous investigation has established that carbonic anhydrase, an essential enzyme for the release of carbon dioxide from the organism, contain, 0.33% zinc. Therefore zinc and iron components of the two protein substances: haemoglobin and carbonic anhydrase are considered as the pivots of gaseous respiration [87].

The serum or plasma zinc concentration is like iron, influenced by various diseases, age and sex [88]. High dietary zinc levels will produce anemia, which can be corrected by the addition of copper and not of iron [89], and reduced tissue iron concentrations. This antagonistic relationship between zinc and iron has been rationalized by the hypothesis that zinc and iron share or compete for common absorptive pathways, but this has not been demonstrated. Since the copper dependent enzyme caeruloplasmin regulates the rate at which Fe^{2+} is released from the liver and is converted to plasma Fe^{3+} transferrrin, zinc could affect iron metabolism by interfering with copper metabolism [73]. There is some evidence that oral iron supplementation, during pregnancy may cause mild zinc deficiency [90]. This finding was confirmed in a study, which showed that the level of antenatal iron supplementation was negatively correlated with maternal plasma zinc level during the third trimester [91, 92]. Zinc bioavailability was also reduced after iron supplementation [93]. On the other hand many investigators have suggested that absorption of zinc is not affected by iron and vice versa [94, 95, 96]. Kordas and Stoltzfus [97], showed that although Fe does seem to reduce the absorption of Zn, DMT-1 is an unlikely site for this absorptive antagonism because Zn is not transported by DMT-1. Zinc-regulated transporter (Zrt) and Iron-regulated transporter Irt) like protein-14 (Zip14) is a transmembrane metal iron transporter that is abundantly expressed in the liver, heart, and pancreas [98]. Holmes et al, [99], studied the effects of multiple micronutrient (MMN) supplementations (15 mg Zn, 65 mg Se, 2 mg Cu) with or without Fe on serum Zn, Se, and Cu concentrations in women from Cambodia. Predominantly anemic nonpregnant women (aged 18–45) received daily 60 mg of Fe (I group); MMN, but no Fe (II group); 60 mg Fe plus MMN (III group); or a placebo (IV group). It was found that 60 mg Fe and the daily MMN formulation may be interfering with the absorption and/or metabolism of supplemental Zn. However, patients took the supplement together with food which was not controlled, and so it is possible that Zn competed for metabolic transporters not only with Fe and minerals contained in the MMN complex but also with elements from the diet.

11. Copper Iron Interactions

Both iron and copper contribute to haemoglobin synthesis and to the formation of some enzymatic systems with a role in the processes of oxidoreductase. Both metals bound to proteins, which are present in the plasma **(87).**

Copper can be mono-and bivalent, therefore like iron it has the capacity to yield and accept electrons a quality which gives it a biologic importance. Copper is found in a proportion of 95% incorporated in Caeruloplasmin, an enzyme with 8 atoms of copper in the molecule. Caerulopasmin favours iron oxidation from the bivalent into the trivalent forms, thus facilitating the fixation of ferric iron on transferrin **(87).** A lack of copper leads to an inhibition of haemoglobin synthesis. In such a case copper deficit can lead to an actual deficit of the iron available for the formation of red blood cells. Abnormalities of iron metabolism may be associated with either a deficit, or an accumulation of iron, or its defective incorporation in haemoglobin **(87).**

An interaction between copper and iron has been known for some time. A lack of dietary copper impairs the utilization of iron in haemoglobin production. This results in an anemia in which the red blood cells are microcytic and hypochromic. The supplementation of copper deficit with iron does not alleviate the anemia, which means that coppers influences, the utilization of iron **(100).**

Copper deficiency results in a hepatic iron overload **(101),** which implies a defective release of iron to the circulation. It is unlikely that the iron defect in copper deficiency is a result of depleted caeruloplasmin because less than 1% of serum caeruloplasmin levels are required for iron incorporation into transferrin **(54).** Iron uptake and haem synthesis has found to be reduced in copper deficiency **(102).** Other studies have shown that haemoglobin biosynthesis is impaired, but the haem biosynthesis enzymes are not affected by copper deficiency **(103).**The iron is essential trace element for collagen synthesis and the conversion of 25-hydroxyvitamin D into an active form **(104).** In a study on zebrafish (*Danio rerio*), Donovan **(105)**, demonstrated that IREG-1 on the surface of placental syncytiotrophoblasts is involved in the transport of Fe from the mother to the fetus. The main protein transporting Fe^{3+} to cells is transferrin **(106).** Equally important in Fe turnover is lactoferrin found in breast milk—a source of Fe^{3+} for newborns and infants **(107).** Another important source of Fe in the body is macrophages, which recover Fe^{2+} from erythrocytes **(108).** Macrophagesdegrade Hgb, resulting in the release of Fe^{2+}, which is then transported by the transmembrane ferroprotin transporter (FPN1) and oxidized by ceruloplasmin to $Fe3^{+}$, to finally bind to transferrin **(109).**

Table 1

Mean, S.E, C.V% and significant differences of all parameters at different stages of the pregnant control group compared to non - pregnant control group.

	Non Pregnant control n=100		Pregnant control n=90					
			1 St trimester n=25		2nd trimester n=35		3rd trimester n=30	
Parameters	X±S.E	C.V%	X±S.E	C.V%	X±S.E	C.V%	X±S.E	C.V%
SIL µg/dl	75.170 ± 1.63 *a*	21.88	71.220 ± 1.79 *a*	19.69	67.250 ± 2.50b *a*	17.06	60.230 ± 2.07 *ba*	13.37
HbL mg/dl	13.105 ± 0.07 *a*	3.49	11.710 ± 0.05 *c*	3.10	10.630 ± 0.07 *b*	2.99	11.940 ± 0.07 *b*	3.70
PCV %	41.364 ± 0.34 *a*	6.18	39.500 ± 0.58 *a*	5.85	38.910 ± 0.35 *a*	4.26	40.080 ± 0.37 *a*	3.29
MCHC %	31.741 ± 0.14 *a*	2.88	29.640 ± 0.37 *d*	3.50	29.510 ± 0.08 *b*	1.30	27.310 ± 0.38 *cb*	4.50
SCuL µg/dl	146.270 ± 5.88 *b*	28.94	150.380 ± 8.45 *b*	16.84	162.080 ± 9.30 *ab*	9.77	163.910 ± 5.29 *ba*	4.72
SZnL µg/dl	92.770 ± 2.91 *b*	20.33	80.390 ± 2.56 *b*	19.63	61.650 ± 1.40 *cb*	17.80	55.670 ± 2.81 *c*	11.28
Cu/Zn Ratio	1.430 ± 0.05 *a*	2.60	1.850 ± 0.1 *a*	14.11	2.300 ± 0.08 *a*	4.20	2.870 ± 0.25 *a*	6.59

