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COPPER-ZINC RATIO WITH PROGESTERONE LEVEL CAN BE MARKERS TO DIAGNOSE ECTOPIC PREGNANCY

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ABSTRACT

Background: Ectopic pregnancies have increased in recent years, with morbidity and mortality being known. Early diagnosis is crucial to avoid complications, and changes in copper, zinc, and copper/zinc ratio are associated with various gynaecological and obstetrical disorders. **Aim:** To evaluate the role of serum copper, zinc, copper/zinc ratio, and progesterone as a biomarker for the diagnosis of ectopic pregnancy. **Patients and Methods:** Beginning on January 1, 2022, and ending on January 1, 2023, a case-control research was carried out. Two groups of one hundered pregnant women were selected for the present study and admitted to the Department of Gynecology and Obstetrics at Al-Emamain Al-Kadhymain Medical City in Baghdad, Iraq. The control group comprised 50 pregnant women in good health, while the study group comprised 50 pregnant women who presented with an ectopic pregnancy. **Results:** The research groups differed significantly in terms of progesterone levels, copper, zinc, and copper/zinc ratio (all P-values were less than 0.001). The optimal cut-off values for ectopic pregnancy diagnosis were 1.2 for the copper/zinc ratio, 70 µg/dL for zinc, and 76 µg/dL for copper. The cut-off points showed that the copper/zinc ratio has an 82% sensitivity and a 76% specificity in diagnosing ectopic pregnancy. **Conclusion:** Ectopic pregnancy patients show decreased copper levels, increased zinc levels, and a decreased copper/zinc ratio, making these non-invasive tests useful for diagnosis.

KEYWORDS: Copper-Zinc Ratio, Ectopic Pregnancy, Progesterone.

INTRODUCTION

Ectopic pregnancy is a condition where the blastocyst implants in the endometrial lining of the uterine cavity after fertilization and fallopian tube transit. It occurs in 97-98% of ectopic pregnancies within the fallopian tube, with up to 7% outside the tube.[1] Heterotopic pregnancy is defined as multiple gestations. In the western world, the prevalence is around 2%, but can be as high as 20% in patients with tubal surgery or previous ectopic pregnancy.[1,2] In the United States, the incidence is estimated at 1% to 2%. Ectopic pregnancy accounts for 2.7% of pregnancy-related deaths. Acquired defects, intrauterine contraceptive device failure, smoking, previous pelvic surgery, infertility history, in vitro fertilization and embryo transfer, endometriosis, emergency contraception failure, and strenuous physical exercise are other risk factors. Ectopic pregnancy is one of the top leading causes of maternal mortality in the first trimester and accounts for 10-15% of all maternal deaths.[3,4]

Copper, a ductile metal with high thermal and electrical conductivity, is crucial for the human body's functioning due to its interaction with metabolic processes like haemoglobin synthesis, neurotransmitter function, iron oxidation, cellular respiration, antioxidant peptide formation, pigment formation, and connective tissue formation.[5] It is also essential for growth, defense mechanisms, bone mineralization, maturation of red and white blood cells, iron transport, cholesterol metabolism, myocardial contractility, glucose metabolism, and brain development. Copper deficiency can result from major burns, renal replacement therapy, parental nutrition, and gastric bypass procedures. It is primarily absorbed in the duodenum, with some absorption in the stomach and distal part of the small intestine.[5,6]

Zinc is the second most abundant trace element, essential for all living organisms, with antioxidant and anti-inflammatory properties. It regulates T- and B-lymphocyte function, maintaining normal immune function and resistance to infection. Zinc is found in over 2700 enzymes and is present in almost all body cells.[7,8] Zinc deficiency can occur due to inadequate diet, absorption, altered demand, or increased loss. Zinc homeostasis is primarily regulated by intestinal absorption, with zinc transporters on the apical and basolateral membrane of enterocytes engaging and regulating cellular and body zinc homeostasis. Zinc transport proteins are essential for its physiology, divided into zinc-regulated transporters (Zrt)/iron-regulated transporter (Irt)-related proteins (ZIPs) and transporters (ZnTs). Plasma zinc levels in healthy individuals range from 12 to 16 µM, representing less than 1% of whole-body zinc. Serum is a rapidly exchangeable zinc pool, while skeletal muscle and bone contain zinc with lower turnover and slower availability for systemic zinc homoeostasis.[9]

