Risk Factors for Neural-Tube Defects Detected in Utero: A Prospective Community-Based Study from Addis Ababa

Abenezer Tirsit[1,2], Mahlet Yigaramu[3], Daniel Zewdneh[4], Winner Kucha[5], Seifu Hagos[6], Bilal Shikur[6], Tsegazeab Laeke[1,2], Bente E. Moen[7], Rolv T. Lie[7,8], Morten Lund-Johansen[2,9], Rupavathana Mahesparan[2,9]

■ BACKGROUND: **A recent community-based study from Addis Ababa identifying Neural Tube Defect (NTD) cases by ultrasound examination of pregnant women showed a higher prevalence of 17 per 1000 fetuses. The risk factors behind the high prevalence remain unclear.**

■ METHODS: **Altogether 891 of the 958 women participated in the ultrasound examination. Thirteen with unaffected twin pregnancies were excluded. Among 878 singleton pregnancies, 15 NTD cases were identified. Serum Folate, vitamin B12, and homocysteine levels were measured in case-mothers and a sub-set of 28 noncase mothers. Because of the modest sample size, exact logistic regression analysis was used to estimate associations between risk factors and NTDs.**

■ RESULTS: **Serum vitamin status was generally poor for participants in the study. Still, relatively higher values of folate or vitamin B12 in serum, appeared to be protective for NTD (odds ratio [OR] = 0.61 per ng/ml, 95% Confidence interval [CI]: 0.42–0.85 and OR = 0.67 per 100 pg/ml, 95% CI: 0.41–1.02, respectively). High serum homocysteine was associated with higher risk of NTD (OR = 1.3 per μmol/l, 95% CI: 1.02–1.8). Women aged 30 years or more had an OR of 3.5 (95% CI: 1.1–12) for having a NTD child, and families with NTD children had lower household income. Women in the NTD group also had more spontaneous abortions or stillbirths in previous pregnancies. Self-reported intake of folate did not appear to protect against NTDs.**

■ CONCLUSIONS: **Within this high-prevalence community, poor vitamin status was identified as a risk factor for NTDs detected at ultrasound examination. Improving food security and fortification of foods or food ingredients could be alternative measures.**

BACKGROUND

A report from the World Health Organization[1] suggests that low- and middle-income countries (LMICs) contribute to 94% of all birth defects and 95% of deaths from birth defects globally. Neural tube defects (NTDs) are the second most common category of birth defects after cardiac anomalies.[1,2] NTDs develop around the 28th gestational day due to the failure of neurulation or alterations in the morphogenesis or histogenesis of the nervous tissue.[3] Surgery for NTD and hydrocephalus are the most commonly performed neurosurgical procedures in Ethiopia.[4] In a recent retrospective study, investigating the outcomes of surgically treated NTDs in Addis Ababa, 26.1% had immediate wound complications, and the mortality at four years of age was 41% in this patient group.[4] In addition, most of surviving children had lifelong disabilities.[5] In a prospective study 38% of the patients were readmitted and 55% had wound

Key words
- Community-based prospective study
- High prevalence
- Intrauterine NTD
- Neural tube defects

Abbreviations and Acronyms
NTDs: Neural tube defects
CI: Confidence interval
ANC: Antenatal care
OR: Odds ratio
5MTHF: 5-Methyltetrahydrofolate

From the [1]Division of Neurosurgery, College of Health Science, Addis Ababa University, Addis Ababa, Ethiopia; [2]Department of Clinical Medicine, University of Bergen, Bergen, Norway; [3]Department of Gynecology and Obstetrics, [4]Department of Radiology, [5]Department of Biochemistry, and [6]School of Public health, College of Health Science, Addis Ababa University, Addis Ababa, Ethiopia; [7]Department of Global Public Health and Primary Care, Centre for International Health, University of Bergen, Bergen, Norway; [8]Centre for Fertility and Health, Norwegian Institute of Public Health, Bergen, Norway; and [9]Department of Neurosurgery, Haukeland University Hospital, University of Bergen, Bergen, Norway

To whom correspondence should be addressed: Rupavathana Mahesparan, M.D., Ph.D. [E-mail: rupavathana.mahesparan@helse-bergen.no]

Citation: World Neurosurg. (2024) 185:e683-e690. https://doi.org/10.1016/j.wneu.2024.02.108

*Journal homepage: **www.journals.elsevier.com/world-neurosurgery***

*Available online: **www.sciencedirect.com***

related complications. Hydrocephalus (80%) and open defects (88%) were identified as risk factors for readmission. Hydrocephalus and younger age were significantly associated mortality.[6]

