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PhD in Clinical Anatomy and Neurobiology

Unveiling the Hidden Burden of Neural Tube Defects Crisis: A Triple-Lens Investigation of Prevalence, Risk Factors and Maternal Folate Deficiency in War-Torn Tigray, Northern Ethiopia

PhD Dissertation

By: Birhane Alem Berihu (PhD candidate)

A Thesis Submitted to Mekelle University, College of Health Sciences, Institute of Biomedical Sciences, Department of Anatomy for the Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Clinical Anatomy and Neurobiology

DECLARATION BY THE STUDENT

DECLARATION BY THE STUDENT

I, Birhane Alem Berihu, hereby certify that I have personally carried out the work presented in this dissertation entitled:

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APPROVAL OF THE DISSERTATION BY EXAMINERS

The dissertation entitled:

"Unveiling the Hidden Burden of Neural Tube Defects Crisis: A Triple-Lens Investigation of Prevalence, Risk Factors and Maternal Folate Deficiency in War-Torn Tigray, Northern Ethiopia"

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Abstract

Background: Neural tube defects (NTDs) are among the most severe congenital anomalies globally, with disproportionately high prevalence in low-resource settings. The Tigray regional stateinnorthern Ethiopia, already facing one of the highest NTD burdens in sub-Saharan Africa, has endured an extensive armed conflict for several decades and as recently as in 2020, resulting in the collapse of its healthcare system, widespread famine, and profound maternal health challenges. Despite anecdotal reports of increased birth defects during the conflict, empirical data have been lacking.

Objective: This dissertation aimed to assess the prevalence, and associated factors of NTDs in war-affected areas of Tigray. It also evaluated maternal red blood cell (RBC) folate levels in NTD high-risk regions to explore biochemical risk factors contributing to these anomalies.

Methods: A mixed-methods approach was employed across three interrelated studies. Quantitative data were obtained through hospital-based retrospective chart reviews, prospective observational assessments, and laboratory analyses of RBC folate concentrations in first-trimester pregnant women. Sociodemographic, obstetric, nutritional, and biochemical variables were analyzed to identify risk factors associated with NTDs.

Results: The findings revealed an alarmingly high prevalence of NTDs (262 per 10, 000 births) in the conflict-affected health facilities of Tigray, significantly exceeding national and regional estimates. Risk factor analysis of our study showed strong links between NTDs and factors such as young maternal age, rural residence, unplanned pregnancy, poor prenatal care, low education, high birth order, and lack of folic acid use. Additional risks included poor folate awareness, food insecurity, low dietary diversity, violence exposure, and limited healthcare access. Biochemical assays showed widespread deficiencies in RBC folate among pregnant women in high-risk areas, with levels frequently falling below WHO-recommended thresholds for NTD prevention, with dietary diversity strongly influencing folate status, emphasizing the key role of nutrition in NTD prevention.

Conclusion: This study demonstrates an alarming surge in NTDs in war-affected Tigray, primarily driven by widespread folate deficiency, poor dietary diversity, and the collapse of maternal health services. Additional risk was compounded by key socio demographic factors such as low education attainment, rural residence, and limited reproductive autonomy. These findings underscore how conflict magnifies preventable health crises through the convergence of nutritional, medical, and social failures. Hence, efforts should prioritize the restoration of antenatal care services, targeted folic acid supplementation for women of reproductive age, and community-based nutrition education to improve dietary diversity. In the longer term, food fortification with folic acid should be considered once health systems stabilize. These targeted interventions fall within the study's evidence base and are feasible within conflict-affected and resource-limited contexts.

Key words: neural tube defectcs, Folate deficiency, Risk fctors, War, Siege, Humanuterian crisis, Tigray, Ethiopia

Acknowledgments

I would like to express my sincere gratitude to my principal advisor, Dr. Hayelom K Mekonen, for his exceptional guidance, constructive feedback, and continued support throughout the course of my doctoral studies. I am also deeply grateful to my co-advisors, Professor Afework Mulugeta and Professor Tony Magana, for their valuable insights, mentorship, and scholarly contributions that greatly enhanced the quality and rigor of this work.

I gratefully acknowledge the financial and institutional support provided by Mekelle University, the School of Graduate Studies and Research, and Central Washington University Deaprtment of Health sciences, Ellensburg, Washington. I am particularly thankful to Dr. Tafere Gebreegziabher Belay for his generous financial assistance and professional guidance, which were critical to the successful completion of this research.

My appreciation also extends to the Department of Anatomy, the Institute of Biomedical Sciences, and the College of Health Sciences for providing the academic environment, facilities, and resources necessary to undertake this study.

