ORIGINAL RESEARCH

Correlation between Maternal Serum Copper levels and Congenital Malformed foetus in pregnancy

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Abstract
Structures or functions that are aberrant at birth are referred to as congenital anomalies or malformations. 7% of infant fatalities are caused by congenital abnormalities. During pregnancy, trace elements may have a significant impact. Although copper deficiency and occasional excess are linked to a number of illnesses, it is unclear how these factors affect foetal development in utero. Quantitative analysis of trace element copper can identify whether cu deficiency or cu excess is related to congenital abnormalities. This study aimed to compare the copper (Cu) content in serum samples from mothers carrying healthy and congenitally deformed foetus. In the current study, 200 antenatal women participated, with 100 of them carrying a congenitally deformed foetus and 100 of them carrying a normal foetus at a comparable gestational age. A history and clinical examination were performed on all antenatal women. Blood samples were taken, and copper levels in µg/dl were determined using a colorimetric kit. Based on Quantitative analysis, the difference between the mean serum copper levels in the both groups was statistically significant (p 0.001), and it was 110.3$\pm$23.9 g/dl and 208.6$\pm$29.6 µg/dl, respectively with a normal reference range is 80-155µg/dl. The odds ratio found to be more than 1. The development of foetal congenital abnormalities may be significantly influenced by copper deficiency during conception or embryogenesis. The quantitative serum concentration of copper is of diagnostic significance in depicting congenital aberrations.

Keyword: Serum copper level, Trace element, Congenital malformation, Pregnancy, CNS malformation, Anencephaly, micronuterients, structure abnormality

Introduction

Congenital anomalies are abnormalities in structure or function (metabolic diseases) that develop during intrauterine life and can be seen during pregnancy, at birth, or in certain circumstances, may not be seen until later in infancy, such as hearing problems. About 7% of newborn mortality and between 25.3 and 38.8 million disability years of adjusted life years are caused by congenital anomalies [1]. In India, there were 182 congenital abnormalities for every 10,000 live births [2]. Genetic predispositions, diet, environment, socioeconomic, and demographic factors are all part of the multifactorial aetiology. Congenital abnormalities still have a 50% aetiology that is unknown [3]. Foetal growth and development are directly impacted by maternal nutrition. Copper (Cu), zinc, calcium, magnesium, iron, and iodine are just a few examples of trace elements that have been linked to adverse effects [4]. Copper is a necessary micronutrient for healthy physiology. The daily need for copper rises to 1 mg during pregnancy [5]. It plays an important role in the antenatal period as shown in. Cu is necessary for collagen metabolism, iron transport control, and bone formation. Cu plays a significant part in the synthesis of collagen and elastin; their lack results in a decrease in the tensile strength of the foetal membranes, which causes early membrane rupture and preterm births [6]. Additionally, Cu functions as a coenzyme in oxidation-reduction reactions, RNA synthesis, melanin formation, and fatty acid metabolism [7]. Pancytopenia and hypodermic anaemia caused by Cu

deficiency are resistant to iron administration [8]. Moderate serum copper levels are crucial for bone health. Lower copper levels are strongly linked to decreased bone mineral density, while higher levels are linked to an increased risk of bone fracture [9]. Several mechanisms have been hypothesised as causing teratogenicity linked to copper deficiency. These include direct mechanisms like altered extracellular matrix, for example, low lysyl oxidase activity, excessive oxidative damage, for example, low superoxide dismutase activity, lowered cytochrome-c oxidase activity, and impaired energy production. Indirect mechanisms such as copper deficiency-induced changes in maternal metabolism. Although it may be uncommon for a primary copper deficiency to manifest during pregnancy, secondary or conditioned deficiencies can develop for a variety of reasons, including genetic factors that may affect an individual's requirement, nutritional interactions, drugs, chemicals, and a variety of disease states that can result in conditioned deficiencies. Menkes syndrome (progressive degeneration of the brain and spinal cord, hypothermia, failure to thrive), and occipital horn syndrome, both of which result from mutations in the copper-transporting ATPase gene, are examples of genetic illnesses in humans that can affect antenatal development [8,11]. Huppke-Brendel syndrome is distinguished by hypoplasia, hypomyelination, and psychomotor impairment; this is likely because the ATP7A protein is not acetylated. Patients with extremely low levels of ceruloplasmin and cu pass away in infancy [12]. A mutation in a gene on chromosome 7's long arm causes MEDNIK syndrome, another illness of cu metabolism. Patients experience hearing, mental and physical dysfunction, severe keratosis, and epidermal exfoliation, among other dermatological abnormalities [13]. The adverse effects of too much copper include delayed development, deformities, and spontaneous miscarriages [14]. A high copper level can result in macrosomia, intrauterine hypotrophy, birth abnormalities, and miscarriages in addition to gestational diabetes 15]. Reactive oxygen species (ROS), which are produced when there is an excess of copper, can react and cause oxidative stress and cellular damage [16].As there are insufficient data to support the danger, the link between maternal copper concentrations and birth abnormalities in offspring is uncertain.

