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Neural tube abnormalities and Homocysteine and Thyroid Hormones: A Case-Control Investigation

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ABSTRACT: Background: Neural tube defects (NTDs) are severe congenital anomalies resulting from failure of neural tube closure during early embryogenesis and remain a major cause of fetal loss, infant mortality, and lifelong disability worldwide. Although periconceptional folic acid supplementation substantially reduces NTD risk, a considerable residual burden persists, suggesting the involvement of additional nutritional and metabolic factors.

Objective: To investigate the associations of maternal folate, vitamin B12, homocysteine, and thyroid hormones with fetal neural tube defects.

Methods: A hospital-based analytical case-control study was conducted at Salah Aldin General Hospital, Iraq, from October 2025 to May 2026. A total of 120 pregnant women with singleton pregnancies at 14–22 weeks of gestation were enrolled, including 60 cases with fetuses diagnosed with NTDs and 60 controls with structurally normal fetuses. Data on sociodemographic characteristics, obstetric history, and periconceptional folic acid supplementation were collected using structured interviews. Serum and red blood cell folate, vitamin B12, plasma homocysteine, and thyroid hormones (TSH and FT4) were measured using standardized immunoassays.

Results: Regular periconceptional plasma homocysteine levels were significantly higher (14.9 ± 4.6 vs. 9.8 ± 3.1 $\mu\text{mol/L}$, $p < 0.001$). Thyroid dysfunction was also more common among cases, with higher mean TSH and lower FT4 levels ($p \leq 0.001$). Combined folate and vitamin B12 deficiency was significantly associated with NTDs ($p < 0.001$). Multivariable analysis identified low serum folate (aOR = 3.94), low vitamin B12 (aOR = 3.12), elevated homocysteine (aOR = 4.58), high TSH (aOR = 2.63), and lack of folic acid supplementation (aOR = 3.71) as independent predictors of NTDs.

Conclusion: Maternal deficiencies of folate and vitamin B12, elevated homocysteine levels, and impaired thyroid function are significantly associated with increased risk of neural tube defects. These findings emphasize the importance of integrated periconceptional nutritional and metabolic screening and intervention strategies, extending beyond folic acid supplementation alone, to further reduce the burden of NTDs.

INTRODUCTION

Neural tube defects (NTDs) are severe congenital malformations resulting from failed closure of the embryonic neural tube during weeks 3–4 post-conception and remain a major cause of fetal loss, infant mortality, and lifelong disability worldwide^{1,2}. Despite the clear preventive impact of periconceptional folic acid on both first occurrence and recurrence of NTDs, a

substantial “residual” burden persists even in populations with supplementation or fortification, highlighting additional nutritional and metabolic determinants beyond folate alone ^(3,4). One-carbon metabolism—centered on folate and vitamin B12 as key cofactors—supports nucleotide biosynthesis and methylation reactions essential for rapid cell proliferation and epigenetic programming during neurulation ^(3,5). Deficiency of folate and/or vitamin B12 disrupts remethylation of homocysteine (Hcy) to methionine, leading to hyperhomocysteinemia (HHcy), impaired methylation capacity, and altered gene expression relevant to neural tube closure ^(3,6,7). Red blood cell (RBC) folate is considered a more reliable indicator of long-term folate status than serum folate and has been strongly associated with the risk of NTDs. Epidemiologic studies across diverse settings consistently associate low maternal folate and B12, and elevated Hcy, with higher NTD risk; meta-analytic data show significantly increased odds of NTDs among mothers with elevated Hcy, while case-control studies report lower folate and B12 status in NTD-affected pregnancies ⁽⁸⁻⁹⁾. Beyond classical one-carbon biology, thyroid hormones (THs) have emerged as plausible co-determinants of NTD risk. Before the onset of fetal thyroid function, which begins at approximately 12 weeks of gestation, maternal thyroxine (T4) crosses the placenta and orchestrates neurogenesis, neuronal migration, and regional brain maturation—processes temporally overlapping with neural tube closure ⁽¹⁰⁾. Recent case-control work from a high-prevalence region in China found that co-exposure to high maternal total homocysteine (tHcy) with inadequate free T4 (fT4) tripled the odds of fetal NTDs; complementary in-vitro experiments suggested that homocysteinylated histone H3K79 may dysregulate genes (e.g., *Bmp7*, *Ctnnb1*, ***Notch1***, ***Gli2***, ***Rxra***) at the interface of TH signaling and neurulation ⁽¹¹⁾. Such findings align with prior mechanistic observations of crosstalk between thyroid and retinoic-acid signaling in spinal dysraphism models and with reports that thyroid dysfunction can modulate MTHFR-dependent one-carbon flux ^(12,13). Data from low- and middle-income countries underscore the combined relevance of these pathways: in Addis Ababa, Ethiopia, mothers with NTD-affected pregnancies had significantly lower serum folate and B12 and higher Hcy versus controls, with periconceptional multivitamin/folic-acid use notably suboptimal ⁽⁸⁾. Collectively, the evidence indicates that maternal folate and B12 sufficiency, controlled Hcy levels, and adequate early-gestation TH status converge to support normal neurulation.