I am indebted to the pregnant mothers who generously consented to participate in the study. I also wish to thank the health facilities and the dedicated data collectors for their collaboration and support during the data collection process.

Finally, I extend my heartfelt thanks to my wife, Hiwot Asreshey, and to my children, Firtuna Birhane, Mussie Birhane, and Feruz Birhane, for their unwavering love, patience, and encouragement. Their support has been a cornerstone throughout this academic journey.

Dedication

In loving memory of all the heroic martyrs of Tigray who gave their lives in the struggle for a brighter future of our people. Your sacrifice, dedication and courage will never be forgotten. Your memory lives on in my heart and in the very purpose of this work.

May you rest in peace.

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List of original papers

This dissertation is based on three papers which are listed below:

1. Berihu BA, Mulugeta A, Magana T, Tessema M, Gebreegziabher T, Berhe Y, et al. Neural tube defects in a war-torn Tigray regional state of Ethiopia: a retrospective study of 54,626 deliveries. BMC Pregnancy Childbirth. 2025;25(1):108. doi:10.1186/s12884-025-07206-3

2. Berihu BA, Mulugeta A, Magana T, Belay TG, Yang P, Mekonen HK. Risk factors for neural tube defects in the war and siege-affected Tigray regional state of Ethiopia: a case-control study. BMC Pregnancy Childbirth. 2025;25(1):1–3. doi:10.1186/s12884-025-07865-3

3. Berihu BA, Mulugeta A, Magana T, et al. Near-universal folate deficiency in first trimester pregnant women from Tigray, Ethiopia: implications for neural tube defect prevention. BMC Pregnancy Childbirth(Under review).

List of abbreviation

ANC	Antenatal Care
BD	Birth Defect
DOHaD	Developmental Origins of Health and Disease
EMT	Epithelial-to-Mesenchymal Transition
FAO	Food and Agriculture Organization (of the United Nations)
IFA	Iron and Folic Acid
LMICs	Low- and Middle-Income Countries
MHP	Median Hinge Point
MNCH	Maternal, Newborn, and Child Health
MTHFR	Methylenetetrahydrofolate Reductase
NTD	Neural Tube Defect
PCP	Planar Cell Polarity
RBC	Red Blood Cell
RMNCH	Reproductive, Maternal, Newborn, and Child Health
SGBV	Sexual and Gender-Based Violence
SHH	Sonic Hedgehog
SSA	Sub-Saharan Africa
MTHFR	Methylenetetrahydrofolate Reductase
MTRR	Methionine Synthase Reductase

Chapter one- Introduction

1. Background

Maternal and child health serves as one of the most sensitive indicators of a population's overall wellbeing. In 2021, birth defects (BDs) ranked among the leading causes of infant and child mortality, second only to preterm birth and low birth weight [1]. Birth defects—defined as structural or functional abnormalities that may be detected before birth, at delivery, or later in life—can result from genetic, environmental, or multifactorial causes and may recur across generations [2,3]. Globally, an estimated 8.5 million new cases of birth defects were reported in 2019, with nearly one-third occurring in sub-Saharan Africa. These conditions contributed to approximately 473,400 deaths among children under five years old, accounting for 9.4% of global mortality in this age group [4].

Neural tube defects (NTDs) are among the most severe congenital malformations of the central nervous system [5, 6,7]. They result from the incomplete closure of the neural tube during early embryonic development, typically within the first four weeks of gestation. Conditions such as spina bifida, anencephaly, and encephalocele represent the most common forms of NTDs and are associated with high rates of neonatal death, lifelong disability, and substantial social and economic burden [5, 6,7]. Although NTDs have a multifactorial origin, inadequate folate status during the periconceptional period has been conclusively identified as one of the major risk factors [5,6]. NTDs disproportionately affect low- and middle-income countries (LMICs), where access to folic acid supplementation, food fortification, and prenatal screening remains limited.

While developed nations have reported significant declines in NTD prevalence following mandatory folic acid fortification programs, the condition continues to pose a serious public health challenge in developing regions, with reported incidences ranging between 10 and 110 per 10,000 births [7]. In sub-Saharan Africa, including Ethiopia, NTDs remain under-recognized and under-prioritized within maternal and child health policies despite their significant contribution to infant morbidity and mortality [8, 9]. In Ethiopia, the burden is particularly high. A systematic review estimated the pooled prevalence of NTDs to be between 71.48 and 86.58 per 10,000 births [8], which is nearly twice the rate reported for East Africa [9]. Additionally, approximately 104 out of every 10,000 births in Ethiopia are estimated to result in neonatal death due to NTDs [9]. According to Mekonen et al. [10], of 12,225 neonates

admitted to nine government hospitals, 383 (3.13%) had congenital anomalies, with NTDs accounting for 68.7% of these cases. Previous studies in Tigray reported NTD prevalence rates ranging from 1.31% to 2.15% of total births examined [10,11].