Several studies have reported a high prevalence of NTD in Ethiopia[7-10]; a recent prospective community-based ultrasound prevalence study in Addis Ababa estimated a prevalence of 17 per 1000 (95% CI: 10–27).[11] It is not clear which risk factors that contribute to this high prevalence. Folate deficiency, other nutritional factors, genetic factors, women's age during pregnancy, spontaneous abortions or stillbirth in previous pregnancy, having a child or a sibling with NTD, intake of certain drugs during pregnancy, maternal comorbidities, and low socioeconomic status are all suggested risk factors for NTDs.[12-18]

Folate supplementation in the first trimester reduces NTD development.[19] In Ethiopia, however, there is no national strategy to ensure early folate administration to pregnant women.[20] Iron/folate supplements are usually given to pregnant women in antenatal care (ANC) clinics, but not until the second trimester.[21,22] Consequently, this inadequate folate intake does not reduce NTD prevalence.[14,16]

Low socioeconomic status is prevalent among the Ethiopian population and nutritional deficiencies are common.[15] It is likely that dietary issues are related to the prevalence of NTDs, and that fortification of foods with folate may prevent NTDs and stillbirths in Ethiopia.[23-25]

Aneuploidy of meiotic origin increases dramatically with maternal age.[26] Thus, the risk for spontaneous abortions or carrying a child with congenital malformations increases accordingly. A meta-analysis showed that African women older than 30 years are at 1.5 times higher risk of having a baby with NTD.[14] Another Spanish meta-analysis showed maternal age above 40 years and below 19 years as a risk factor[17] and a maternal cohort from England also showed increased risk with increasing maternal age.[18]

As summarized here, these risk factors are known or have been discussed to be of importance for NTD development. However, it is not known for us whether these factors contribute to the higher prevalence of NTDs we have in our specific population in Ethiopia.

In addition, most of the studies attempting to identify risk factors for NTDs in Ethiopia are hospital-based and not likely to be representative for the general population. According to the 2016 National Ethiopian Demographic and Health Survey, only 29% of births occurred in health facilities.[21] In this study, we attempt to identify and verify maternal risk factors related to NTDs detected by ultrasound in a geographically defined population with a particularly high prevalence in Addis Ababa, Ethiopia.

METHODS

Study Population and NTD Screening

To obtain a representative sample, we included pregnant women from health centres in Addis Ababa according to a previously published method.[11]

We invited pregnant women at gestational age 9–22 weeks into the study from first of October 2018 to 30th of April 2019 when they came for routine ANC follow-up. Consenting women signed a written form. Illiterate women gave their oral consent, witnessed by another person.

Health professionals interviewed all the participants at the health centre by using standardized forms. (**See appendix** for details).

The women were invited to an obstetric ultrasound examination for NTDs screening at the university hospital in Addis Ababa 1 to 2 weeks later. Final year radiology residents under supervision by consultant radiologists did the examinations. Neural-tube defects were defined as a cystic swelling behind the yolk sac at or above 9 weeks of gestation age. For the rest of the pregnancy, the diagnosis was made when there was a spinal defect in continuity.

Patient and Public Involvement

The project plan was submitted to and communicated with the Ministry of Health in Ethiopia. Furthermore, the project plan was presented at a meeting of the Ethiopian Neurosurgical Society, where representatives of parents with children affected by NTDs provided valuable input on the study plan. As data collection commenced in 2019, the neurosurgical society organized a dedicated conference on NTDs, with active participation from parents through their formal association.

Biochemical Markers

A laboratory technician took one sample of 3–5 ml venous blood from all women carrying a foetus with NTD and from 28 women carrying foetuses assessed as normal by US. Samples were centrifuged at room temperature and stored at −80°C in the core lab of Black Lion Hospital. Folate, vitamin B12, and homocysteine serum levels were analysed by enzyme-linked immunosorbent assay (ELISA). The analyses were conducted at the Immunology laboratory of the Armauer Hansen Research Institute in Addis Ababa.

For description and tabulation, folate levels were classified into 3 categories: 1. Deficiency (<3 ng/ml); 2. Possible deficiency (3–5.99 ng/ml); and 3. Normal (6–20 ng/ml) (23). Levels of vitamin B12 were classified into 3 categories: 1. Severe deficiency (<150 pg/ml); 2. Moderate deficiency (150–200 pg/ml), and 3. Normal (>201pg/ml) (24). Homocysteine concentrations of <12 μmol/l are considered optimal, 12–15 μmol/l as borderline, and >15–30 μmol/l are considered as moderate hyperhomocysteinemia (25).

Statistical Analysis

Data entry and descriptive analyses were conducted using Statistical Package for Social Science version 21 (IBM Corporation, Armonk, New York, USA). Regression analyses were performed using StataCorp, College Station, Texas 77,845, USA v.17.