Copper and zinc homeostasis is linked to iron and zinc homeostasis, with dysregulation of one metal potentially affecting the others. Copper plays a role in ferroxidases, while zinc inhibits copper absorption, leading to hypo-cupremia. Deficiency can disrupt cellular functions, affect growth, metabolism, and immune system. High copper/zinc ratios are associated with inflammation, infection, aging, stress, and chronic degenerative diseases.[10-12]

Progesterone is a crucial hormone in reproduction, produced by the adrenal cortex and gonads. It drives anterior pituitary production of gonadotropins, including luteinizing hormone and follicle-stimulating hormone, which promote follicle development, ovulation, and the formation of the corpus luteum. Progesterone is produced by the corpus luteum for the first 10-12 weeks of gestation and the placenta and adrenal glands for the rest of the pregnancy.[13] It can bind to the progesterone receptor, which slows luteinizing hormone pulses. Progesterone plays a crucial role in maintaining normal pregnancy through mechanisms such as modulating maternal immune response, suppressing inflammatory response, reducing uterine contractility, and improving uteroplacental circulation and luteal phase support. Serum progesterone has been used as an adjunct to β-hCG levels and ultrasound, but its usage is limited due to difficulties in interpretation and correlation with clinical findings.[14] The current study aimed to evaluate the role of serum copper, zinc, copper/zinc ratio, and progesterone level as a biomarker for the diagnosis of ectopic pregnancy.

PATIENTS AND METHOD

A case-control study was conducted in the Department of Gynecology and Obstetrics/ Al-Emamain Al-Kadhymain Medical City/ Baghdad/ Iraq during the period from the 1st of January 2022 to the 1st of January 2023.

A sample of 100 pregnant women was enrolled in the current study and consisted of two groups.

1. **Study group:** Consisted of 50 pregnant women who were admitted as a case of ectopic pregnancy.
2. **Control group:** Consisted of 50 healthy pregnant women matched with the case group for age, body mass index (BMI), and gestational age.

Women between the ages of 18 and 44 with a BMI of 18.5–30 kg/m^2 and a gestational age of 6–8 weeks are the study's target participants. Chronic drug use, women who refuse participation, smokers, patients on copper or zinc supplements, patients with a history of pelvic/genital surgery, endometriosis, prior ectopic pregnancy, or pelvic inflammatory illness are among the exclusion criteria. The goal of the study is to offer insightful information on how lifestyle decisions affect health. Age, parity, gravidity, gestational age, prior medical history, and past surgical history were among the information gathered from the patient's medical history. The BMI was computed using the following formula once the weight and height were measured: BMI=weight (Kg)/(height (m)).[2]

Each participant provided a sample of 10 ml of blood, which was centrifuged for 10 minutes at 4000g. The serum samples were sent to a private laboratory for measurement of the levels of progesterone, copper, and zinc after being kept at -80 °C. Following 1/4 dilution with 5% glycerol for determining the zinc level and 1/2 dilution with 10% glycerol for determining the copper level, the copper and zinc measurements were carried out using the flame spectrophotometry technique. Every participant received assurances on information confidentiality and anonymity. The study's purpose was explained to the women, and their verbal agreement was obtained.

Statistical analysis

The statistical package of social science version 22 and Microsoft Excel 2016 were used for data entry and analysis. The descriptive analysis concentrated on percentages and frequencies. Mean (± Standard Deviation (SD)) was used to represent continuous variables. To determine if the differences between the groups were significant, the Chi-Square and t-tests were employed. P-values below 0.05 were regarded as statistically significant.

RESULTS

There were no significant differences between the healthy pregnant women and those with ectopic pregnancy regarding age and BMI (P-values were 0.176 and 0.372 respectively). As shown in table 1.