NTDs have long been a significant public health concern in the Tigray region of northern Ethiopia, an area frequently affected by humanitarian crises, recurrent drought, and food insecurity. The region has also suffered the devastating impacts of prolonged war and siege, leading to widespread displacement of over two million people, increasing hunger, and heightened rates of sexual and gender-based violence (SGBV) [12–18]. Persistent insecurity and the breakdown of law and order have severely disrupted the regional health system [12–18]. Health facilities have been looted or destroyed, and essential medical and nutritional services have collapsed.

Since the outbreak of the 2020 war in Tigray, health infrastructure, supply chains, and public health programs have been extensively damaged [12–18]. Even prior to this conflict, Tigray had a history of war and famine that strained its health system. The recent conflict has intensified existing challenges, restricting access to antenatal care, micronutrient supplementation, diagnostic services, and adequate nutrition. Pregnant women have become particularly vulnerable due to malnutrition, psychological distress, and limited access to maternal healthcare [12–18].

Although there have been anecdotal and clinical reports suggesting a rise in adverse pregnancy outcomes, including NTDs, during the conflict period, empirical data remain scarce. Moreover, the complex interactions between war, nutritional deprivation, and congenital anomalies have received little scientific attention in humanitarian contexts. Therefore, this dissertation seeks to assess the prevalence and associated factors of NTDs in war-affected areas of Tigray, northern Ethiopia, and to evaluate maternal red blood cell (RBC) folate levels in high-risk populations. By employing an integrated research approach, this study aims to generate evidence on how the war and siege and humanitarian crisis have influenced NTD occurrence and to provide data-driven insights for developing targeted preventive and interventional strategies for use within Tigray’s health system and similar conflict-affected settings.

1.2 Statement of the problem

Neural tube defects (NTDs) remain a critical yet insufficiently addressed public health challenge in war-affected settings, particularly in the Tigray regional state of Northern Ethiopia. These congenital anomalies, which are strongly linked to maternal folate deficiency, have devastating consequences for neonatal survival and long-term health outcomes. Despite their preventability, NTDs have received minimal attention in the context of humanitarian crises.

The recent war and siege in Tigray (2020–2022) have profoundly disrupted the region's already fragile health system. Health facilities have been damaged or rendered non-functional, supply chains for essential medicines and supplements have been interrupted, and access to antenatal care has been severely curtailed. At the same time, widespread food insecurity has likely intensified maternal undernutrition, including deficiencies in critical micronutrients such as folate. Collectively, these conditions create a perfect storm for the emergence or worsening of neural tube defects among newborns.

Hospital-based reports and clinical observations suggest a possible surge in the incidence of NTDs across the region. However, robust epidemiological data are critically lacking. The breakdown of surveillance systems in conflict zones makes large-scale data collection difficult, and this gap in reliable, population-level information significantly impairs efforts to understand the true scale of the problem. In particular, there is a glaring absence of data on maternal red blood cell (RBC) folate concentrations—an established biomarker of folate status and a direct indicator of NTD risk.

This lack of comprehensive and empirical data on NTDs and their determinants in Tigray not only limits targeted interventions but also reflects a broader blind spot in global health responses to congenital anomalies in conflict-affected populations. The current gap in evidence prevents policymakers, humanitarian actors, and health system leaders from designing informed, effective strategies for prevention, early detection, and care.

Therefore, there is an urgent need for systematic, multidisciplinary research that integrates prevalence study, risk factor, and biomarker assessment. By generating high-quality evidence on the prevalence of NTDs, associated maternal and environmental risk factors, and folate status among pregnant women, this dissertation seeks to fill this critical knowledge gap. Ultimately, the goal is to inform the design of context-appropriate, evidence-based

interventions and policy responses both within the Tigray health system and in similar conflict-affected settings globally aimed at improving maternal nutrition, strengthening antenatal care, and preventing future cases of neural tube defects.

1.3 Rationale of the Study

This study is considered timely and important in light of existing reports suggesting a potential surge in the burden of NTDs and the unprecedented humanitarian crisis in Tigray. The research is justified on the following grounds:

Public Health Relevance: Understanding the burden and determinants of NTDs in war-affected regions is crucial for developing effectiveprevention and interventional strategies. Since many of the risk factors such as folate deficiency-are modifiable, identifying them can inform both immediate and long-term public health responses.