Different letters a, b, c indicate that the mean are different significantly at $p< 0.01$

Table2

Mean, S.E, C.V% and significant differences for all parameters in pregnant control compared to non - pregnant controls whole groups

	Non- Pregnant control n=100		Pregnant control n=90	
Parameters	X+S.E	C.V%	X+S.E	C.V%
SIL µg/ dl	75.170 + 1.63 a	21.88	69.274 + 2.98 ba	21.39
HbL mg/ dl	13.105 + 0.07 a	3.49	11.810 + 0.05 b	3.10
PCV %	41.364 + 0.34 a	6.18	39.390 + 0.26 b	4.68
MCHC %	31.741 + 0.14 a	2.88	30.000 + 0.07 b	1.65
SCuL µg/dl	146.270 + 5.88 b	28.94	163.710 + 9.12 a	19.77
SZnL µg/dl	92.770 + 2.91 b	20.33	66.620 + 1.75 b	18.77
Cu/Zn Ratio	2.38 + 0.05 a	2.60	2.38 + 0.08 a	3.89

Different letters a, b, c indicate that the mean are different significantly at $p < 0.01$

Table 3

A Comparison of mean, S.D, and 95% C.L. For the mean of all parameters in the three trimesters of pregnant controls

	1 St trimester n= 25			2nd trimester n= 35			3rd trimester n= 30		
Parameters	x	S.D	95% C.L	X	S.D	95% C.L	X	S.D	95% C.L
SIL µg/ dl	71.22	1.34	54.20 + 89.70	67.25	3.64	38.90 + 95.50	60.23	8.28	44.90 + 75.50
HbL mg/ dl	11.71	0.33	10.42+ 11.83	10.63	0.35	10.47 + 11.47	11.94	0.45	11.79 + 12.08
PCV %	39.50	2.3	39.16 +39.80	38.91	1.65	38.19 + 39.62	40.08	1.32	39.39 + 40.76
MCHC %	29.64	1.47	26.70 + 29.95	29.51	0.39	28.90 + 31.28	27.31	1.38	26.60 + 29.41
SCuL µg/dl	150.38	20.4	102.50 + 198.30	162.08	14.93	105.70 + 218.40	163.91	18.38	103.70 + 224.10
SZnL µg/dl	80.39	5.78	68.70 + 92.20	61.65	10.97	53.70 + 69.50	55.67	6.27	52.20 + 59.20
Cu/Zn Ratio	1.85	0.63	0.98 + 2.11	2.3	0.57	0.79 + 2.80	2.87	0.57	1.06 + 2.97

Tabel 4

Mean, S.E, C.V% and significant differences of all parameters at different stages of the p-IDA group compared to pregnant control as whole group.

	Pregnant control n= 90		P - IDA n= 100					
			1 St trimester	n= 35	2nd trimester	n=35	3rd trimester	n=30
Parameters	X+S.E	C.V%	X+S.E	C.V%	X+S.E	C.V%	X+S.E	C.V%
SIL µg/dl	69.274 + 2.98	21.39	65.94 + 2.75 ba	14.39	50.31 + 4.43 ba	14.31	44.38 + 3.90 b	15.55
HbL mg/dl	11.81 + 0.05	3.10	9.00 + 0.11 c	6.64	8.22 + 0.23 f	4.99	9.6 + 0.24 b	5.71
PCV %	39.39 + 0.26	4.68	32.64 + 0.29 cb	5.40	30.69 + 0.69 c	9.59	34.4 + 0.81 b	5.28
MCHC %	30.00 + 0.07	1.65	27.50 + 0.47 d	6.43	26.31 + 0.25 d	4.28	27.9 + 0.22 cb	1.72
SCuL µg/dl	163.71 + 9.12	19.77	180.94 + 10.21 ba	19.42	203.06 + 13.68 ba	14.89	225.94 + 9.37	16.43
SZnL µg/dl	66.62 + 1.75	18.77	50.23 + 1.96 c	18.53	41.64 + 1.09 c	17.88	32.04 + 1.00	12.13
Cu/Zn Ratio	2.38 + 0.08	3.89	3.59 + 0.10 a	5.07	4.85 + 0.30 a	4.10	7.04 + 0.18 a	5.17

Different letters a,b,c indicate that the mean are different significantly at $p < 0.01$

Table 5

Mean, S.E, C.V% and significant differences for all parameters in pregnant control compared to P -IDA as whole groups.

	Pregnant controlS n= 90		P- IDA n= 100	
Parameters	X+S.E	C.V%	X+S.E	C.V%
SIL µg/dl	69.27 + 2.98 ab	21.39	55.812 + 39.00 b	16.87
HbL mg/dl	11.81 + 0.05 b	3.10	8.854 + 21.00 c	4.94
PCV %	39.39 + 0.26 b	4.68	32.615 + 0.52 c	1.70
MCHC %	30.00 + 0.07 b	1.65	27.879 + 0.08 c	3.88
SCuL µg/dl	163.71 + 9.12 a	19.77	210.50 + 7.93 a	15.66
SZnL µg/dl	66.62 + 1.75 b	18.77	40.092 + 1.07 c	17.80
Cu/Zn Ratio	2.38 + 0.08 a	3.89	5.25 + 0.10 a	2.96

Different letters a,b,c indicate that the mean are different significantly at $p < 0.01$

Table 6
A Comparison of mean, S.D, and 95% C.L For the mean of all parameters in the three trimesters of P- IDA.

