Original Article

Maternal Serum Zinc level in pregnant women with second trimester induced abortion with anencephalic fetus

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Summary:

Background: Neural tube defects (NTD) are group of heterogeneous and complex congenital anomalies of the CNS. Commonly included in this group anencephaly, spina bifida and encephaloceles. Anencephaly is the most severe defect; it is always lethal and results in stillbirth or early neonatal demise, is characterized by absence of the brain and cranium above the base of the skull and orbits

Objective: The objective of this study is to assess the relationship between maternal serum zinc level and anencephaly occurrence in women with second -trimester induced abortion due to anencephalic fetus.

Study design and setting: This study is a case- control study, carried out in Baghdad teaching hospital throughout the period between November 2008- November 2009.

Patients and method: This study involves 33 pregnant women whose pregnancies were terminated for anencephaly in the second-trimester that was diagnosed by ultrasound the control group consisted of 66 matched to demographic and obstetrical characteristics and had normal ultrasonographic findings in second-trimester with documented normal fetal outcome. Zinc level determination was made using flame atomic absorption spectrophotometer (AAS).

Results: Medication consumption during the first trimester of pregnancy has a significant relation with the occurrence of NTDs with a p – value = 0.027.

Low serum Zinc level was found in 18 (54.5%) of the cases and 5 (7.6%) of the controls with p- value = 0.0001. There was a highly significant relationship between the presence of NTDs and low serum zinc level.

Conclusions: In this study there was a significant association between NTDs and low serum zinc levels, adding to the evidence about the importance of nutritional and maternal health factors in the etiology of this disease.

Keywords: Neural tube defects, second trimester abortion, serum zinc level.

Introduction:

Neural tube defects (NTD) are a group of heterogeneous and complex congenital anomalies of the CNS. Commonly included in this group anencephaly, spina bifida and encephaloceles. (1) .The incidence of neural tube defects varies from 1/100 live births in certain regions of china to about 1/5000 live birth in Scandinavian countries (2). Worldwide their incidence is 1.4 to2 per 1000 live birth (3). Anencephaly is the most severe defect, it is always lethal and results in stillbirth or early neonatal demise, characterized by the absence of the brain and cranium above the base of the skull and orbits. Cephalocele is a herniation of meanings and brain tissue through a defect in the cranium, typically an occipital midline defect. Spina bifida occurs when one or more vertebral arches fail to fuse, so that meanings (meningocele) or neural tissue plus meanings (meningomyelocele) protrude under the skin (3). Embryonic development of the neural tube is that the entire neural tube formation is completed during Carnegie stage 11 and 12, within a span of about 10 days starting from post fertilization day 18. (4) This critical window of neural tube closure is the period during which any primary failure of neural tube closure must occur. (5) Development of the neural tube is a multi-step process strictly controlled by genes and modulated by a group of environmental factors. It involves gene–gene, gene–environment and gene–nutrient interactions. Despite years of intensive epidemiological, clinical and experimental research, the exact etiology of NTD remains rather complex and poorly understood. However, it is generally agreed that most NTD cases are of multifactorial origin, having a significant genetic component To this etiology that interacts with a number of environmental risk factors (6, 7, and 8) the role of maternal nutrition on normal fetal development and growth is well known. Nutritional status of the parents can affect the quality of the gametes and fertilization capacity (9). A recent study also suggested that periconceptional intake of zinc can reduce NTD risk, and other study reported methionine use decreases NTD risk (10, 11). Maternal serum alfa fetoprotein(AFP)has been used as a method of screening for NTD in areas with a high incidence of these malformations detailed ultrasound must be performed (12). Inability to obtain a view of the biparietal diameter raises the

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suspicion of anencephaly and if visualization is adequate in the 2nd trimester, anencephaly can be diagnosed in virtually all cases (3). Spina bifida, on the other hand, requires the systematic detailed examination of the fetal spine (8). Zinc is a trace element and it is essentiality for growth and well-being of plants and animals is well established...Normal value of serum zinc is 80-120 µg /dl (10.7-18.4 µmol /L) in adult human. (13) Zinc deficiency most often occur when zinc intake is inadequate or poorly absorbed ،or when the body̓ s requirement for zinc increases (14, 15) ·Signs of zinc deficiency include growth retardation, delayed sexual maturation and impotence, eye and skin lesions, and loss of appetite. (16) In the U.S.A ،.the Dietary Reference Intake (DRI) for zinc is 8 mg/ day for women. Pregnancy increases zinc requirements by 3 mg/d. (17) Zinc is an integral component of nearly 300 enzymes in different species of all phyla (18).Zinc has close interrelationship with the endocrine system. It is essential for growth and reproductive function. Zinc also plays a major role in protein synthesis .Severe zinc deficiency causes inhilution of immune function (19). In normal pregnancy, maternal serum zinc concentration varies with the gestational age and it declined progressively with each trimester (20, 21). Zinc deficiency during pregnancy can negatively affect both the mother and fetus (22).