2. Materials and Methods

2.1. Design of study

The current study involved 200 pregnant patients who visited the Department of Obstetrics and Gynaecology over the course of a year at Pt. B. D. Sharma PGIMS, Rohtak. It was an observational case-control study. This study was according to Strobes checklist. The Pt.B.D.SHARMA Post Graduate Institute of Medical Sciences, UHS, Rohtak, Biomedical Research Ethics Committee, and these institutions gave their approval for this study.

Sample Size Calculation-

Considering mean (±SD) serum copper level in mothers of congenitally malformed babies (cases) and normal pregnant women (control) as µg/L (2831.1 microg/L (+/- 1017 g/L vs. 2402 microg/L vs. 1035.7µg/L±299.8µg/L P=0.004, Confidence interval (CI) of 95 percent, power 80 per cent, based on a study conducted in 2009[20], the sample size in each group will be estimated to be 68 patients and rounded to 70 which http://www.openepi.com/SampleSize/SSCohort.htm calculates. We include a sample size of 100 in the present study group.

2.2. Research Participants

As a study group, 100 women carrying congenitally deformed foetuse in the second or third trimester of pregnancy (i.e., before labour induction or during the early stages of labour) were included. As a control group, there were 100 more women in the same region carrying healthy foetus with comparable gestational ages.

2.3. Exclusion criteria

1. Pregnant women with medical complications such renal disease, trophoblastic disease, heart disease, chronic hypertension, diabetes mellitus, multiple pregnancies, urinary tract infections, or epilepsy
2. Any patient taking copper dietary supplements.
3. Women with any other obstetric complications like GDM, PIH, APH
4. Women with a history of smoking and alcohol

2.4. Methodology

Before selecting the study participants, detailed history and a comprehensive clinical examination were conducted. With the patient's informed written consent, a questionnaire was used to collect personal and clinical data including age, gestational age, socioeconomic status, education, dietary habits, and occupation. All of the study participants' blood was drawn for normal laboratory tests such haemoglobin, blood group, HIV status, TSH, and GCT, as well as specific tests like serum copper. When women were enrolled in the trial, 6ml of venous blood was drawn into red vacutainers and labelled with their names and hospital identification numbers. Serum was separated by centrifugation at 3000 rpm for 10 minutes after the blood had been allowed to clot at room temperature for 30 minutes. Centrifugation was used to separate the serum, which was kept at -20°C until analysis. Copper levels in samples were measured using colorimetric kits and expressed as g/dl. The information was gathered and statistically examined. Using the unpaired Student t-test and Chi-square test, results were presented as means and standard

deviations. Odds ratio and p-value were calculated. A 0.05 p-value was regarded as significant. For statistical analysis, the Statistical Package for Social Sciences (SPSS) version 21.0 was utilised.