Since the sample size was modest, we decided to use exact logistic regression to estimate the odds ratio (OR) of the association between each risk factor and NTDs. All estimates (except for maternal age) were adjusted for maternal age (≥30, yes/no). Precision of the OR estimates is represented by 95% confidence intervals. Since the outcome (NTD) was relatively rare, the ORs are good approximations of relative risks. Continuous variables were kept continuous in our analyses to retain statistical power.

Ethical Approval

Ethical approval was given from IRB of Addis Ababa Health Bureau (Ref. no. Addis Ababa Health bureau 7029/227) and college of Health Science, Addis Ababa University (Protocol no. 088/17/Surg) and the Regional Committee for Medical and Health Research Ethics, Norway (REK Vest, project no. 103230).

RESULTS

Altogether 958 pregnant women consented and were enrolled. Of these, 891 (93%) came for ultrasound examination. Among 891 women, 13 had twin pregnancy. We identified 15 NTD cases among 904 fetuses, corresponding to an ultrasound-based prevalence of 166 per 10,000.[11]

In comparing the cohort of 15 women with NTD fetuses to their counterparts, the former exhibited higher age (53% of the women were age 30 or above) and a greater history of spontaneous abortions (20% of the pregnant women with NTD fetuses had spontaneous abortion), as indicated in Table 1. Importantly, none of the women in the NTD group had a prior occurrence of NTD in their offspring. Notably, a majority (53%) of women in the NTD group had completed only primary school; however, no significant educational differences were observed between the groups. Similarly, small variations were noted in the categories of occupation. Additionally, the mean family income was lower for the NTD group (2675 ETB in the NTD group vs. 4734 ETB in the non-NTD group).

A higher proportion (73%) of women in the NTD-group reported to have taken iron/folate supplementation during the pregnancy. However, women in both groups typically reported to start their folate intake around 2 months after they became pregnant. The dose of folate that was reported to be taken routinely was 87 mg of iron with 300 µg of folic acid daily. Only a couple of women in the NTD-group had comorbidities or used medication like anti-convulsant or deworming drugs for intestinal parasites in the NTD group (Table 2).

We then analysed serum folate, vitamin B12 and homocysteine levels in the 15 pregnant women in the NTD group and in 28 control-women from the non-NTD group. The distribution of levels of these measurements is shown in Table 3. Vitamin status was generally poor for participants in the study. Within the measured range, we still found higher values of folate to be protective for NTD (OR=0.61 per ng/ml, 95% CI: 0.42–0.85) (Table 4). A similar association was seen for vitamin B12 (OR=0.67 per 100 pg/ml, 95% CI: 0.41–1.02). All 4 women with Vitamin B12 deficiency in the NTD group, and 3 out of 7 women with Vitamin B12 deficiency in the non-NTD group, had additional folate deficiency. High serum homocysteine level was associated with higher risk of NTD (OR=1.3 per µmol/l, 95% CI: 1.02–1.8). It should also be noted that the 3 women in the NTD group with hyperhomocysteinemia all had normal serum folate and vitamin B12 levels.

Estimated ORs for associations between risk factors and NTD with 95% confidence intervals and P-values are presented in Table 4. These ORs may be interpreted as relative risks. Women aged 30 or more had a 3.5-fold risk of an NTD (95% CI: 1.1–12). A previous spontaneous abortion doubled the NTD risk, but the confidence interval of the OR did not exclude 1. The analysis suggested that higher family income reduced the risk, but again the confidence interval did not exclude 1. Taking a folate supplement had an apparent effect in the opposite direction than expected, but the confidence interval included values consistent with an expected protection.

DISCUSSION

In this prospective study from Addis Ababa, Ethiopia, we recruited and interviewed 956 women and were able to record results from an ultrasound examination of NTDs for 891 (93%) pregnant women. Among these 891 women, 13 had twin pregnancy. We identified 15 NTD cases among 904 fetuses, yielding an ultrasound-based prevalence of 166 per 10,000 (95% CI: 100–274).[11]

In this population, we found an increased risk of NTDs among babies for women aged 30 years or more. In general, the pregnant women in our study were younger in comparison with other studies.[18,26] The association with maternal age was stronger in our data than in previous Ethiopian studies,[14-16] but consistent with other reports from Africa. Another case-control study from Addis Ababa did not find an association between maternal age and risk of NTD, with a mean age in both the case and the control group of 26 years.[8] However, this study was hospital based, and the selection of women participating might differ from our present study and might be the reason for these different results.