Patients and methods:

This study was designed as a case- control studyconducted at the department of obstetrics and gynaecology of Baghdad Teaching Hospital from first of November 2008 till first of November 2009 . The case group comprised 33 pregnant women whose pregnancies were terminated because of second-trimester ultrasonic diagnosis of neural tube defects (anencephaly). The control group consisted of 66 women who were selected according to matching demographic and obstetrical characteristics and had normal ultrasonic findings in second-trimester ultrasound of normal fetal development. Inclusion criteria involve all pregnant women who had induced abortion in their second trimester for fetal NTD diagnosed by ultrasound in the period of this study while exclusion criteria include women who had history of epilepsy with or without antiepileptic treatment, diabetics, anemic patients and those who did not receive their folic acid supplementations .The collection of data for each patient is done by direct interview using Questionnaire including, demographic factors: as mother's age, BMI(BMI was measured for every woman in the case and control groups at the same time of collection of blood sample by using the BMI equation , body weight in kg / height in m^2. Obstetrical history, i.e. Parity & previous history of abortions , family history of congenital anomalies and mother's history of exposure to any type of drug during the first trimester of pregnancy .

Measurement of serum Zinc:Three milliliters of venous blood were obtained from each case and control women by venipuncture using disposable syringes at the time of interview in the 2nd trimester. Blood was collected in a disposable plastic tube and left to stand at room temperature for (15) minutes. Tubes were then centrifuged and sera were separated immediately using disposable pipette. The serum of each individual sample was put in sterile tube and was stored at -20º c. Serum zinc was measured by flame atomic absorption spectrophotometry Values below 70 µg/dl were used for the statistical analysis of this parameter.

Results:

We studied 33 pregnant women with NTD fetuses in their second trimester compared with 66 pregnant women with healthy fetuses as controls. Table (1) shows the demographic distribution of cases and control

Table. 1: Demographic distribution of cases and controls

Maternal age (years)	NTD cases		Controls		P value
	No	%	No	%	
<18	-	-	3	4.5	0.369
18—24	9	27.3	24	36.4	
25—29	5	15.2	9	13.6	
30—34	14	42.4	17	25.8	
≥ 35	5	15.2	13	19.7	
Mean±SD (Range)	28.97±6.71 (18-47)		27.08±7.20 (15-47)		
Parity	NTD cases		Controls		P value
	No	%	No	%	
Multiparity (≥4)	12	36.4	13	19.7	0.072
P 0-3	21	63.6	53	80.3	
Mean±SD (Range)	3.06±2.60 (0-11)		1.83±1.97 (0-7)		
BMI (Kg / m2) Mean±SD (Range)	27.09 ± 8.794		26.23 ± 8.754		0.942

Table2: show the relation between history of drug intake, previous abortion, and previous congenital anomalies in both groups:

Exposure to drugs	Cases	Controls		P - value	
	No.	%	No.	%	
Not exposed	28	84.8	64	97	0.027 (s)
Exposed	5	15.2	2	3	
Salbutamol	1	3.03	-	-	-
Methyldopa	1	3.03	1	1.5	
Amoxicillin	2	6.06	1	1.5	
Propranolol	1	3.03	-	-	
Previous congenital anomalies	NTD cases	Controls		P value	
	No	%	No	%	-
yes	-	-	1	1.5	
NO	33	100	65	98.5	-
Previous abortion	NTD cases	Controls			
	No	%	No	%	P value
Yes	7	21.2	10	15.2	0.451
No	26	78.8	56	84.8	
history of NTD	NTD cases	Controls			
	No	%	No	%	P value
Positive	-	-	2	3.0	-
Negative	33	100	64	97.0	

Table (2) shows the effects of drug exposure during 1st trimester on the occurrence of NTDs. Where 15.2% of the cases were exposed to drugs (salbutamol, methyldopa, amoxicillin, and propranolol) during their 1st trimester, while 3% of the control group were exposed to drugs (methyldopa and amoxicillin), which means there is a significant statistical difference between the two groups with *P* – value = 0.027.Previous history of congenital anomalies in both groups have no significant difference as described above where there was no previous history of congenital anomalies in the cases group, while 1.5% of the control group had previous cong. anomalies(tetra logy of fallot). Also history of previous abortions have no statistical significance between the two groups with *p* – value = 0.451 .also there was no history of NTDs in the cases group, while there was 3% of the controls have this history . Mean serum zinc level was 75.45 µg / dl in the cases, while it was 90.83µg / dl in the controls. So there was statistically significant difference between the cases and the controls with a *P*- value= 0.0001.