	1st trimester		n= 35	2nd trimester		n=35	3rd trimester		n= 30
Parameters	x	S.D	95% C.L	X	S.D	95% C.L	X	S.D	95% C.L
SIL µg/ dl	65.94	1.64	26.49 - 67.2	50.31	2.78	32.04 - 68.57	44.38	4.45	21.94 + 66.81
HbL mg/ dl	9.00	0.89	8.56 - 9.44	8.22	1.01	7.5 - 8.7	9.6	0.54	9.53 + 9.67
PCV %	32.84	1.77	32.71 +32.96	30.69	2.94	29.28 + 32.1	34.4	1.81	34.30 + 34.50
MCHC %	27.50	2.86	26.00 + 28.9	26.31	1.12	26.12 + 26.49	27.9	0.48	26.90 + 28.80
SCuL µg/dl	180.94	17.32	118.65 + 243.22	203.06	16.4	142.38 + 263.77	225.94	16.19	121.04 + 330.83
SZnL µg/dl	50.23	6.3	43.43 + 57.03	41.64	7.81	35.9 + 47.36	32.04	3.88	29.75 + 34.32
Cu/Zn Ratio	3.59	0.45	0.95 + 1.67	4.86	0.67	0.43 + 1.67	7.04	0.68	0.72 + 1.96

Table 7
Mean, S.E, C.V% and significant differences for all parameters in 1st trimester pregnant control compared to the 1st trimester P-IDA.

	1st trimester Pregnant controlS n= 25		1st trimester P- IDA n= 35	
Parameters	X+S.E	C.V%	X+S.E	C.V%
SIL µg/dl	71.22 + 1.79 a	19.69	65.94 + 2.75 ba	14.39
HbL mg/dl	11.71 + 0.05 c	3.10	9.0 + 0.11 e	6.64
PCV %	39.5 + 0.58 a	5.85	32.84 + 0.29 cb	5.40
MCHC %	29.46 + 0.37 b	3.5	27.5 + 0.47 d	6.43
SCuL µg/dl	150.38 + 8.5 b	16.84	180.94 + 10.21 b a	19.42
SZnL µg/dl	80.39 + 2.56 b	19.63	50.23 + 1.96 c	18.53
SCu/Zn Ratio	1.85 + 0.1 a	14.11	3.59 + 0.1 a	5.07

Different letters a,b,c,d indicate that the mean are different significantly at $p < 0.01$

Table 8

Mean, S.E, C.V% and significant differences for all parameters in 2nd trimester pregnant control compared to the 2nd trimester P-IDA.

	2nd trimester Pregnant controls n= 35		2nd trimester P- IDA n= 35	
Parameters	X+S.E	C.V%	X+S.E	C.V%
SIL µg/ dl	67.25 + 2.5 ba	17.06	50.31 + 4.43 ba	14.31
HbL mg/ dl	10.63 + 0.07 b	2.99	8.10 + 0.23 f	4.99
PCV %	38.91 + 0.35 a	4.26	30.69 + 0.69 c	9.59
MCHC %	29.51 + 0.08 b	1.3	26.31 + 0.25 d	4.28
SCuL µg/dl	162.08 + 9.3 ba	9.77	203.06 + 13.68 b a	14.89
SZnL µg/dl	61.65 + 1.4 c b	17.80	41.64 + 1.09 c	17.88
SCu/Zn Ratio	2.30 + 0.08 a	4.20	4.85 + 0.3 a	4.10

Different letters a,b,c,d indicate that the mean are different significantly at $p< 0.01$

Table 9

Mean, S.E, C.V% and significant differences for all parameters in 3rd trimeste pregnant control compared to the 3rd trimester P-IDA.

	3rd trimester Pregnant controlS n= 30		3rd trimester P- IDA n= 30	
Parameters	X+S.E	C.V%	X+S.E	C.V%
SIL µg/ dl	60.23 + 2.07 ba	13.37	44.38 + 3.9 b	15.55
HbL mg/ dl	11.94 + 0.07 b	3.70	9.6 + 0.24 d	5.71
PCV %	40.08 + 0.37 a	3.29	34.4 + 0.81 b	5.28
MCHC %	27.31 + 0.38 cb	4.5	27.9 + 0.22 cd	1.72
SCuL µg/dl	163.91 + 5.29 ba	4.72	225.49 + 9.3 a	16.43
SZnL µg/dl	55.67 + 2.81 c	11.28	32.04 + 1.0 d	12.13
SCu/Zn Ratio	2.87 + 0.25 a	6.59	7.04 + 0.18 a	5.17

Different letters a,b,c,d indicate that the mean are different significantly at $p< 0.01$

Table 10
Correlation coefficent matrix and signignificant levels for all parameters in1st trimeter pregnant controls

Parameters	SIL	HbL	PCV %	MCHC	SCuL	SZnL	SCu/Zn Ratio	
SIL	1.0	NS	NS	0.44 *	NS	- 0.43 *	0.5 *	
HbL		1.0	NS	NS	NS	NS	NS	
PCV %			1.0	NS	NS	NS	NS	
MCHC				1.0	NS	NS	NS	
SCuL					1.0	NS	NS	
SZnL						1.0	NS	
SCu/Zn Ratio							1.0	

Table 11
Correlation coefficent matrix and signignificant levels for all parameters in 2nd trimeter pregnant controls

Parameters	SIL	HbL	PCV %	MCHC	SCuL	SZnL	SCu/Zn Ratio
SIL	1.0	- 0.06 **	- 0.67 **	0.064 ***	- 0.11 *	NS	- 0.15 ***
HbL		1.0	NS	NS	- 0.097 ***	023***	- 0.15 ***
PCV %			1.0	NS	NS	NS	- 0.15 ***
MCHC				1.0	NS	NS	- 0.17 ***
SCuL					1.0	NS	NS
SZnL						1.0	NS
SCu/Zn Ratio							1.0

Table 12
Correlation coefficent maxtrix and signignificant levels for all parameters in 3rd trimeter group II pregnant control.