Our study suggested that a higher proportion of women with previous spontaneous abortion had a foetus with an NTD, but the estimate had a wide confidence interval. Other studies have also reported previous abortions and perinatal or neonatal mortality as risk factors for NTD, but the findings are not consistent.[8]

The women in the NTDs group in our study appeared to have lower educational level compared to the non-NTD group, but we could not be sure that our OR estimate was different from 1. Lack of knowledge may be a risk factor for NTD in our study population, as this might be connected to dietary issues. In addition, the average monthly income was lower in the NTD group than in the non-NTD group. This supports that the NTD group has fewer resources than the others. These findings suggest that a lower socio-economic status may be a risk factor for NTD. Previous studies have also reported low socio-economic status as risk factors for NTD.[14,16] Low socio-economic status may be related to food insecurity and micronutrient deficiency in this population.

Studies have shown that folate deficiency is a preventable risk factor for development of NTD.[19] Guidelines recommend folate intake from preconception until 3 month after conception.[27] We did not find self-reported folate intake to contribute to less NTDs. However, the women in our study usually started to take the folate two months after they became pregnant, and the dosage of folate (<500 µg) intake was lower than what is recommended to prevent NTDs. Thus, the folate supplement may have been taken too late and at insufficient doses. This is a likely reason for the lacking relation between the reported folate supplementation and NTD in our study. Although estimated associations with risk factors from the interview are consistent with estimates from other studies, none of them are likely to explain the high prevalence in our study.

Folic acid is not the only nutrient that may increase the risk for NTDs if deficient.[28] Deficiency in vitamin B12 during pregnancy is

Table 1. Characteristics of 878 Singleton Pregnancies From Addis Ababa with Completed Ultrasound Examination in a Study of NTDs

	Fetus with NTD (n = 15)	Unaffected (n = 863)	Total (n = 878)
Maternal age			
< 30 years	7 (47%)	639 (74%)	646 (74%)
≥ 30 years	8 (53%)	208 (24%)	216 (25%)
Missing	0	16 (2%)	16 (2%)
Marital status			
Married	14 (93%)	815 (94%)	829 (94%)
Single	1 (7%)	35 (4%)	36 (4%)
Divorce/separated/ widowed	0	10 (1%)	10 (1%)
Missing	0	3 (0.4%)	3 (0.4%)
Outcome of previous pregnancy			
Live birth	6 (40.0%)	397 (46%)	403 (46%)
Still birth	0 (0%)	18 (2%)	18 (2%)
Spontaneous abortion	3 (20%)	95(11%)	98 (11%)
Induced abortion	1 (7%)	50 (6%)	51 (6%)
No previous pregnancy	4 (27%)	237 (27%)	241 (27%)
Missing	1 (7%)	66 (8%)	67 (8%)
Educational level			
Cannot read and write	0	87 (10%)	87 (10%)
Can read and write	1 (7%)	64 (7%)	65 (7%)
Completed primary school	8 (53%)	290 (34%)	298 (34%)
Completed secondary school	5 (33%)	264 (31%)	269 (31%)
Completed college	1 (7%)	156 (18%)	157 (18%)
Missing	0	2 (0.2%)	2 (0.2%)
Maternal occupation			
Unemployed	1 (7%)	125 (14%)	126 (14%)
Student	0	9 (1%)	9 (1%)
House wife	6 (40%)	296 (34%)	302 (34%)
House maid/local drink seller	0	30 (3%)	30 (3%)
Civil servant/merchant/ self employed	8 (53.3%)	400 (46%)	408 (46%)
Missing	0	3 (0.4%)	3 (0.3%)
Mean monthly household income (SD)	2675 ETB* (1172)†	4734 ETB* (6013)‡	
Folate during pregnancy			
Yes	11 (73%)	472 (55%)	483 (55%)
No	4 (27%)	387 (45%)	391 (45%)
Missing	0	4 (0.5%)	4 (0.5%)

NTD, neural tube defect.
*Ethiopian birr.
†Information was missing for 7 women.
‡Information was missing for 345.

also a known risk factor for NTDs. It is important in the formation of the amino acid methionine; which is important for neurulation and development of the nervous system.[28] Most reports have shown that vitamin B12 and folate deficiencies are associated, as conversion of homocysteine to methionine needs 5-methyltetrahydrofolate as a donor, which is a bioactive form of folate.[28,29]

Our study was supplemented with objective measurements of serum folate, B12 and homocysteine in case-mothers and a set of controls to enable estimation of general levels and potential associations with case-status. Vitamin status was generally poor for participants in the study, but even poorer for case-mothers. All 4 women with Vitamin B12 deficiency in the NTD group and 3 out of 7 women with Vitamin B12 deficiency in the non-NTD group also had folate deficiency. In addition, homocysteine, a hazardous metabolite, accumulates in the presence of folate deprivation, and elevated homocysteine levels have long been associated with cardiovascular disease and birth defects, such as NTDs.[29] We found only 3 women in the NTD group with hyperhomocysteinemia, and all 3 had normal serum folate and vitamin B12 levels.