Scientific Contribution: This dissertation will contribute new data on the epidemiology of NTDs in a conflictaffected setting. It also integrates a biochemical assessment of maternal micronutrient status, thus bridging clinical observation with underlying nutritional pathology.

Policy and Programmatic Impact: Findings of the study will be communicated to national and regional healthauthorities, humanitarian agencies, and NGOs working in maternal and child health. Data generated from this study can support folic acid supplementation programs, antenatal nutrition interventions, and birth defect surveillance systems.

ContextSpecific Knowledge: By focusing on a war-torn region, the study acknowledges the unique vulnerabilities and environmental stressors affecting maternal health. The findings will also provide background information to other conflictaffected areas in the world.

1.4 Significance of the study

This dissertation holds significant implications across multiple domains:

Anatomy of Human development/Embryonic and fetal anatomy/Embryological and morphogenetic science

Understanding Neural Tube Defects (NTDs) within the context of a conflict-affected regions such as like Tigray region, provides valuable insights into how environmental and socio-economic factors influence embryonic development. NTDs result from the disruptions in the

early stages of neural tube formation. Examining their prevalence and associated risk factors in such a unique setting enhances our understanding of how external stressors- such as nutritional deficiencies, infections, and maternal health disparities- impact embryogenesis. This research contributes meaningfully to the fields of developmental anatomy and embryology by identifying potential new factors affecting neural tube closure and provides a rare opportunity to explore how these factors may vary under conditions of instability and conflict.

Community Relevance

The findings from this study are crucial for the local communities in Tigray. The high rates of NTDs can lead to significant physical and psychological burdens for affected families and communities. By identifying the prevalence and determinants of NTDs in the region, the study will help local health authorities and non-governmental organizations to design tailored interventions to address the specific needs of affected populations. Improved understanding and management of NTDs can enhance community health outcomes, reduce disability, and alleviate the socio-economic impacts of these conditions on families.

The public health significance

The public health significance of this study is profound. Armed conflict often led to disruptions in health services, exacerbating existing health issues and creating new challenges. By providing an epidemiological perspective on NTDs amidst such conditions, the research will highlight gaps in healthcare delivery and identify specific barriers to effective maternal and child health services. The results will be instrumental in shaping public health policies, improving prenatal care practices, and ensuring that essential preventive measures, such as folic acid supplementation, are accessible even in conflict zones. Addressing NTDs effectively can lead to a reduction in the overall burden of these conditions, contributing to better health outcomes and a more resilient healthcare system.

Scientific Community

For the scientific community, this study offers a critical contribution to the understanding of how conflict and environmental stressors influence congenital conditions. It narrows a significant gap in the literature by providing data on NTDs from conflict-affected health systems, a context that has been underrepresented in existing research. The findings will be valuable for epidemiologists, public health researchers, and policy makers interested in the

intersection of environmental factors and congenital anomalies. Additionally, the study's methodologies and results can serve as a model for similar research in other conflict-affected areas, broadening the scope of epidemiological and developmental studies globally.

1.5 Research Question

What is the magnitude and distribution of spina bifida, anencephaly, encephalocele, among births in the war-torn Tigray health care system?

What maternal, environmental, and health system-related factors are associated with an increased risk of neural tube defects in newborns delivered in resource-constrained public hospitals in Tigray?

What are the levels of RBC folate among pregnant women in NTD high-risk areas of Tigray, and how do these levels compare with established deficiency thresholds?

1.6 Hypothesis

There is a high burden of neural tube defects (spina bifida, anencephaly, encephalocele) among births recorded in health facilities of war-torn Tigray.

Mothers with inadequate periconceptional folate intake, poor antenatal care access, exposure to war-related stress, and malnutrition are at significantly higher risk of delivering newborns with neural tube defects in Tigray.

Pregnant women in NTD high-risk areas of Tigray have RBC folate levels below the WHO-recommended thresholds, indicating a high prevalence of folate deficiencies that may contribute to NTD risk.

1.7 Objective of the Dissertation

1.7.1 General objective

- To investigate the Prevalence, type, and associated factors for neural tube defects in the Tigray regional state to better understand the different aspects of disease and develop better prevention and treatment strategies.