3. Results

The demographic breakdown of the research and control groups is shown in Table 1. The majority of the women in the current study, 52 (52%) in the study group and 54 (54%) in the control group were between the ages of 21 and 25. The study group's average age was compared to the control group, it was 23.92.8 years against 24.73.7 years. There was no discernible difference between the two groups. In the study group, 53 (53%) of the women were nulliparous to a maximum of 62 (62.0%) in the control group. In both categories, the majority of the women were unbooked. Both groups shared similar socioeconomic, educational, and employment statuses. 97% of the women in the control group and all of the subjects in the study group were stay-at-home. In the control group, there were only 3 (3.0%) female employees. The study group's mean BMI was determined to be 20.9 1.8 kg/m2, while the control group's mean BMI was 21.3 1.8 kg/m2. In terms of BMI, there was no statistically significant difference between the two groups. No women in the study group or the control group had a history of radiation exposure, any congenital abnormalities, or a fever illness during the first trimester. There were only 5 (5.0%) women in the study group who reported having consumed folic acid before conception while there were none in the control group. According to Table 2, out of the 100 women in the study group, congenital malformations most frequently affected the central nervous system (CNS) (74%), followed by the gastrointestinal tract (6%), numerous congenital defects (4%), and the cardiovascular system (2%). The majority of CNS abnormalities, 39% of which were anencephaly, were neural tube anomalies.

Table 3. shows a comparison of the study group's and control group's serum copper levels. The difference between the mean serum copper levels in the study group and the control group was statistically significant (p 0.001), and it was 110.323.9 g/dl and 208.629.6 g/dl, respectively. When compared to other deformity categories, copper levels in patients with CNS abnormalities were considerably lower (p 0.05).

Table 3 displays, 22 women in study group had s.copper level less than 80 g/dl that is the lowest value in normal range (80-155g/dl) of s. copper.The odds ratio with value is 57.6115, 95%CL is 3.4409 to 964.6037, z statistic is 2.819 and p value is 0.0048. 56 women in study group had s. copper level less than 115g/dl that is middle value in normal range. The odds ratio with value is 255.2022, 95%CL is 15.402 to 4223.5542, z statistic is 3.871 and p value is 0.0001. Only 3 women in study group have copper levels more than 155g/dl that is the highest value of normal range.The Odds Ratio >1 (more than 1) signifies that lower serum copper level is strongly associated with gross congenital anomaly in antenatal women.

Table 1.
Sociodemographic Distribution in study and control group

	Study Group (100)	**Control group(100)**	**p-value**
Age (years) <21	19 (19%)	16 (16%)	
Age (years) 21-25	52 (52%)	54 (54%)	0.916
Age (years) 26-30	25 (25%)	27 (27%)	
Age (years) 31-35	4 (4%)	3 (3%)	
Mean ± SD (Range)	24.7±3.7 (18 – 35)	23.9±2.8 (20 – 34)	0.087
Median (IQR)	25 (22 – 27)	25 (21 – 26)	0.529
Parity (P0)	53 (53%)	62 (62%)	
Parity (P1)	28 (28%)	30 (30%)	
Parity (P2)	17 (17%)	0 (0%)	
Parity (P3)	1 (1%)	8 (8%)	
Parity (P4)	1 (1%)	0 (0%)	
Booked	21 (21%)	36 (36%)	p – value = 0.019
Unbooked	79(79%0	64(64%)	Chi Square Value =
Total	57(28.5%)	143 (71.5%)	5.521
Body Mass Index (BMI) (Kg/m^2)	20.9 ± 1.8	21.3± 1.8	P value 0.116

Both study and control group are comparable

Table: 2 Distribution of fetal malformations among study population (n=100)

System affected	CNS	Renal	GIT	CVS	Fetal hydrops	genital	Others
percentage	74%	11%	6%	2%	2%	1%	4%

Table : 3 Mean values of Serum Copper levels in study and control.

