Original Article

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Maternal Folate and Vitamin B_{12} Status and Neural Tube Defects in Northern Iran: A Case Control Study

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Abstract

Objective: This study was conducted to determine the serum level of folic acid and vitamin B12 in neural tube defects pregnancies (NTD) and healthy controls in Northern Iran.

Methods: This case-control study was performed on women with neural tube defects pregnancies and controls with unaffected pregnancies in Northern Iran during 2006. Twenty three pregnant women whose pregnancies were diagnosed as NTD by a second-trimester ultrasonographic examination were recruited as cases. The control group (n=23) consisted of women who were selected among socio-economic status (SES) matched women who had a normal targeted ultrasound during the second trimester with documented normal fet al outcome. Fetal NTD was suspected with targeted second-trimester ultrasound during the 16th week of gestation and confirmed with high maternal serum α-fetoprotein levels. Folate, vitamin B_{12}, homocysteine and alpha fetoprotein were evaluated after target ultrasonography.

Findings: Serum alpha fetoprotein level (mean ± SD) in cases and controls was 120.2±64.1 and 50±33.5 iu/ml, respectively (P<0.05). The mean ± SD folate in cases and controls was 8.4±4.2 versus 9.3±4.2 ng/ml, respectively. This difference was not significant. Folate deficiency was found in 30.4% of the cases and 13% of the controls (OR = 2.9, 95%: 0.54–19.8). Vitamin B12 deficiency was found in 13 % of cases and 17.7% of the controls (OR=0.7, 95%: 0.1-4.9).

Conclusion: This study showed that the probability of having a newborn with NTDs in maternal folate deficiency is three times higher than with normal folate in Northern Iran.

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Key Words: Neural tube defects; Folic acid deficiency; Vitamin B_{12}; Pregnancy; Iran

Introduction

Neural tube defects (NTDs), manifesting as anencephaly or spina bifida, are to some degree preventable developmental malformations [1]. The incidence varies from 1/100 live births in certain regions of China to about 1/5000 live birth in Scandinavian countries[2]. Previous study

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showed that the rate of NTDs in Gorgan, North of Iran, was 2.88 per 1000 births [3]. Etiology of NTD is considered multifactorial, with genetic, environmental and nutritional factors playing some role[4,5,6,7,8]. Folic acid deficiency may play a role in causation of NTD in the offspring[9].

Some studies have documented lower maternal serum folic acid levels in women with neural tube defects pregnancies in comparison with unaffected pregnancies [10,11]. On the other hand, some studies suggest that nutrients other than folate may also be essential to neural tube closure and have a potential role in risk reduction, with vitamin B_{12} mentioned most often [11-15]. Vitamin B_{12} is a necessary cofactor for two enzymes in DNA synthesis: folate–dependent methionine synthesis and folate–independent methyl malonyl–CoA mutase [16].

Over the last twenty years, evidence for the independent role of B_{12} in NTD development has accumulated from case-control studies on maternal and fetal serum and amniotic fluid B_{12} levels. With two exceptions [17,18], studies on maternal serum B_{12} showed lower levels in mothers who have had NTD–affected pregnancies than in mothers without such a history [11,14,15,19-27].

Although various studies were done on the role of folate and vitamin B_{12} in different countries, regarding the high prevalence of neural tube defects[3] and the lack of documented studies about the role of maternal serum folic acid and vitamin B_{12} in neural tube defects in this area, we conducted this study to look for an association of maternal folic acid and vitamin B_{12} deficiency and neural tube defects in Gorgan, Northern Iran.

Subjects and Methods

This prospective hospital based case control study was conducted during 2006 at Dezyani Hospital with a labor facility in Gorgan, a capital city in the Golestan province in northern Iran.

This hospital is a referral hospital with an annual rate of more than 6000 deliveries accounting for the largest portion (80%) of deliveries in Gorgan. This study was approved by the Institutional Ethical Committee of Gorgan University of Medical Sciences.

Sample and study participants: Twenty three pregnant women whose pregnancies were diagnosed as NTD based on ultrasonographic examination during the 16th week of gestation were recruited as cases. The control group (n=23) consisted of women who were selected among gestational age matched women who had a normal targeted ultrasound during the second trimester (during the 16th wk of gestation) with documented normal fetal outcome. Fetal NTD was suspected, diagnosed with targeted second-trimester ultrasound during the 16th wk of gestation and confirmed with high maternal serum α-fetoprotein levels.

Normal fetus in control (healthy) Mothers were confirmed by ultrasound and maternal serum α-fetoprotein test during the 16th wk of gestation. A peripheral blood sample was collected from mothers in case and control groups after targeted second-trimester ultrasound during the 16th wk of gestation and the serum samples were analyzed for folic acid, B_{12}, alpha-fetoprotein, homocysteine level by radioimmunoassay using Gamma counter (Genesis – USA, and kits from Kavoshyar Company). Serum folate, vitamin B_{12} and homocysteine of less than 5 ng/ml, 160 pg/ml and 5 micromole/liter respectively, were considered as the cut-off value. Also the cut-off level of alpha fetoprotein in sera samples of mothers were considered as >120 iu/ml.