A recent case-control study in pregnant women from 3 hospitals in Addis Ababa reported significantly lower median concentration of folate and vitamin B12 in the mothers with NTD children than controls, while homocysteine concentration was significantly higher in the cases than the controls. Similar to our study, they concluded that lower folate and vitamin B12, and higher homocysteine levels were associated with NTDs.[30]

Our study is unique by being prospective and community based and may provide less biased estimates for risk factors that are subject to recall bias or biased by selection. Our study also included collection of blood samples. A recently published systematic review and meta-analysis of previous retrospective hospital based studies of risk factors of NTDs from Ethiopia included 611,064 participants from 42 studies.[31] Similar to our study, this study report strong association between maternal age ≥35 (AOR 1.90, 95% CI [1.13–3.25]) and maternal low educational status (AOR 1.60, 95% CI [1.13–2.24]) with having NTDs.[31] In addition, lack of antenatal care (AOR 2.26, 95% CI [1.30–3.94]), preconception intake of folic acid (AOR 0.41, 95% CI (0.26–0.66)), having chronic medical illness (AOR 2.06, 95% CI [1.42–

Table 2. Health Conditions Among 878 Pregnant Women From Addis Ababa with Completed Ultrasound Examination

	Fetus with NTD (n = 15)	Unaffected (n = 863)	Total (n = 878)
Maternal diseases (number affected)			
Diabetes mellitus	0	10 (1%) (4 missing)	10 (1%) (4 missing)
Hypertension	0 (7 missing)	37 (4%) (304 missing)	37 (4%) (311 missing)
Febrile illness during pregnancy	3 (20%)	137 (15%) (12w. missing)	131 (15%) (4 missing)
Maternal drug intake during pregnancy (number affected)			
Anticonvulsant Drugs	0	5 (1%) (5 missing)	5 (1%) 5 missing)
Deworming medicine	1 (7%)	243 (3%) (2 missing)	25 (3%) (2 missing)
Methotrexate	1 (7%)	31 (4%) (3 missing)	32 (4%) 3 missing)
NTD History			
NTD in previous pregnancy	0 (8 missing)	3 (0.4%) (346 missing)	3 (0.3%) (354 missing)
Family member with NTD	0	11 (1%) (32 missing)	11 (1%) (3 missing)

2.99]), drinking alcohol (AOR 2.70, 95% CI [1.89–3.85]), smoking cigarette (AOR 2.49, 95% CI [1.51–4.11]), chewing khat (AOR 3.30, 95% CI [1.88–5.80]), exposure to pesticides (AOR 3.87, 95% CI [2.63–5.71]), and residing in urban areas.

(AOR 0.75, 95% CI [0.58 to 0.97]) and family history of NTDs (AOR 2.51, 95% CI [1.36–4.62]) were found to risk factors associated with NTD cases.[31] While some risk factors were not within the scope of our study, we conducted an analysis of vitamin levels in blood samples. Our findings underscore the necessity for more comprehensive investigations into nutritional status.

Our data suggest that a low household income may be associated with NTDs, although the difference was not significant. Most women in our study reported that their household was suffering from some kind of food shortage. Encouraging people to eat food enriched with folate as a possible preventive measure may therefore not be efficient in our population. For this reason, it has been argued that fortification of foods is a better option than supplementation in Ethiopia.[25]

Table 3. Serum Measurements of Folate, Vitamin B12, and Homocysteine for 15 Pregnant Ethiopian Women with Foetuses Affected by NTD and 28 Singleton Controls

Serum level	NTD Pregnancy n = 15	Control Pregnancy n = 28
Folate		
Deficiency	3 (20%)	0
Possible deficiency	5 (33%)	8 (29%)
Normal	7 (47%)	20 (71%)
Vitamin B12		
Severe deficiency	1 (7%)	2 (7%)
Moderate deficiency	3 (20%)	5 (18%)
Normal	11 (73%)	21 (75%)
Homocysteine		
Hyperhomocysteinemia	3 (20%)	0
Borderline	7 (47%)	8 (29%)
Normal	5 (33%)	20 (71%)

STRENGTH

Our study recruited pregnant women from the communities by contacting them at the early ANC-visits. This makes the results more relevant for a larger population of Addis Ababa and less affected by various selection mechanisms. The study was based on ultrasound examination of all pregnant women in the study. Since we include all NTD-cases found early in the pregnancies, our study is less affected by possible selection from spontaneous or induced abortions. Ultrasound examinations were performed at the university hospital and the blood analyses were made in a quality laboratory. The very high prevalence may represent an opportunity to find key risk factors in this population.