Table 1: Age and body mass index distribution.

Age and body mass index	Study group	Control group	P-value*
Age (years) Mean± SD	29.04±5.2	27.60± 5.2	0.176
Body mass index (kg/m^2) Mean± SD	24.64±2.2	25.06±2.4	0.372

**t-test*

There were no significant differences between healthy pregnant women and those with ectopic pregnancy regarding gravidity, abortion, and gestational age (P-values were 0.891, 0.458, and 0.122, respectively), as shown in table 2.

Table 2: Distribution of the gravidity, abortion and gestational age according to the study groups.

Obstetrical history		Study group	Control group	P-value
Gravidity No. (%)	≤3	21 (42.0)	19 (38.0)	0.891*
	4-6	25 (50.0)	26 (52.0)	
	≥7	4 (8.0)	5 (10.0)	
Miscariage No. (%)	0	38 (76.0)	42 (84.0)	0.458*
	1	7 (14.0)	6 (12.0)	
	2	5 (10.0)	2 (4.0)	
Gestational age (weeks) Mean±SD		6.54±0.37	6.42±0.36	0.122**

**Chi-Sauqre test; ** t-test*

There was a significant difference between healthy pregnant women and those with ectopic pregnancy regarding the progesterone level (P-value was <0.001). There was no significant difference between healthy pregnant women and those with ectopic pregnancy regarding the haemoglobin and β-hCG levels (P-values were <0.707 and 0.136, respectively). As shown in table 3.

Table 3: Distribution of the haemoglobin and hormones.

Investigations	Study group	Control group	P-value
Haemoglobin (g/dL) Mean ±SD	11.5±0.7	11.4± 0.6	0.707
Progesteron (ng/mL) Mean± SD	8.3±1.3	36.0±6.7	**<0.001**
β-hCG (mIU/mL) Mean ±SD	4081.4±718.5	4304.6±764.7	0.136

**t-test*

The copper level was significantly lower in pregnant women with ectopic pregnancy than in healthy pregnant women (P-value was <0.001). The zinc level was significantly higher in pregnant women with ectopic pregnancy than in healthy pregnant women (P-value was <0.001). The copper/zinc ratio level was significantly lower in pregnant women with ectopic pregnancy than in healthy pregnant women (P-value was <0.001). As demonstrated in table 4.

Table 4: Distribution of the investigation results according to the study groups.

Investigations	Group A	Group B	P-value*
Copper (µg/dL) Mean (±SD)	67.0±7.3	84.1±10.1	**<0.001**
Zinc (µg/dL) Mean (±SD)	75.8±9.6	59.7±8.8	**<0.001**
Copper/Zinc ratio Mean (±SD)	0.91±0.23	1.44±0.28	**<0.001**