The diagnosis was evidenced by outcome observation after termination of pregnancy.

Data collection: Questionnaire forms were completed with direct interviews with the mothers, carried out by a nurse. Questionnaires given to the mothers included family history, birth place, age, place of residency, as well as mother's gynecological and obstetrical history (parity and outcome of pregnancies, preterm and term births, abortions and stillbirths). Any history of congenital malformations was also recorded. Mothers with diabetes mellitus or epilepsy during the first trimester of the pregnancy were excluded from the study.

Data Analysis: Categorical data were compared by Chi-square and Fisher's exact test. Mann-

Whitney U test and independent Student's t test were used for comparison of means. Presence of neural tube defect was considered as the dependent factor in multivariable logistics regression analysis.

Independent factors included in the analysis were parity, history of abortions, maternal drug exposure, and folate deficiency as dichotomous variables. Data were analyzed using SPSS 11.5 and STATA SE/8.

Findings

The means ± SD of Maternal age in case and control group was 24.0±4.6 and 26.5±5 (P=0.08). Number of pregnancy of mothers (mean±SD) in case and control group was 1.6±1 and 2.6±1.5 (P=0.012).91% of Mothers in cases and 57 % of Mothers in controls had not history of abortion (P=0.08).91% of mothers in cases and 78% in controls had 20-34 years old ranges.

Serum alpha fetoprotein level (means ± SD) in case and controls was 120.2±64.1 and 50±33.5 iu/ml, respectively (P<0.001). The mean concentration of serum folate did not differ appreciably between cases and controls across the entire study period (8.4 versus 9.3 ng/ml, P=0.97) (Table 1).

Overall 30.4% of cases and 13% of the controls in this study had folate deficiency (*P*=0.002). Our findings showed that maternal folate deficiency can increase the probability of a newborn to have NTDs three times more than those of pregnancies with normal folate, although Multivariate logistic regression analysis showed no association between the presence of NTD and folate deficiency (OR=2.9, 95% CI: 0.54-19.8) (Table 2).

The mean concentration of serum B_{12} did not differ between cases and controls in this study (238 ± 65 versus 227± 85 pg/ml, P =0.61) (Table 1).

Overall 13% of cases and 17% of the controls had B_{12} deficiency. Logistic regression analysis showed no Association between the presence of NTD and B_{12} deficiency (OR =0.7, 95% CI: 0.1-4.9) (Table 2).

The mean ± SD of serum homocysteine in case and control women was 8.١ ± 4 and 10.4 ± 4 micmol/lit respectively, but this difference was not significant (Table 1 and 2).

Discussion

The findings of this study showed that serum folic acid concentration was lower in affected pregnancies compared to control pregnancies, although this difference was not statistically significant. Our findings are similar to pervious studies such as Wald[10], Smithills[28] and Mills[26] (Table 3). Wald et al reported that serum folate level in cases (twenty seven women) was lower than in controls (108 women) but the comparison was not significant [10].

On the other hand Bunduki et al[9] in France and Thorand et al[15] in Germany reported that Serum folate level in their cases was significantly lower than in controls.

Also Gaber et al reported that the median serum folate concentrations were similar in cases and controls in Egyptians patients [29].

Given the substantial reduction in risk achievable, it may be surprising that the differences in serum folic acid levels between affected and unaffected pregnancies are too little. An explanation could be that the range of

Table 1: Level of folic acid, B_{12}, αFP and homosysteine maternal serum (Mean±SD) in cases and controls

	Cases (n=23)	Controls (n=23)	*P*-value
Folic Acid (ng/ml)	8.4±4.2	9.3±4.2	t =0.72 , 0.79
Vitamin B_{12} (pg/ml)	238.7±65.6	227.1±85.5	t =0.514 , 0.61
αFP (iu/ml)	120.2±64.1	50±33.5	t =4.6 , <0.001
Homocysteine (micromole/lit)	8.1±4.5	10.4±4.4	t =1.73 , 0.091

αFP: alpha fetoprotein

Table 2: Logistic regression analysis of the variables included in the study

Risk factor	Crude OR (CI 95%)	Adjusted OR (CI 95%)
Low levels of serum folic Acid	2.9 (0.7-13.1)	1.1 (0.11-10.6)
Low level of serum vitamin B_{12}	0.7 (0.14-3.6)	1.1 (0.13-9.6)
Low level of serum αFP	28.6 (3.3-249.7)	28.6 (2.8-296.8)
Low level of serum homocysteine	1.6 (0.24-10.4)	1.3 (0.12-13.1)

OR: Odds Ratio; CI: Confidence Interval; αFP: alpha fetoprotein

values in a population is relatively narrow; so much of the variation in risk reflects background genetic predisposition rather than folic acid differences. Also geographical, nutritional and biological factors could be involved.