LIMITATIONS

Although our study was designed to represent the communities of Addis Ababa, our sample was based on attendance at the health centers providing antenatal care and may be biased toward women with better knowledge about antenatal care and with concerns about complications during pregnancy. As recent reports show that less than half (32%) of pregnant women receive optimal antenatal care visits in Ethiopia.[32] In addition, we did not collect specific details on the number of antenatal care visits or their previous history. Retrieving compiled data retrospectively at the health center posed logistical challenges. However, it's worth noting that a substantial proportion of pregnant women in our study were in their first or early second trimester, suggesting that they likely had their first visit around the time of recruitment.

Despite the high prevalence, our sample size was modest for estimation of associations. Still, our community-based estimates will add to the growing evidence from multiple studies. Some of the women who were enrolled in the study (7%) did not come for ultrasound examination at the university hospital. These may in

Table 4. Estimated Associations Between NTD and Potential Risk Factors Including Serum Measurements

Risk Factors	OR*	95% Confidence Interval	*P* Value
Maternal age ≥30 years	3.5	(1.1–12)	0.03
Single, divorced, separated or widowed mother	1.2	(0.03–8.2)	0.99
Previous spontaneous abortion†	2.5	(0.41–12)	0.35
Educational level (continuous by level)	1.0	(0.66–1.7)	0.96
Unemployed	0.46	(0.01–3.1)	0.76
Family income (continuous by 1000 ETB)	0.75	(0.46–1.03)	0.12
Took folate supplement	2.4	(0.71–10)	0.19
Fever during pregnancy	1.9	(0.33–7.4)	0.53
Serum measurements‡			
Serum folate (per ng/ml)	0.61	(0.42–0.85)	0.002
Serum B12 (per 100 pg/ml)	0.67	(0.41–1.02)	0.06
Serum homocysteine (per μmol/l)	1.3	(1.02–1.8)	0.03

*Odds ratio estimates from exact logistic regression. Estimates are adjusted for maternal age (≥30 years, yes/no).
†Based on 636 women with a previous pregnancy.
‡Analyses based on serum measurements in 15 case-pregnancies and 28 controls.

various ways be different from those who participated. The need for travel to get to the referral university hospital for obstetrics ultrasound examinations may for example have contributed to selection. There may also have been selection ahead of recruitment since not all pregnant women attend the health centers for antenatal care and since some women did not consent. Also, it was a weakness that we did not plan for blood samples from the start in this study. We cannot be sure that the control mothers are representative of all non-case mothers. Although the statistical power was limited by the number of cases, better planning might have given larger numbers of participants for the blood sample analyses and more precise estimates. We acknowledge the importance of achieving statistical significance and will enhance our statistical approach to ensure a more robust and conclusive analysis in future studies.

Identification of risk factors requires that there is adequate variation in risk factor levels within the study sample. The generally poor social conditions and poor nutritional status may partly explain the high prevalence of NTDs. These risk factors may still not show up as significant risk factors within the study if study participants are relatively similar. Moving forward, we recognize the importance of expanding our research to include a more extensive exploration of potential hidden risk factors within the community. This insight will guide future studies and contribute to a more comprehensive understanding of the factors influencing NTDs in our target population. This will involve a targeted investigation into factors unique to this demographic, supplementing our analysis of established risk factors.

CONCLUSION

We attempted to find risk factors among women giving birth in a community with a remarkably high prevalence of NTDs. As expected, maternal age of 30 or above was a significant risk factor for NTDs. Low socioeconomic status and low family income were associated with NTD, but with imprecise estimates. Food security and vitamin status appeared to be generally poor in the community, but higher serum-folate and serum B12 were still protective for NTD. Both dosage and timing of intake of folate supplements appeared to be inadequate. The results indicate a need for revision of current practice of folate supplementation, as well as the importance of integrating preconception counselling with obstetrics care in Ethiopia. However, improving the situation may require improvements in the food security and socioeconomic situation for the families. Fortification of foods or food ingredients used in all households is a measure that should be considered.

CRediT AUTHORSHIP CONTRIBUTION STATEMENT

Abenezer Tirsit: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Data curation, Conceptualization. **Mahlet Yigaramu:** Writing – original draft, Supervision, Methodology, Investigation, Data curation, Conceptualization. **Daniel Zewdneh:** Methodology, Investigation, Conceptualization. **Winner Kucha:** Investigation, Data curation. **Seifu Hagos:** Methodology, Investigation, Data curation, Conceptualization. **Bilal Shikur:** Methodology, Data curation. **Tsegazeab Laeke:** Data curation. **Bente E. Moen:** Writing – original draft, Validation, Supervision, Methodology, Conceptualization. **Rolv T. Lie:** Writing – review & editing, Writing – original draft, Supervision, Methodology. **Morten Lund-Johansen:** Writing – review & editing, Writing – original draft, Validation, Supervision, Conceptualization. **Rupavathana Mahesparan:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Data curation, Conceptualization.