Parameters	SIL	HbL	PCV %	MCHC %	SCuL	SZnL	SCu/Zn Ratio
SIL	1.0	NS	NS	NS	NS	NS	NS
HbL		1.0	NS	NS	NS	NS	0.7 ***
PCV %			1.0	NS	NS	NS	0.68 ***
MCHC %				1.0	NS	NS	0.12 ***
SCuL					1.0	NS	NS
SZnL						1.0	NS
SCu/Zn Ratio							1.0

Table 13
Correlation coefficent maxtrix and signignificant levels for all parameters in1st trimeter P-IDA.

Parameters	SIL	HbL	PCV %	MCHC %	SCuL	SZnL	SCu/Zn Ratio
SIL	1.0	0.28 *	NS	NS	NS	NS	NS
HbL		1.0	NS	0.87 ***	NS	NS	NS
PCV %			1.0	0.63 ***	NS	NS	NS
MCHC %				1.0	NS	NS	NS
SCuL					1.0	0.49 *	0.87 ***
SZnL						1.0	NS
SCu/Zn Ratio							1.0

Table 14
Correlation coefficent maxtrix and signignificant levels for all parameters in2nd trimeter P-IDA.

Parameters	SIL	HbL	PCV %	MCHC %	SCuL	SZnL	SCu/Zn Ratio
SIL	1.0	NS	NS	NS	NS	NS	NS
HbL		1.0	0.97 ***	0.85 ***	NS	NS	NS
PCV %			1.0	0.71 ***	NS	NS	NS
MCHC %				1.0	NS	NS	NS
SCuL					1.0	NS	0.83 *
SZnL						1.0	- 0.84 *
SCu/Zn Ratio							1.0

Table 15
Correlation coefficent maxtrix and signignificant levels for all parameters in3rd trimeter P-IDA.

Parameters	SIL	HbL	PCV %	MCHC %	SCuL	SZnL	SCu/Zn Ratio
SIL	1.0	NS	NS	0.89 *	NS	NS	NS
HbL		1.0	0.95 **	NS	NS	NS	NS
PCV %			1.0	0.71 ***	NS	NS	NS
MCHC %				1.0	NS	NS	NS
SCuL					1.0	NS	0.99 ***
SZnL						1.0	NS
SCu/Zn Ratio							1.0

12. Results and Discussion

SIL in group II (Pregnant Controls)

In the present study there is a gradual decrease in the SIL at different stages of the pregnant controls. The greatest changes occur during the 2nd and 3rd trimesters (table 1, 2, 3). These finding agree with the results observed by many other investigators [34, 110, 55, 111].Also a slight gradual decrease in SIL during different stages of pregnancy reaching its lowest values during the last trimester (table 1, 3). These findings are consistent with results found by other investigators [112,113,114]. Also a gradual decrease in the SIL at 2nd and 3rd trimesters of the pregnant controls compared with that in the nonpregnant controls is observed (table 1). This too, is in agreement with previous studies [34,114].

Although iron constitutes less than 0.01 of 1% of the body's total weight it has several major roles. It assists in the transport of oxygen and carbon dioxide throughout the body erythropoiesis, thermoregulation, humoral and cellular immunity and it aids in the production of red blood cells. Two thirds of the body's iron is found in the molecule of hemoglobin, transferrin, serum ferritin and myoglobin [115,116]. In a healthy iron replete person, stored iron accounts for the remaining one third of the body's total iron [116].

A total of (1 gm.) of iron is required during a normal term pregnancy. The present study shows that in the 1st trimester of pregnant controls, there is a difference in SIL compared with that in-group I (non-pregnant controls), but this difference is statistically non-significant. This difference may be attributed to the fact that the mother has sufficient iron reserves at the beginning of pregnancy [117]; besides the iron demands during the 1st trimester of pregnancy are actually lower than they are prior to pregnancy [118]. Also fetal demands for iron increases progressively during the 2nd and 3rd trimesters. Other studies [116, 125, 126], showed that elemental iron requirement increased from 1.5 to 2 mg per day up to 5 to 7 mg by the late 2nd trimester and throughout the 3rd trimester. About half of this iron is needed for the increased maternal blood volume; the remainder is used for fetal and placental growth or is lost during the normal heightened excretion that is associated with pregnancy [1I3, 116, 118]. The erythroid activity starts early in pregnancy and may exhaust the iron stores before fetal demands for iron can be met [119]. Another factor, which also affects the SIL, is that fetoplacental demand deposition of iron increases markedly during the 3rd trimester [120]. It has been suggested that the placenta with its avidity for maternal circulating iron through its richness in transferrin receptors protects the fetus extracted iron in amounts proportional to the level available in the mother, [121,114], however, it appears that the capacity of iron to transfer from the placenta to the fetus is limited by a threshold mechanism [122]. In the plasma, iron is transported bound to β1-globulin fraction of the portion Apo transferrin, forming transferrin [52]. The plasma concentration of transferrin is measured as the TIBC [117]. Under normal conditions very little iron is absorbed [34]. Normally when the body has become saturated with iron so that essentially all the Apo ferritin in the iron storage areas has become saturated with iron. It becomes difficult for transferrin to release iron to the tissues. As a consequence, the transferrin which is normally one - third saturated with iron now becomes almost fully bound with

iron [117,55], so that the transferrin accepts almost no new iron from mucosal cells and the rate of absorption of iron from the intestinal mucosa becomes greatly decreased [34,123,55]. Another factor that many help and control absorption of iron is that iron transports protein apotransferrin (iron free transferrin) which not only transports iron in the blood but also enters the mucosal cells and transports iron through the cytosol of these cells as well.