Variable	**Study group(n=100)**	**Control group (n=100)**	**P-value**
Mean Copper level (µg/dl)	110.3±23.9	208.6±29.6	<0.001
Range	70-168	166.1-263.9	
a. odds Ratio	255.2022		0.0001
95%CL	15.402 to 4223.5542		
z statistic	3.871		
b. odds Ratio	57.6115		
95%CI	3.4409 to 964.6037		
z statistic	2.819		

Discussion

Copper is an essential micronutrient which plays a key role in embryonic and fetal development. Variations in copper levels in pregnancy have been to be associated with fetal malformations in the development of the embryo and foetus. Pregnancy-related variations in copper levels have been linked to foetal abnormalities. The goal of the present study was to determine whether there was any correlation between maternal copper levels and foetal congenital abnormalities. The central nervous system (CNS) accounted for 74% of the congenital malformations identified in the current study, followed by renal abnormalities (11%) gastrointestinal tract (6%), multiple congenital defects (4%) and cardiovascular system (2%). The results of the present research were in line with those of Sachdeva et al., who examined gross congenital malformations at a government hospital and discovered that the incidence of GCMF was 16.4 per 1000 consecutive singleton births (>28 weeks), with the three most common malformations being anencephaly (44.68%), talipes equinovarus (17.02%), and meningomyelocele (10.63%).[3] Bhinde et al. examined the prevalence of congenital abnormalities in a South Indian mother cohort in Pune, in contrast to the current study. Congenital heart defects (CHDs) were the most prevalent, according to the data. The rate of musculoskeletal malformations was 49.40 per 10,000 newborns, and the rate of urinary system malformations was 38.42 per 10,000 births. Less commonly, abnormalities in the respiratory system, genitalia, and digestive system were observed [17].The difference between the mean serum copper levels in the study group and the control group was statistically significant (p 0.001) at 110.323.9 g/dl and 208.629.6 g/dl, respectively. According to Buamah et al., who calculated the levels of copper in maternal serum during 244 normal and 15 abnormal pregnancies, the mean levels of copper in maternal serum were lower in anencephalic pregnancies (mean 25.57.7 mol/L) than in normal pregnancies (32.55.5 mol/L) overall. The difference between the two groups t-test analysis results was p 0.001, which is very significant. Therefore, a lack of copper may contribute to the aetiology of congenital malformations [18]. Karolina Rak and colleagues looked at the correlation between the levels of specific mineral elements in pregnant women's blood and the levels of 30 nitrotyrosine (30NT), a marker of oxidative stress, in babies' umbilical cord blood. A statistically significant negative correlation was observed between the concentration of Cu in the maternal serum and the concentration of 3′NT in the Umbilical cord serum, but only in male newborns [19].In research by Zeyrek [20] et al. and Cengiz [21] et al., in contrast to the present study, it was discovered that mean copper content was greater in mothers of congenitally deformed foetuses than in normal pregnant women. When Wilson et al. examined the plasma levels of trace elements (Cu, Zn, Se, and Fe) in pregnant women at 15 weeks and monitored them for pregnancy complications, they observed that the mean plasma copper level at 15 ± 1 weeks' gestation was higher in women whose pregnancies became complicated compared to those whose pregnancies remained uncomplicated.. Compared to pregnant women whose Cu blood concentration was within the 1st and 2nd tertile, those with higher blood Cu levels (3rd tertile) had a significantly increased risk of prenatal problems (pre-eclampsia, gestational diabetes, early birth, and/or SGA) [22].

Conclusion

In the current investigation, it was discovered that the study group's serum copper levels were significantly

lower. The development of foetal congenital abnormalities may be significantly influenced by copper deficiency during conception or embryogenesis. Therefore, it can be recommended that testing for copper levels during pregnancy be done in order to gain more knowledge about the aetiology of congenital abnormalities. As the odds ratio in this stduy, we can recommend serum copper in all antenatal women who had history of congenital malformations in her previous pregnancies if we find copper deficiency we correct it timely. We recommend more large scale research for the effect of copper not only in congenital malformed fetus but also in developing other medical disorder/conditions (pre-eclampsia, gestational diabetes,SGA) in antenatal women that is great contribution in public health to find out causative factor in all above mention conditions. This study has certain restrictions. Due to its limited sample size, it is unknown how much copper was present during the prenatal and postpartum periods of embryogenesis. The findings of the current study offer hints for investigating the potential etiological significance of copper in foetal congenital malformations and identify a promising topic for future treatment trials in pregnant women carrying congenitally deformed foetus. The cause-and-effect association between copper levels and congenital abnormalities should be confirmed through multicentric large-scale research including geographic differences.

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