ACKNOWLEDGMENTS

The research team would like to acknowledge all pregnant women enrolled into the study and their families. Also, the health professionals working in all the health centers and the university hospital especially Prof. Daniel Seifu from the department of Biochemistry and Afrah Mohammed for their input during planning the project. At the department of International Collaboration (DIC) in Haukeland University Hospital, Jon Wigum Dahl, Grete Marie Slotterø Eilertsen and Camilla Skjelbred, for their continuous support during the PhD training of the first author.

REFERENCES

1. Organization WHO. Congenital anomalies: fact sheet 370 2012. Available at: http://www.who.int/mediacentre/factsheets/fs370/en/. Accessed May 30, 2022.
2. Organization WHO. Global health estimates (GHE) disease burden; 2015. Available at: http://www.who.int/healthinfo/global_burden_disease/estimates/en/index2.html. Accessed April 14, 2015.
3. Moore KL, Persaud TVN. The developing human. Clinically oriented Embryology. In: *Chapter: Congenital anomalies of the brain and spinal cord*. 8th edition. Philadelphia: Saunders; 2008.
4. Asfaw ZK, Tirsit A, Barthélemy EJ, et al. Neurosurgery in Ethiopia: a New chapter and future prospects. *World Neurosurg*. 2021;152:e175-e183.
5. Getahun S, Masresha S, Zenebe E, Laeke T, Tirsit A. Four-year treatment outcomes of children operated for neural tube defect in Addis Ababa, Ethiopia: a retrospective study. *World Neurosurg*. 2021;148:e695-e702.
6. Tirsit A, Bizuneh Y, Yesehak B, et al. Surgical treatment outcome of children with neural-tube defect: a prospective cohort study in a high volume center in Addis Ababa, Ethiopia. *Brain Spine*. 2023;3:101787.
7. Taye M, Afework M, Fantaye W, Diro E, Worku A. Magnitude of birth defects in central and northwest Ethiopia from 2010-2014: a descriptive retrospective study. *PLoS One*. 2016;11:e0161998.
8. Gedefaw A, Teklu S, Tadesse BT. Magnitude of neural tube defects and associated risk factors at three teaching hospitals in Addis Ababa, Ethiopia. *BioMed Res Int*. 2018;2018:4829023.
9. Sorri G, Mesfin E. Patterns of Neural tube Defects at two teaching hospitals in Addis Ababa, Ethiopia. A three years retrospective study. *Ethiop Med J*. 2015;53:119-126.
10. Berihu BA, Welderufael AL, Berhe Y, et al. High burden of neural tube defects in Tigray, Northern Ethiopia: hospital-based study. *PLoS One*. 2018;13:e0206212.
11. Tirsit A, Zewdneh D, Yigeremu M, et al. Prevalence of neural tube defects among pregnant women in Addis Ababa: a community-based study using prenatal ultrasound examination. *Childs Nerv Syst*. 2023;39:2423-2431.
12. Zaganjor I, Sekkarie A, Tsang BL, et al. Describing the prevalence of neural tube defects worldwide: a systematic literature review. *PLoS One*. 2016;11:e0151586.
13. Atlaw D, Tekalegn Y, Sahiledengle B, et al. Magnitude and determinants of neural tube defect in Africa: a systematic review and meta-analysis. *BMC Pregnancy Childbirth*. 2021;21:426.
14. Oumer M, Tazebew A, Silamsaw M. Birth prevalence of neural tube defects and associated risk factors in Africa: a systematic review and meta-analysis. *BMC Pediatr*. 2021;21:190.
15. Bitew ZW, Worku T, Alebel A, Alemu A. Magnitude and associated factors of neural tube defects in Ethiopia: a systematic review and meta-analysis. *Glob Pediatr Health*. 2020;7:2333794X20939423.
16. Gashaw A, Shine S, Yimer O, Wodaje M. Risk factors associated to neural tube defects among mothers who gave birth in North Shoa Zone Hospitals, Amhara Region, Ethiopia 2020: case control study. *PLoS One*. 2021;16:e0250719.
17. Vieira AR, Castillo Taucher S. Edad materna y defectos del tubo neural: evidencia para un efecto mayor en espina bífida que anencefalia [Maternal age and neural tube defects: evidence for a greater effect in spina bifida than in anencephaly]. *Rev Med Chil*. 2005;133:62-70.
18. Bound JP, Francis BJ, Harvey PW. Neural tube defects, maternal cohorts, and age: a pointer to aetiology. *Arch Dis Child*. 1991;66:1223-1226.
19. Yi Y, Lindemann M, Colligs A, Snowball C. Economic burden of neural tube defects and impact of prevention with folic acid: a literature review. *Eur J Pediatr*. 2011;170:1391-1400.
20. Kancherla V, Koning J, Biluts H, et al. Projected impact of mandatory food fortification with folic acid on neurosurgical capacity needed for treating spina bifida in Ethiopia. *Birth Defects Res*. 2021;113:393-398.
21. Central statistical agency (CSA) [Ethiopia] and ICF. 2016. Ethiopia demographic and health Survey 2016: key indicators report. Addis Ababa, Ethiopia, and Rockville, Maryland, USA. CSA and ICF.
22. Mamuye Azanaw M, Gebremariam AD, Teshome Dagnaw F, et al. Factors associated with numbers of antenatal care visits in rural Ethiopia. *J Multidiscip Healthc*. 2021;14:1403-1411.
23. Haidar j, Melaku U, Pobocik RS. Folate deficiency in women of reproductive age in nine administrative regions of Ethiopia: an emerging public health problem. *S Afr J Clin Nutr*. 2010;23:132-137.
24. Welderufael AL, Berihu BA, Berhe Y, et al. Nutritional status among women whose pregnancy outcome was afflicted with neural tube defects in Tigray region of Ethiopia. *Brain Dev*. 2019;41:406-412.
25. Dixon M, Kancherla V, Magana T, Mulugeta A, Oakley GP. High potential for reducing folic acid-preventable spina bifida and anencephaly, and related stillbirth and child mortality, in Ethiopia. *Birth Defects Res*. 2019;111:1513-1519.
26. Chiang T, Schultz RM, Lampson MA. Meiotic origins of maternal age-related aneuploidy. *Biol Reprod*. 2012;86:1-7.
27. Ami N, Bernstein M, Boucher F, Rieder M, Parker L. Canadian Paediatric Society, Drug Therapy and Hazardous Substances Committee. Folate and neural tube defects: the role of supplements and food fortification. *Paediatr Child Health*. 2016;21:145-154.
28. Guideline. *Optimal Serum and Red Blood Cell Folate Concentrations in Women of Reproductive Age for Prevention of Neural Tube Defects*. Geneva: World Health Organization; 2015.
29. Wolff T, Witkop CT, Miller T, Syed SB, U.S. Preventive Services Task Force. Folic acid supplementation for the prevention of neural tube defects: an update of the evidence for the U.S. Preventive Services Task Force. *Ann Intern Med*. 2009;150:632-639.
30. Kucha W, Seifu D, Tirsit A, et al. Folate, vitamin B12, and homocysteine levels in women with neural tube defect-affected pregnancy in Addis Ababa, Ethiopia. *Front Nutr*. 2022;9:873900.
31. Tesfay N, Hailu G, Habtetsion M, Woldeyohannes F. Birth prevalence and risk factors of neural tube defects in Ethiopia: a systematic review and meta-analysis. *BMJ Open*. 2023;13:e077685.
32. Hailemariam T, Atnafu A, Gezie LD, et al. Utilization of optimal antenatal care, institutional delivery, and associated factors in Northwest Ethiopia. *Sci Rep*. 2023;13:1071.