The most important factor affecting transferrin synthesis is the level of iron stores [124,125,126]. When the iron stores have been depleted during pregnancy, a natural sequence of compensatory mechanisms occurs and the body becomes more efficient in iron adsorption [34]; iron stores are also responsible for the physiological increase in the transferrin concentration that occurs during pregnancy [127, 34, 55]. There is a positive correlation between Transferrin Saturation (TS) and SIL, so that the low percentage of TS accompanies a low SIL, while a significant negative correlation is found between TS and TIBC at different stages of pregnant controls group [34, 55]. During pregnancy as iron stores have been depleted, SIL decreases and the rate of iron absorption becomes greatly accelerated in an attempt to compensate for this deficiency [117]. The iron, which is absorbed, is then transported by transferrin in the plasma to other parts of the body where it is needed [110, 55].

SIL in-group III (P-IDA)

Anemia is a common hematological complication of pregnancy and is associated with increased rates of premature birth; low birth weight and prenatal mortality. IDA decrease the amplitude of adaptation of the pregnant women. It might be responsible for abortion or preterm labour as well as disarrangement of myo- material activity during labour and puerperium. Congenital fetal malformations and intrauterine growth retardation might be also stimulated by iron deficiency [118,128,115,129]. Women are at greatest risk of this type of anemia [130]. It is concluded that IDA during pregnancy adversely affects the iron endoment of the infant at birth [125].

ID and its consequent anemia are recognized as the most common specific nutrient deficiency in the world including Iraq [131,118,128,132]. It is estimated that about 2.15 billion people are iron deficient [133], and 41.5% of the women were found to be anemic Hb < 11 gm\dl [52], and that this deficiency in Namibia severs enough to cause anemia in 1.2 billion people globally [134]. While on the basis of Hb <12 gm\dl, 37%of the women were considered anemic. IDA was present in 76% of the anemic in spite of its fairly good sociodemographic and nutritional conditions. Gestation age was the factor most strongly associated to IDA [135].

In an inner-city sample of pregnant women, IDA was associated with a higher risk of preterm delivery and low birth weight [136,129,137]. About 90% of all anemic cases are due to lack of iron, affecting mostly the developing world where nearly 1/3 of the population is iron deficient. Roughly, 47% of nonpregnant women and 60% of pregnant women are anemic worldwide [138], in the industrial world as whole anemia prevalence during pregnancy oscillates between 9 and 14% for the same age-sex categories although the poor among these societies are more affected [118]. ID defined as an insufficient supply of iron to the cells of the body after iron reserves have been exhausted, since this is a late manifestation of chronic ID [139].

In the present study a lower SIL is found in P-IDA compared to their values in pregnant controls with haemodilution that occurs naturally (table 4, 5, 7, 8, 9,). These observations agree with the prvious studies **(34,116,118).** Also a marked prpgressive decrease in SIL during different stages of P-IDA have been observed in the 3**rd** trimester (table 6), which is consistent with the finding of other finding of other investigators **(34,118).** It is well known that in IDA, iron stores are depleted, and iron absorption becomes more efficient in an attempt to compensate; therefore the liver synthesizes more apotransferrin, and plasma iron binding protein transferrin increases to transfer iron to the different parts of the body, which results in a progressive decline in TS in P-IDA, **(34).**

(HbL), (PCV) & (MCHC) In-Group I (Non-Pregnant Controls)

In the present study the non-pregnant control females exhibited mean values for HbL, PCV and MCHC which are consistent with values reported in other development countries **(137).**

(HbL), (PCV) & (MCHC) In-Group II (Pregnant Controls)

A study of 90 pregnant normal controls shows a minor decline in HbL, PCV, and MCHC in the 1st,2nd and 3rd trimesters than in non-pregnant controls (table1) which accords with the observations made by other researchers **(109,116,118).** In the 1**st** and 2**nd** trimesters there is a slight decrease in the HbL, PCV, and MCHC in contrast with the 3**rd** trimester (table 1)**,** which shows a slight increase according with other workers **(116,118).** The changes in HbL and MCHC during 2**nd** and 3**rd** trimesters were statistically non-significant but it was statistically significant compared with 1st trimester, the changes in PCV were statistically non-significant.

Few studies have been made on zinc metabolism in human's anemia **(114,140,141,142).** In this present study a significant positive correlation is found between HbL and SZnL during 2**nd** trimester pregnant controls. As SZnL declines progressively with increasing gestational age, it results in the lowering of HbL. To the best of my knowledge, this is the first time such as observation has been made and no indication of this relationship is to be found in the literature in Iraq. So far the possible explanation is that a decrease in SZnL is regarded as another cause of anemia in pregnancy which is related to the essential role of zinc in haemoglobin synthesis **(143,144).** It has been found that the red blood cells are rich in both iron and zinc **(114,145).** Zinc is like iron and copper in that it has an important role in haematopoiesis and hemoglobin synthesis **(142),** and its decrease causes a type of anaemia, which resembles that of iron deficiency and it might be called "Zinc Deficiency Anemia". Abnormal haematological findings in low SZnL are particularly relevant to the human clinical situation. HbL is indicative of anemia (<10.0 gm/100 ml).

It is suggested here that an aetiological relationship exists between low SZnL and anemia of pregnancy. Some connections with impaired haemsynthsis owing to a defect in **δ**-Amino Laevulinic Dehydrase (**δ**-ALA-D) are possible. This is a zinc metalloenzyme which catalysis the condensation of **δ** -ALA to porphobilinogen (PBG) in human red blood cells, which is activated by zincs **(146,147,148).** Activity of this enzyme is increased by oral zinc supplementation in man **(140).** If haemsynthsis is increased by oral Zinc deficiency, the

damage to this synthesis will affect δ-Amino Laevulinic Dehydrase Porphobilinogen (δ-ALA-PBG) step, therefore haemoglobin synthesis will be impaired and anemia occurs.

Low zinc in pregnant women increases maternal morbidity and involves a higher risk to the fetus **(140).**

(HbL),(PCV) & (MCHC) In-Group III (P-IDA).