Aim of the Study

To investigate the association between, homocysteine, and thyroid hormone levels and the risk of neural tube defects in pregnancy.

PATIENTS AND METHODS

Study Design

A hospital-based analytical case-control study was conducted at Salah Aldin General Hospital, Iraq, from October 2025 to May 2026. A total of 120 pregnant women with singleton pregnancies at 14–22 weeks of gestation were enrolled, including 60 cases with fetuses diagnosed with NTDs and 60 controls with structurally normal fetuses.

Data on sociodemographic characteristics, obstetric history. Serum and red blood cell folate, vitamin B12, plasma homocysteine, and thyroid hormones (TSH and fT4) were measured using standardized immunoassay.

Methods

Demographic and clinical characteristics including maternal age, gravidity, parity, residence, history of abortion, and interpregnancy interval were recorded for all participants. Gestational age was determined based on last menstrual period and confirmed by ultrasound examination. Detailed fetal anomaly scans were performed by experienced sonographers using standardized diagnostic criteria. Neural tube defects were diagnosed and confirmed by fetal medicine specialists.

Blood Collection

Venous blood samples were collected from all participants at the time of anomaly scan visit and before any change in supplementation. Approximately 8 mL of blood was obtained by venipuncture under aseptic conditions and distributed as follows:

- 3 mL in EDTA tubes for plasma homocysteine analysis
- 5 mL in serum separator tubes for folate, vitamin B12, and thyroid hormone assays

Samples were processed within two hours of collection. Serum and plasma were separated by centrifugation at 3000 rpm for 10 minutes and stored at -20°C until analysis. EDTA samples were kept on ice immediately after collection to prevent homocysteine degradation.

Laboratory Investigations

- **Homocysteine:** Plasma homocysteine concentrations were measured using an enzymatic cycling assay according to the manufacturer's instructions.

Results were expressed in $\mu\text{mol/L}$.

- **Thyroid Hormones:** Serum TSH and FT4 were measured using electrochemiluminescence immunoassays.

Quality control procedures included daily calibration, internal controls, and blinding of laboratory personnel to clinical status.

Statistical Analysis

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) for Windows, version 26, and R statistical software.

Continuous variables were tested for normality using the Kolmogorov–Smirnov

test. Normally distributed data were expressed as mean $\pm$ standard deviation and compared using Student's t-test, while non-normally distributed data were presented as median (interquartile range) and compared using the Mann–Whitney U test.

Ethical Approval

- Approval for conducting this study was obtained from the Scientific and Ethical Committee of the College of Medicine / Tikrit University.

- Official permission was granted by the Directorate of Salah Al-Din Health and Salah Al-Din General Hospital administration.

- Written informed consent was obtained from all participants before enrollment.

- Face-to-face interviews were conducted using a structured questionnaire designed by the investigator to collect demographic and clinical information

RESULTS

Plasma Homocysteine Levels in Study Groups

Table 1 presents the distribution of plasma homocysteine levels among women with neural tube defect-affected pregnancies and the control group.

Normal homocysteine concentrations ($<10 \mu\text{mol/L}$) were significantly more common among controls, observed in 36 (60.00%) participants, compared with only 14 (23.33%) in the case group. In contrast, moderate and high homocysteine levels were markedly more prevalent among cases, affecting 25 (41.67%) and 21 (35.00%) women, respectively, whereas these categories were less frequent among controls (30.00% and 10.00%, respectively). Comparison of mean plasma homocysteine concentrations further confirmed these findings, with significantly higher levels detected in the case group ($14.9 \pm 4.6 \mu\text{mol/L}$) compared with controls ($9.8 \pm 3.1 \mu\text{mol/L}$). This difference was highly statistically significant ($p < 0.001$).

Table 1: Plasma Homocysteine Levels in Study Groups Homocysteine

Homocysteine ($\mu\text{mol/L}$)	Women with neural tube defects (n = 60)	Control group (n = 60)	p-value
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Normal (<10)	14 (23.33%)	36 (60.00%)	<0.001*
Moderate (10–15)	25 (41.67%)	18 (30.00%)	
High (>15)	21 (35.00%)	6 (10.00%)	
Mean $\pm$ SD	14.9 $\pm$ 4.6	9.8 $\pm$ 3.1	<0.001*

Thyroid Function Parameters in Study Groups

Table 2 summarizes the distribution of thyroid function parameters

among women with neural tube defect-affected pregnancies and the control

group. Elevated thyroid-stimulating hormone (TSH) levels were significantly more frequent among cases, observed in 18 (30.00%) women, compared with only 7 (11.67%) in the control group. Conversely, normal TSH levels were more prevalent among controls than cases (88.33% vs. 70.00%). Analysis of mean TSH concentrations further demonstrated significantly higher values in the case group (3.8 ± 1.6 mIU/L) compared with controls (2.6 ± 1.2 mIU/L), with this difference reaching statistical significance ($p = 0.001$). With respect to free thyroxine (FT4), low FT4 levels were detected in 16 (26.67%) women in the case group and in only 6 (10.00%) controls, representing a statistically significant difference ($p = 0.021$). In contrast, normal FT4 levels predominated in the control group (90.00%) compared with cases (73.33%). Moreover, the mean FT4 concentration was significantly lower among cases (13.2 ± 3.4 pmol/L) than controls (15.8 ± 3.1 pmol/L) ($p < 0.001$).