Furthermore, in this study, we did not observe any relation between the serum vitamin B_{12} and NTD. The results of our study are similar to pervious researches[15,17,18,19,21,25,26]. The results of previous studies about the level of B_{12} in NTD pregnancy and healthy pregnancy are depicted in Table 4.

Molloy[18] reported that in early pregnancy no significant differences were found between the affected mothers and the controls in the median values and frequency distributions of vitamin B_{12} concentrations.

Mills study[26] in 89 pregnancies resulting in NTD offspring and 178 control pregnancies showed that in the NTD mothers , the mean ± SD level of B_{12} was not significantly lower than in control mothers (482.8 ± 161.1 vs 520.3 ± 191.9 pg/ml) (1.05, 95% CI 0.92 to 1.19). His population-based investigation in a low rate area demonstrated no relationship between maternal vitamin B_{12} level during pregnancy and the risk for NTDs.

Moreover, some studies reported to find an association between maternal serum B_{12} levels and NTD[19,29,30]. A study in Texas-Mexico border population found a strong association between maternal serum B_{12} levels and NTD risk[17]. Also Gaber et al showed that low maternal serum values of vitamin B_{12} can be considered an important etiologic factor for the development of neural tube defects in Egyptian mothers[29].

Zhang[30] in China reported that serum vitamin B_{12} concentration was lower in 84 NTD-affected pregnant women than in 110 controls (P<0.01). Furthermore a recent study reported that deficient or inadequate maternal vitamin B_{12} status is associated with a significantly

Table 3: Summary of findings from previous studies of maternal serum folic acid and NTDs mean differences between cases and controls

Study		No of Pregnancies	Mean (*Median) SFA (ng/ml)	Difference	Statistical significance
Smithells [26]	Affected	5	4.9	-1.4	No
	Unaffected	953	6.3		
Mills [24]	Affected	89	4.1	-0.2	No
	Unaffected	172	4.3		
Wald [8]	Affected	16	4.3*	-1.4	No
	Unaffected	36	5.7*		
Economides [23]	Affected	8	9.8	2.4	No
	Unaffected	24	7.4		
present study	Affected	23	8.4	-1.1	No
	Unaffected	23	9.3		

* Median of SFA(ng/ml)

Table 4: Summary of findings from previous studies of maternal serum B_{12} and NTDs mean differences between cases and controls

Study(first author)	Time of vein puncture	#Cases / controls	Cases (pg/ml)	Controls (pg/ml)	*P*<0.05
Schorah[13]	1st trimester	6/48	309	417	no
Molloy[16]	Early pregnancy	28/363	297	277	no
Economides[23]	Mid-trimester	8/24	205	230	no
Mills[24]	1st or 2nd Trimester	78/150	483	520	no
Kirke[9]	Pregnancy	81/247	243	296	yes
Steegers–Theunissen[21]	Pregnancy	27/31	297	323	no
Thorand[15]	Pregnancy	30/62	256	237	no
Stoll[19]	1st trimester	6/340	430	440	no
Present study	2nd trimester	23/23	238	227	no

increased risk for neural tube defects[31]. According to findings of our study serum homocysteine concentration was not significant between cases and controls, but Mills[32] reported that mothers of children with neural-tube defects had significantly higher homocysteine values (8.62 [SD 2.8] micromole/ lit).

Also Ratan study[33] showed that high plasma homocysteine in both parents had an association with NTD. Ratan concluded that NTD may not only be due to nutritional deficiency in the mothers but also due to more intricate gene-nutrient interaction defects in the affected families, probably some abnormal folate-homocysteine metabolism.

These variations in different studies could be explained by the influence of racial, ethnic and socioeconomic, geographical, nutritional and biological factors in various parts of the world. Other reasons for these variations in the differences are the sample size and time of sampling.

Despite about two decades past the administration of folic acid in reproductive age women and in programmed pregnancy by the health system, some factors such as low doses of consumption, gastrointestinal disorders and lack of knowledge about the importance of folic acid in normal development of fetus can affect the success of this important program.

However, this study has certain limitations. Firstly the sample size was small. Secondly, we are not aware how many cases or controls were taking folic acid or B_{12}–containing supplements periconceptionally (ie, before and after conception).

Conclusion

This study showed that maternal folate deficiency can increase the probability of a fetus to have NTDs as compared to pregnancies with normal folate. In our opinion, folate supplements are appropriate to prevent recurrence of NTD in infants of high–risk women. Finding of lower folate levels in case mothers than in control mothers would have supported the use of folate supplements to prevent NTDs in low-risk populations. This finding focuses attention to the importance of nutritional factors in addition to genetic role in the etiology of neural tube defects. Further studies should be carried out with large sample size to verify the cause-effect relationship of homocysteine metabolism with NTD.

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Conflict of Interest: None

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