** t-test*

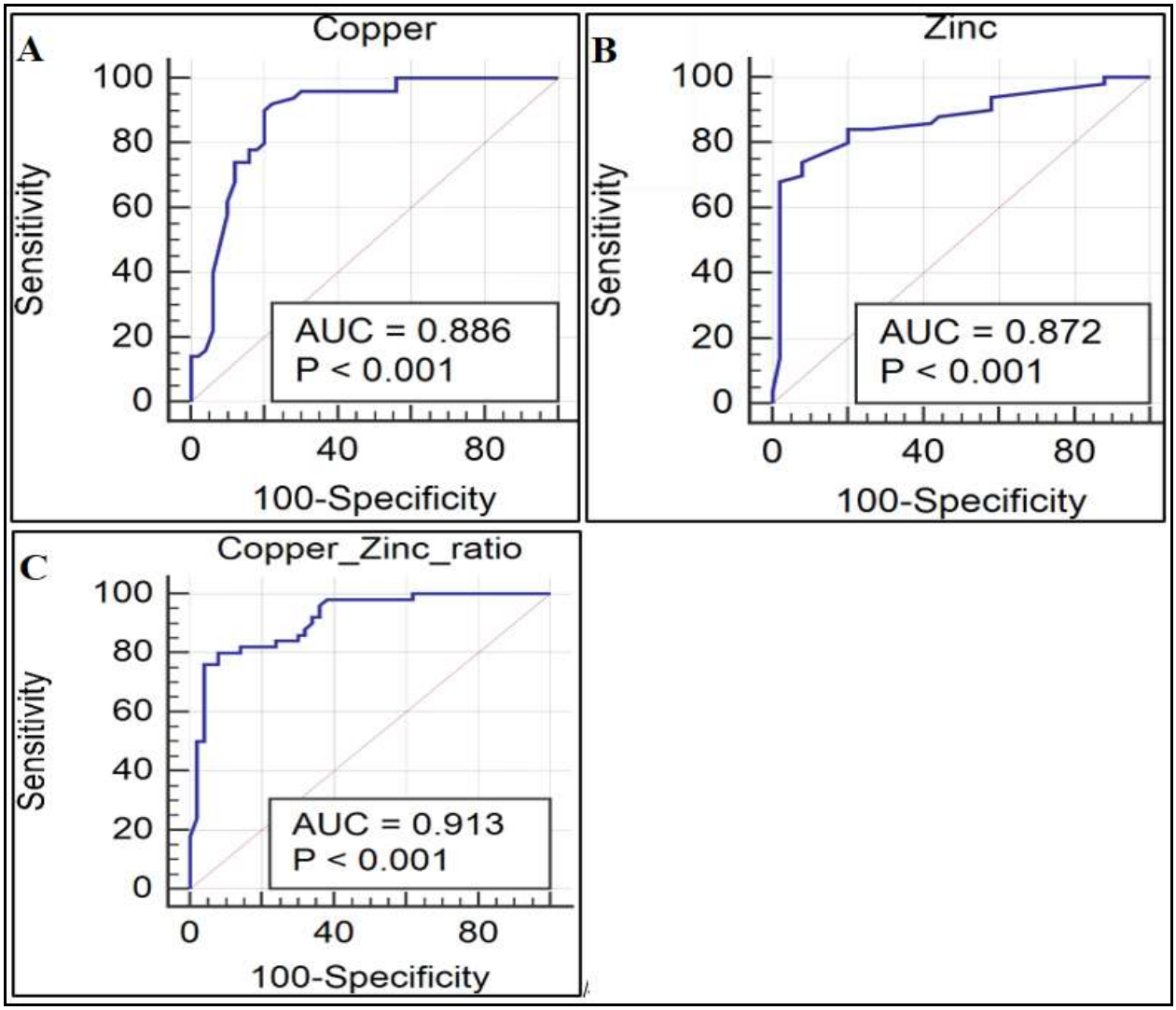

Figure 1: Receiver operating characteristic curve for diagnosis of ectopic pregnancy. A: copper, B: Zinc, C: Copper/zinc ratio.

As shown in figure 1, according to the Receiver operating characteristic (ROC) curve, the best cut-off points for diagnosis of ectopic pregnancy were 76 Copper µg/dL for copper, 70 µg/dL for zinc, and 1.2 for copper/zinc ratio. According to these cut-off points, the sensitivity, specificity, positive predictive value, and negative predictive value of the copper/Zinc ratio in the diagnosis of ectopic pregnancy were 82%, 76%, 77.3%, and 80.8%, respectively, as shown in table 5.

Table 5: Accuracy of the copper, zinc, and copper/zinc ration.