In the present study there is a severe progressive decline of HbL ,PCV and MCHC at different stage of the P-IDA compared with that in pregnant controls (table7,8,9), which is in accordance with results observed by previous workers **(87,71,126,129,137139,144).** The lowest values are found in the 2**nd** trimester compared to the 1**st** and 3**rd** trimesters of the P-IDA, while a slight elevation in HbL, PCV and MCHC is observed in the 3**rd** trimester which agrees with the results of previous studies **(71,170,171).**

Serum Copper Level (SCuL) in GroupII (pregnant controls)

The healthy pregnant control in the present study showed a significant increase in SCuL compared to non-pregnant control. This is in agreement with previous studies **(71, 218, 153)**. The rise in SCuL was continuos throughout the gestation, and the increase was most marked in 2nd trimester, but it was a non-significant increase at different stages of pregnancy compared to non-pregnant controls. This agrees with the findings of other workers (21,218,166). The results of this study show that among pregnant control as a whole group, the maternal SCuL is more than that of non-pregnant control women.

The role of Copper in biological processes has not yet been clearly established; of the various cations, copper levels in women fluctuate; the cation increasing most significant during pregnancy, is copper **(149, 69).** A decrease in Cu and Fe concentration in the duodenal mucosa was found. Simultaneously, diminution in concentration of two cited metals and Caeruloplasmin Level (SCL) activity was noted, significant correlation was observed between fetal liver Cu contents and SCL activity and Cu concentration in serum, SCL activity is related to reduced availability of Cu and Fe in Fetuses. It is suggested that the mechanism of Cu and Fe deficit content in fetuses is based on the diminution of absorption of these metals by intestine expose cadmium on the reduction of metal concentration in blood serum, and in consequence, they're decreasing availability fetuses **(151).** The exact mechanism responsible for the increase in the SCuL in pregnant controls is not clearly understood. One of the main possibilities for hypercupreamia during gestation is the increase in the production of hormones by the placenta particularly oestrogens**, (149, 68)**, which is essential for the continuation of the pregnancy. The daily production of placental oestrogen progressively increases in blood serum as pregnancy advances. It reaches its peak value in the 3**rd** trimester that of normal women at the end of pregnancy **(123).**

A more recent approach has used zinc, which blocks the absorption of copper and increase copper excretion in the stool. Zinc acts to remove accumulated copper from the body as well as prevent its reaccumulation **(152).** In the present study, a significant negative correlation is found between SCul and HbL in the 2**nd** trimester of the pregnant controls, while a significant positive correlation is found between SCuL and HbL in the 1**st** and 3**rd** trimesters of the pregnant controls(table 1,3). So hypercupraemia may be due to decreased HbL in the whole blood during 2**nd** trimester. This is the first time such a finding has been

made and to the best of my knowledge it has not been previously reported. The explanation is based on the facts that copper and iron play essential roles in haematopoiesis and interrelationship between these two trace elements in the synthesis of hemoglobin. Since at the beginning of the last century, it has been realized that the metabolism of iron and copper is interrelated [153]. Copper has a catalytic action on the synthesis of haemoglobin and in the production of the erythrocytes. Because of its bio catalytic properties on the cell, it also plays an important role in the processes of growing [150]. It has been postulated that copper is essential for the stroma of red blood cells or the release of erythrocyte from bonemarrow. Furthermore, copper is essential for the mobilization of iron from the tissues and its conversion in to haemoglobin [116]. Both iron and copper play a major role in a variety of enzyme activities, such as cytochrome C oxidase which transfers electrons to the molecular oxygen at the terminal end of the mitochondrial electron transport chain [52].

The role of copper in maintaining haemoglobin levels lies in intracellular iron metabolism. Copper appeared to enhance the rate of iron uptake from transferrin in the cells having more transferrin receptors. Also copper appears to enhance the storage of intracellular iron and as a result, to reduce the rate of cellular turnover of the iron [100], Copper has an important role in maintaining the plasma membranes functions, and it is found that changes in the membranes of erythrocytes and reticulocytes occur in copper deficient animals [157] . Reticulocytes take up a majority of iron via transferrin-mediated endocytosis [158]. So the rate of release of iron from transferrin and its uptake by reticulocyte are dependent upon an active electron transport chain [159]. Copper appears to influence the retilization of iron from old erythrocytes Normally, the reticulocytes of the spleen phagocytose old erythrocytes and release the iron back in to the circulation. Copper and cytochrome C oxidase are involved in the movement of the iron. In the absence of cytochrome C oxidase the synthesis of haem from Fe^{+3} and protoporphyrin will be impaired [160].

So in this study as HbL is decreased during pregnancy, utilization of caeruloplasmine in erythropoiesis as iron transferase is also decreased and hypercupraemia and hypercaeruloplasminaemia resulted. Since the results of the present study shows hypercupraemia and copper appears to enhance the rate of uptake and the storage of intracellular iron it also reduces the rate of cellular turnover of iron. But the studied group shows a decrease in SIL, therefore the lack of iron would reduce the availability of intracellurar iron for haemoglobin synthesis, So HbL is decreased and anaemia occurs.

Hypercupraemia is the usual response to hypoferraemia. In the present study, as iron falls, the copper content tends to rise, and this agrees the findings of other workers [116,161]. It must be pointed out that this study has been done on whole blood and since blood iron is present principally in the haemoglobin molecule it will be expected that whatever anaemia is present, the total blood iron would diminish. It does not necessarily follow that there is a reciprocal relationship between copper and iron [116], though the majority of investigators show that there is an inverse relationship between SCuL and SIL [87, 100,116] and (table11). Although the concentration of SIL and copper is intimately connected with erythropoiesis, some authors could not find any relationship between HbL and SIL, in the whole blood, as

these values depend partly upon the rate of deposition in the tissues, partly upon the amount of intestinal absorption, and partly upon the TIBC of plasma proteins [103]. Other investigators have observed that the elevation in SCul would seem to be independent of the change in the iron, since there is no correlation in time or degree between these two variables. It seems strange, however that the high copper content of the fetal tissues should be associated with a low fetal plasma copper, while high iron content of the fetal tissue is accompanied by a high SIL in the fetus and a low SIL in the mother [102], So hypercupraemia are associated with hypoferraemia in the present study but this association is statistically non- significant.