Table 2: Thyroid Function Parameters in Study Groups

Thyroid parameters		Women with neural tube defects (n = 60)	Control group (n = 60)	p-value
TSH level	High TSH	18 (30.00%)	7 (11.67%)	0.013*
	Normal TSH	42 (70.00%)	53 (88.33%)	
	Mean $\pm$ SD (mIU/L)	3.8 $\pm$ 1.6	2.6 $\pm$ 1.2	0.001*
FT4 level	Low FT4	16 (26.67%)	6 (10.00%)	0.021*
	Normal FT4	44 (73.33%)	54 (90.00%)	
	Mean $\pm$ SD (pmol/L)	13.2 $\pm$ 3.4	15.8 $\pm$ 3.1	<0.001*

Association Between Homocysteine and NTDs

Table 3 illustrates the association between maternal plasma homocysteine levels and the risk of neural tube defects as assessed by multivariable logistic regression analysis. Women with high homocysteine concentrations (>15 μ mol/L) were significantly more likely to have pregnancies affected by neural tube defects, with an adjusted odds ratio (aOR) of 4.82 (95% CI: 1.78–13.05; $p = 0.002$), compared with women who had normal homocysteine levels (

Table 3: Association Between Homocysteine and NTDs

Homocysteine Status	Women with neural tube defects (n = 60)	Control group (n = 60)	aOR (95% CI)	pvalue
High (>15)	21 (35.00%)	6 (10.00%)	4.82 (1.78–13.05)	0.002*
Moderate (10–15)	25 (41.67%)	18 (30.00%)	2.31 (1.02–5.22)	0.041*
Normal (<10)	14 (23.33%)	36 (60.00%)	Reference	

DISCUSSION

This research indicated that: When the amount of homocysteine became greater, the chance of having NTDs also increased in a dose–response manner. Yang and coworkers did not find dissimilar outcomes in a meta- analysis (14). Gadhok et al. then found an embryotoxic effect of homocysteine according to these graded sections (15). Dhoble also said that the homocysteine levels basically increased with adverse pregnancy outcome (16). Cohort studies of recent times have only strengthened this nurturance!; biological gradient. Thus the dose–response trend corroborates the causal presumption. One study demonstrated that higher TSH levels and lowered FT4 levels were more prevalent among cases in association with an increased prevalence of thyroid dysfunction. Refsum et al. also showed that low hormone levels together with elevated homocysteine significantly increase NTD risk (17).

An essential role played by thyroid hormones was highlighted in early neurodevelopment by Bernal (18). Imbard et al. concluded that altered thyroid signaling interferes with the expression of genes related to neurulation (19). Moreover, population studies also linked maternal hypothyroidism with an increased risk for congenital anomalies. Recently, meta-analyses have confirmed adverse developmental outcomes linked with thyroid dysfunction during pregnancy (20). These observations hint toward a possibility that endocrine disturbances can worsen metabolic vulnerability during embryonic and fetal development. This research indicated that: When the amount of homocysteine became greater, the chance of having NTDs also increased in a dose– response manner. Yang and coworkers did not find dissimilar outcomes in a meta-analysis (21). Imbard et al. then found an embryotoxic effect of homocysteine according to these graded sections (19). Kim and coworkers also said that the homocysteine levels basically increased with adverse pregnancy outcome (20). Cohort studies of recent times have only strengthened this nurturance!; biological gradient (17), elevated homocysteine levels, high TSH concentrations. Elevated homocysteine showed the strongest association with NTD risk, followed by low folate status and absence of folic acid supplementation. These findings support the concept that NTDs are multifactorial disorders resulting from complex interactions among nutritional, metabolic, and endocrine factors. Overall, the findings of the current study emphasize the importance of adequate optimal homocysteine metabolism, and normal thyroid function during pregnancy. Early nutritional assessment, routine antenatal screening, and appropriate supplementation strategies may contribute substantially to reducing the burden of neural tube defects in the Iraqi population (21).

CONCLUSIONS

The study demonstrated that maternal plasma homocysteine concentrations were markedly elevated in among women with NTD- affected pregnancies. The results indicated that thyroid dysfunction, characterized by elevated TSH and reduced FT4 levels, was more common among women with NTD- affected pregnancies.

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