Investigations		Stduy group	Control group	Total
Copper	Abnormal (<76 µg/dL)	45 (90.0)	10 (20.0)	55 (55.0)
	Normal (≥76 µg/dL))	5 (10.0)	40 (80.0)	45 (45.0)
Sensitivity=90%, Specificity=80%, Positive predictive value=81%, Negative predictive value=88%, P-value=0.001				
Zinc	Abnormal (>70 µg/dL)	41 (82.0)	10 (20.0)	51 (51.0)
	Normal (≤70 µg/dL)	9 (18.0)	40 (800)	49 (49.0)
Sensitivity=82%, Specificity=80, Positive predictive value=80%, Negative predictive value=81%, P-value=0.001				
Copper/ Zinc ratio	Abnormal <1.2	41 (82.0)	12 (24.0)	53 (53.0)
	Normal ≥1.2	9 (18.0)	38 (76.0)	47 (47.0)
Sensitivity=82%, Specificity=76%, Positive predictive value=77.3%, Negative predictive value=80.8%, P-value=0.001				

DISCUSSION

There were insignificant differences between women with ectopic pregnancy and healthy pregnant women regarding age and BMI. This resulted from matching between the study group and control group regarding these variables. No significant difference was obtained between healthy pregnant women and those with ectopic pregnancy regarding gravida and abortion. In agreement, Wang et al. concluded an insignificant association between gravida and ectopic pregnancy.[15]

In the current study, there was no significant difference in the haemoglobin level between the study group and the control group. In agreement, the results of eight women with unruptured ectopic pregnancy who were enrolled in another study in Australia revealed normal haemoglobin levels.[16] Ciprian et al. reported a case of ectopic pregnancy with a normal haemoglobin level (13.4 g/dL).[17] These results might be due to that most of the patients in the present study were stable on admission, except one who was haemodynamically unstable on admission.

Detecting fFN in amniotic fluid discharges as an indication of disruption of the choriodecidual interface, which provides a clue to the contraceptive technique that promotes vaginal birth. Compared to the Bishop score, the fFN test is a better predictor of a successful induction of labor. A positive fibronectin test has a sensitivity of 76% and a specificity of 60.3%. While Nagihan et al. reported that serum β-hCG levels in the normal pregnancy group were statistically significantly higher than in ectopic pregnancy.[19] This probably contributed to the difference in the number of patients between different studies.

Only 30% of ectopic pregnancies have a normal β-hCG course throughout a typical pregnancy, according to Alkatout et al. A-hCG levels grow more slowly and plateau or even show a decline in serum levels in 70% of ectopic pregnancies. An ectopic or no longer intact gestation is strongly suggested by an aberrant β-hCG pattern.[20] Probably most cases may belong these 30% of ectopic pregnancies which had normal β-hCG may be implanted in the vascular area.

The current study's key conclusion was that the case group's progesterone levels were noticeably lower than those of the control group. In contrast, a different research conducted in China by Chun et al. found that patients with ectopic pregnancy had considerably lower levels of progesterone than those with normal pregnancies.[21] This agreed with another study that was done by Stephen et al. in the United Kingdom which concluded that serum progesterone has been shown to be lower in ectopic pregnancy compared to a viable pregnancy.[22] In the same line, Nagihan et al. concluded that serum progesterone levels in normal pregnancies were statistically significantly higher than the levels in an ectopic pregnancy.[19] In addition, Fatmir et al. revealed that the progesterone level in ectopic pregnancy is lower than in normal intrauterine pregnancy.[23]

The study's primary findings were that patients with ectopic pregnancy had significantly lower serum copper, significantly higher serum zinc, and significantly lower copper/zinc ratios than those with normal pregnancies. Similar results were found in another study conducted in Turkey by Abdullah et al., which revealed that ectopic pregnancy cases had considerably higher serum zinc levels and lower serum copper levels than healthy pregnant control cases. Furthermore, a substantial drop in the serum copper/zinc ratio was seen, which might potentially aid in the detection of ectopic pregnancy cases.[24]

CONCLUSION

Ectopic pregnancy is a condition where the blood levels of copper, zinc, copper/zinc ratio, and serum progesterone are significantly lower than normal pregnancy. Copper levels decrease below 76 µg/dL, while zinc levels increase above 70 µg/dL. The copper/zinc ratio also decreases, with a cut-off point of 1.2. Serum progesterone levels also decrease in ectopic pregnancy patients compared to normal pregnancy. These findings can be used as valuable markers for ectopic pregnancy diagnosis.

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