Another explanation that has been offered for the elevation of SCuL in the maternal blood among pregnant controls is related to the normal physiological adjustment for the mobilization of the copper from the maternal tissues, especially the liver which acts as a basic storehouse that supplies the pregnant organs with its copper [162]. So that copper may be transported through the placenta to the fetues which has a biological value in the growth and development of the fetues [163]. The increasing content of the maternal copper during pregnancy continously supplies sufficient copper to the developing fetus. The growing fetus needs an increased copper supply. It has been shown that fetal organs especially liver and spleen contain much more copper than in the adult. It has been observed also that the copper content of placental arterial blood is greater than that of placental venous blood by 30% [164]. This suggests that there is a constant withdrawal of copper from the maternal blood through placental and it is stored in the fetus [163]. The mobilization of copper from the maternal tissue especially liver, is hormone dependent mainly oesterogen which is elevated in the maternal blood during pregnancy [135,68], Therefore the rise in SCul during pregnancy is regarded as a result of an increased ability of the organs of the pregnant women to meet the higher demands of the growing fetus for copper [163].

Serum Copper Level (SCuL) in Group III (P-IDA)

In IDA the body is deficient in iron content of the blood as well as iron stores. It therefore, seems quite reasonable to postulate that, in these clinical conditions caeruloplasmin is synthesized at a regular rate but is not utilized for various functions and so possibly because of this, its caeruloplasmin rises in the blood in IDA. Hypercupraemia in the P-IDA in the present study may be due to inhibition in the essential role that copper plays in energy supply at the cellular level [166].

Copper plays an essential physiological role in a number of enzymes such as cytochrome C oxidase which is a cuproenzyme in the mitochondrial electron transport chain, required for the deoxidation of iron from the $Fe^{3+}$to the Fe^{2+}. This enzyme mediates the final step in the electron transport chain for the reduction of molecular oxygen [52]. Also is the structural component of cytochrom C oxidase and it is essentail for its enzymatic activity. Each molecule of this enzyme contains two atoms of copper [168].

Iron- like copper also plays a major role in a variety of enzyme activities involved in the metochondrial electron transport chain such as cytochrom c oxidase, Succinic dehydrogenase and NADH dehydrogenase. The latter two enzymes play an important role at the initial steps of oxidative phosphorylation. All these activities involve a redox change

where by iron alternates between its ferrous and ferric states (23,22%). The presence of iron is necessary for the metabolic process, erythropoiesis, oxygen transport and respiration, thermoregulation and humoral and cellular immunity **(115).** It is becoming increasingly apparent that in ID, the activities of these enzymes are impaired before any of the more traditional features of ID, such as anaemia or changes in HbL**(169).**

ID and anaemia most probably alter many functions in the body during pregnancy, due to the multiple role of iron in enzymes and iron compounds have an oxygen transport and in many redox reactions particularly those involved in molecular oxygen **(170).** These reactions are the backbone of the respiratory chain. It has been documented that clear derangements in energy metabolism include diminished oxidation capacity at the metochondrial levels altered mitochondrial structure, Lactic acid accumulation and restricted oxygen transport **(171,172).** In P-IDA. The elevation of blood copper content occurs as a consequence of the inhibition of energy, which causes accumulation of unused copper in the body.

Serum Zinc Level (SZnL) in Group I (Non-pregnant controls)

The normal value for SZnL in the non-pregnant healthy women varies from one report to another **(149,166,173,174)**. In the present study the control non-pregnant women of childbearing age exhibited SZnL with a mean of 92.77µg/dl (table 1). This agrees with the values reported from other developing countries **(178,176)**. However, the mean value for SZnL in this study, is lower than the mean values in the literatures from western countries **(174)**, but these results could be compared more favorably with the values reported in other developing countries than with the values of western countries, the low SZnL reported in this work be related to zinc deficiency, known to exist in several areas of the Middle East as Iran, Egypt, and Kuwait **(145)**. This zinc deficiency may be attributed to dietary habits prevailing in these countries. Many Asians including Iraqi's continue to eat their traditional diets based on cereal and pulse. This kind of diet with its low zinc, high fiber and phytate content, has resulted in clinically evident Zinc deficiency in some Middle Eastern communities **(183)**. Phytate and fiber impaired absorption and bioavailability of Zinc **(184,145)**. In Iraq most of the population consume large amounts of bread and rice with their meals, which contain phytic acid, which forms a complex substance with Zinc and inhibits its absorption (186) In addition to food, other factors may cause zinc deficiency such as the source of water supply, geographical location and socio-economic and racial status **(186,**145**)**.

Serum Zinc Level (SZnL) in Group II (pregnant controls)

In the present work, a progressive reduction in maternal SZnL with increasing gestational age is observed compared to non-pregnant control group (table 1,2), which confirm the observations of other workers **(107,95,142,174)**. A decline in SZnL begins early in pregnancy and the greatest decrease observed in the 3rd trimester of pregnancy (table 1), several physiological factors may contribute to the decrease in SZnL during pregnancy. One of such factors may be the increase in SCuL. In the present study, a significant inverse relationship is found between SZnL and SCul in different trimesters of normal pregnancy (table 4). SZnL showed a progressive decline while SCuL showed elevation with increasing gestational age. This agrees with those-findings by previous workers **(40, 149, 73, 166)**. Zinc and copper are two of the most metabolically important trace metal nutrients. For this reason

and because of their close chemical similarity and extensive biological interaction; they are often considered together in this research. Studies of the competitive interaction between zinc and copper for intestinal absorption have been largely focused on the possible role of MT [75, 76]. It has been suggested that the antagonism between copper and zinc may involve competition for binding sites on MT [52] .Copper has a depressing effect on zinc absorption and copper has shown to have a higher affinity than zinc for MT [42, 75]. So low SZnL might therefore accentuate any copper excess.