Conflict of interest statement: Addis Ababa University, Ethiopia facilitated the study project, including planning and the data collection. The University of Bergen, Norway granted and sponsored a PhD study for the first author.

Received 12 December 2023; accepted 20 February 2024

Citation: World Neurosurg. (2024) 185:e683-e690. https://doi.org/10.1016/j.wneu.2024.02.108

Journal homepage: ***www.journals.elsevier.com/world-neurosurgery***

Available online: ***www.sciencedirect.com***

APPENDIX

Supplementary Table 1. This interview guide was used during examinations…..explain where and when it was used.

Obstetric Ultrasound, 9—12 weeks	
No of intrauterine pregnancy	
Gestational age using crown-rump length (mm), (for each fetus if multiple)	
Is there is any cystic mass besides the yolk sac?	
Is trans cranial lunacy visible?	
Is the cisterna magna effaced?	
Nuchal translucency distance- (mm)	
Obstetric ultrasound between 18—22	
Gestational age (for each fetus if multiple)	
Is the supra orbital skull vault formed?	
If no,	Cerebral parenchyma absent Present but disorganized
Is there is any spinal defect in continuity?	
If yes, length of spinal defect (in terms of number of spine)	
If yes to 1608, describe the shape of frontal bone	Concave anterior or lemon shaped Oval Crescent shape
Cerebellar shape	
Herniation of cerebellar tonsil below foramen magnum:	Yes No
Is there is any spinal defect?	Yes No
Is overlying skin	Intact Defective
Level of defect	Cervical Thoracic Thoracolumbar Lumbar sacral
Gestational age (for each fetus if multiple)	
Is there is any overlying cystic mass in	

Form used at ultrasound for NTD examination.