Table.3: Comparison between mean serum zinc levels of the cases and controls regarding past obstetrical history and exposure to drugs

Previous history	Serum zinc level Mean±SD		P-value
	Case	Control	
Congenital anomalies Yes	-	90.00±	-
No	75.45±14.60	90.85±12.76	0.0001*
P value	-	-	
NTDs Yes	-	90.00±14.14	-
No	75.45±14.60	90.86±12.74	0.0001*
P value	-	0.897	
Abortion Yes	72.86±11.13	93.00±6.75	0.0001*
No	76.15±15.51	90.45±13.46	0.0001*
P value	0.861	0.756	
Exposure to drugs Yes	72.00±15.36	78.00±10.95	0.721
No	75.45±14.71	86.43±16.20	0.312
P value	0.433	0.103	

Table (3) show mean serum zinc level was 75.45 µg / dl in the cases, while it was 90.83µg / dl in the controls. So there was statistically significance deference between the cases and the controls with a *P*- value= 0.0001. The mean serum zinc levels between the cases and controls regarding past obstetrical history and exposure to drugs show there was no statistically significant difference regarding previous history of NTDs and congenital anomalies between the mean serum zinc level of the cases and controls. But there was statistical significant difference when we compare the previous history of abortion of the cases with that of the controls with *p*– value =0.0001. Exposure to drugs has no statistically significant effect on the mean serum zinc levels when we compare between the cases and the control.

Table.4 correlations between serum zinc level and demographic and obstetrical characteristics of the cases

		Serum zinc level Mean±SD	
		Cases	Controls
Maternal age (years)	r	-0.238	0.084
	P	0.183	0.504
BMI (Kg/m2)	r	-0.218	0.120
	P	0.222	0.422
No of abortions	r	-0.175	0.103
	P	0.331	0.410
Gestational age (weeks)	r	-0.459	-0.010
	P	0.007*	0.938
Parity	r	0.100	0.055
	P	0.581	0.660

Table (4) shows the correlations between serum zinc level and demographic and obstetrical characteristics of the cases and the controls. There was a statistically significant correlation between gestational age and serum zinc level of the cases (*p*-value =0.007). There were no statistically significant correlations between maternal age, BMI, previous abortion and parity with the serum zinc level.

Discussion:

NTDs are one of the most common birth defects, but their causes are not well understood. The formation of the neural tube during development is a complex process, and has genetic and environmental factors that contribute to NTDs(7, 8) In this study the results demonstrate that there is an association between low serum zinc and conception with fetuses of NTDs , which agrees with studies done by Cengiz et al, (2004)23, Zeyrek D et al and Cavdar et al. 2009 (24) Another two studies done in Iran by Golalipour et al. 1st one in (2006) (25) and the 2nd in (2009) (26), showed that there is a relation between low serum zinc and occurrence of NTDs in the fetus, which agrees with this study. While another study done in 1993 by Hambidge M et al. (27) showed that there was no association between serum zinc and neural tube defects, which is contradict with this study. The explanation for this disagreement is that serum zinc levels of cases included in previous studies were measured in the 1st trimester of pregnancy and the study was conducted in a developed country (UK) with higher usual intakes of zinc, so the serum zinc levels were in the normal range at the time of entry to their study**.** Whiteman et al 2000(28) found that women who had more than 4 deliveries have higher risk for NTD .And they state that "women aged more than 35 years had a substantially increased risk of having an NTD-affected pregnancy". These results are in contrast to our results. The cause for this difference may be of our small sample Consumption of medications in the 1st trimester of pregnancy is a factor that is widely accepted as a cause of congenital malformations which tallies with what was found by Mandiracioglu A et al. (2004) (29) who state that "Drug intake during pregnancy was identified as a risk for NTD development which agrees with our study which demonstrate on incidence under the effect of drug exposure. A study done by Whiteman et al 2000(28)showed that there was a significant relation between history of abortions and NTDs- affected pregnancies, contrasting our finding that showed that there was no relation between history of abortions and NTDs occurrence which may be due to the sampling number. Histories of previous NTDs and previous abortion are associated with low serum zinc level as is shown in our study which support the results of a review done by Shah D, Sachdev HP (2006) (30). In our study maternal serum zinc level was found to be decreased when gestational age increased and this can be explained by the physiological changes that occur in pregnancy leading to blood volume expansion, hormonal changes, and increased fetal zinc requirement for new tissue formation. Ashraf M et al 1999(21) found that with each trimester there is a progressive decline in serum zinc level in normal pregnancy.

Conclusions:

In this study there is an association between NTDs and low serum zinc levels, adding to the evidence about the importance of nutritional and maternal health factors in the etiology of this disease.

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