The results of the present study reveal a highly significant negative relationship between SZnL and SCL. There was a progressive elevation of SCL during different stages of pregnancy [71, 186]. The elevation in SCL was accompanied by a progressive decrease in SZnL. A possible explanation is that during the course of pregnancy many biochemical and physiological changes take place and physiological adaptation to these changes has occurred. One of these changes is in the plasma proteins especially globulins which are elevated and albumin which is depressed [34, 184].

Both proteins are synthesized in the liver [72]. Caeruloplasmin is a α_2 globulin, it is a cuproenzyme and most copper in the plasma is bound to this enzyme [52, 61, 155].

Albumin is a zinc carrier protein; normally about 80% of plasma zinc is bound to albumin. Since there is an influx of zinc into the cells and efflux of copper, plasma hypercupraemia and plasma hypozincaemia will occur. hypercupraemia is accompanied by hypercaeruloplasminaemia and hypozincaemia is accompanied by hypoalbuminaemia of pregnancy [34,184]. On the other hand, a decrease in albumin synthesis is accompanied by an increase in globulin synthesis resulting in an increase in SCL and a decrease in SZnL.

Hypozincaemia is also explained depending on the fact that during late pregnancy, the absolute quantity of zinc required by the fetus is greatest; pregnant women excreted more zinc in the urine than non-pregnant control women [91]. As a consequence of an increase in the glomerular filtration rate, which is increased by about 50% during pregnancy,which tends to increase the rate of water and electrolyte loss in the urine [34, 123].

Other investigators have suggested that hypozincaemia and hypoalbuminaemia of normal pregnancy are due to an increase in plasma volume after the 1st trimester. The cause of the gradual fall in SZnL found in this study must be sought among several possible mechanisms, gradual haemodilution or plasma volume expansion during pregnancy [149, 118,150]. It is well known that during pregnancy blood volume begins to increase slowly in early pregnancy; a more marked increase occurs during the 2nd trimester, while in the 3rd trimester, plasma volume reaches about 150% of pregnancy plasma volume [116,118].

Oestrogens have been shown to produce hypozincamia, which are considered to be one of the most important zinc - lowering factors [127, 96,176]. SZnL continues to decline more in the 3rd trimester when estrogen production reaches its peak [166,152,175].

Hypozincaemia in the maternal plasma during pregnancy has been attributed to increased zinc uptake by the fetus and placenta. The accumulation of zinc by fetal tissues is accelerating during the 2nd and 3rd trimesters of pregnancy. The decreased maternal level could be due to an increasing transfer of zinc to the fetus, which is needed for its growth.

[174,176].Other investigators suggest that the decline in SZnL during pregnancy may be due to an increased transfer of plasma zinc to the maternal erythrocyte [174].

SZnL In-Group III (P-IDA)

The role played by zinc in biology is now better known, and numerous biochemical mechanisms, such as immunity or actions on several hormones and more than 200 enzymes, have proved to be zincdependent. Thus many functions are disturbed when this trace metals is deficient, including, for example, taste, and appetite, cell multiplication, growth, pregnancy, fertility, defence against bacteria, brain function, development, and reproduction. However, the effect of poor maternal zinc nutriture, usually measured as plasma zinc, on poor pregnancy outcome has not been consistent. A low zinc intake was associated with approximately at two-fold increase in the risk of low birth weight (<2.5gm) after controlling for calories and other confounding variables. The risk of preterm delivery (<37completed weeks) was also increased [141, 63]. Low zinc intake during pregnancy prevents the normal accumulation of long – chain fatty acids and differentially depletes maternal whole body stores of linoleic and alpha-linolenic acids [179]. Zinc deficiency in pregnant rats, decreases folate bio- availability of folinic acid, and folate polyglutamates [140]. Preconception folic acid supplements do not compromise the zinc status of the mother or the embryo with respect to zinc intake [177].

In the present work, it is found that SZnL severely declines in the PIDA. One of the consequences of this hypozincaemia is hypercupraemia. In the IDA of pregnancy a marked hypercupraemia occurs due to a sever decrease in SIL. It seems that thionein is a normal fetal protein which functions in zinc and probably copper homeostasis during gestation and early post- natal life. The capacity for the synthesis of this protein is retained in the adult and can form a control mechanism when serious disturbances occur in the metabolism of these cations. Zinc and copper - thioneins can act as cation donors and restore functional activity to the apoproteins of appropriate metalloenzyme [76, 147]. Hypozincaemia in the present study may be also due to hypercaeruloplasminaemia, which is more evident in the P-IDA [87], since a significant negative correlation is found between SZnL and SCL at different stages of P-IDA as gestational age is in progress. The SCL reaches its highest level and SZnL its lowest level during the 3rd trimester. Increased SCL, which is a α_2-globulin, during pregnancy with IDA, is often accompanied by the decrease in plasma protein, especially albumin [184].

Cu /Zn Ratio

It has been found by many investigators that not only elevated SCuL reduced SZnL can be an aid in the differential diagnosis of some diseases; The SCu/ Zn Ratio may also be used to asses various pathological conditions [185]. In the present study, assessment of this ratio is tried to prove whether this ratio could be used as an index in predicting and managing IDA in pregnancy.

The SCu / Zn Ratio in P-IDA as a whole group is about four times greater than that of non-pregnant controls and about three times greater than that of pregnant controls as a whole group. These changes indicate the disturbed relationship between SCul (which is elevated)

and SZnL(which is depressed) in P-IDA. The use of this ratio could have an advantage over using SCuL and SZnL alone.

13. Conclusions

The presented literature review suggests that Fe, Cu, and Zn are crucial for the proper course of pregnancy. The results should be approached with caution, but most studies indicate the influence of metals on the parameters of mother and child. Moreover, Fe, Cu, and Zn may be promising biomarkers in predicting complications in pregnancy.

Additionally, the results of the researchers show important relationships between Fe, Cu, and Zn in the body. An increase or decrease of one element may significantly affect the action of the other two. It is particularly significant to note that the elements do not exhibit antagonistic actions against each other when they are within daily reference values.

Fe, Cu, and Zn play a key role in the homeostasis of the body, and any changes in their concentrations can cause interactions that are dangerous to the health of the mother and fetus.

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