1.7.2 Specific objective

1. To estimate the prevalence Neural tube defects in a war-torn Tigray regional state of Ethiopia: a retrospective study

2. To Identify risk factors for neural tube defects in the war and siege-affected Tigray regional state of Ethiopia: a case-control study
3. To invstigate RBC folate status in First Trimester Pregnant Women in NTDs high risk area of Tigray, Ethiopia, cross sectional biochemical analysis

Chapter 2: Literature Review

2.1 Introduction

This chapter presents a review of existing literature relevant to neural tube defects (NTDs), their risk factors, and the role of maternal folate status in the Tigray regional state of Ethiopia. The review is structured according to the three main objectives of the dissertation: (i) to assess the burden of NTDs in a war-torn setting, (ii) to identify risk factors for NTDs in the context of conflict and siege, and (iii) to investigate red blood cell (RBC) folate levels among first-trimester pregnant women in NTD high-risk areas.

2.2 Neural Tube Defects: Concepts and Global Burden

2.2.1 Neural Tube Development

The development of the neural tube, a foundational event in early embryogenesis, is a highly orchestrated process termed neurulation. This begins during the third week post-fertilization, following the formation of the primitive streak and the initiation of gastrulation, which generates the trilaminar germ disc consisting of the ectoderm, mesoderm, and endoderm. Among these, the ectoderm responds to inductive signals from the underlying notochord particularly through the secretion of morphogens such as Sonic Hedgehog (SHH) to form a specialized region known as the neuroectoderm. This neuroectoderm thickens to form the neural plate, marking the first visible step toward central nervous system development [19].

As the neural plate elongates cranially and caudally, its lateral edges begin to elevate, giving rise to the neural folds, which flank a central depression called the neural groove. These folds gradually approach one another and fuse at the midline in a zipper-like manner to form the neural tube, the embryonic precursor of the brain and spinal cord [20]. This fusion does not occur simultaneously along the entire length of the neural plate but begins in the cervical region and progresses both cranially and caudally, eventually closing the anterior and posterior neuropores around days 25 and 27 post-fertilization, respectively [21].

Neurulation is typically divided into two key phases: primary neurulation and secondary neurulation. Primary neurulation pertains to the transformation of the neural plate into the

neural tube by folding and fusion mechanisms and primarily gives rise to the brain and the majority of the spinal cord. In contrast, secondary neurulation involves the formation of the caudal (tail) portion of the spinal cord through the condensation of mesenchymal cells in the caudal eminence, followed by their cavitation to form a tubular structure that seamlessly connects with the neural tube formed during primary neurulation [22].

An essential and often underappreciated aspect of this process is the behavior of the neural crest cells, which originate from the dorsal aspect of the neural tube. These cells undergo an epithelial-to-mesenchymal transition (EMT) and migrate extensively throughout the embryo, contributing to the development of peripheral nerves, melanocytes, craniofacial cartilage, and numerous other structures [23]. Neural crest migration is tightly regulated and shares many signaling pathways and gene regulatory networks with neural tube formation, making these two processes intimately interlinked both developmentally and pathologically.

The morphogenesis of the neural tube is driven by a suite of highly dynamic cellular and molecular mechanisms. One of the earliest events is the elongation of neuroepithelial cells, a process regulated by cytoskeletal elements particularly microtubules and strengthened by intercellular adhesion molecules such as cadherins. These changes contribute to the narrowing and elevation of the neural plate and the eventual formation of neural folds [24]. Central to the shaping of the neural tube is the activity of Sonic Hedgehog (SHH), which is secreted by the notochord and floor plate. SHH induces apical constriction at hinge points in the neural plate, particularly the median hinge point (MHP) and dorsolateral hinge points (DLHPs), facilitating the bending of the neuroepithelium necessary for tube closure [25].

A critical layer of regulation occurs via Planar Cell Polarity (PCP) signaling, which coordinates the mediolateral intercalation of cells, a process known as convergent extension. This movement narrows and lengthens the neural plate and is governed by core PCP proteins including VANGL, CELSR, and DVL. Mutations or disruptions in PCP signaling pathways result in a widened, flat neural plate and failed neural tube closure, often manifesting as severe congenital anomalies like craniorachischisis, where the brain and spinal cord remain exposed [26].

Ciliogenesis: the formation of primary cilia on neuroepithelial cells is another vital component, influencing SHH signaling gradients that direct neural patterning and closure. Defects in ciliary structure or function impair signal transduction and are implicated in severe neural tube defects

such as exencephaly [27]. Cilia also serve a mechanical role in sensing extracellular morphogen concentrations, thus helping orient cells within the neuroepithelium.

Importantly, epigenetic regulation and maternal nutritional status intersect significantly with these genetic pathways. DNA methylation, histone modifications, and chromatin remodeling complexes tightly regulate the expression of neural tube-related genes. For instance, proper methylation of the PAX3 gene is essential for neural crest differentiation and neural fold elevation. These epigenetic processes are directly influenced by maternal folate status, as folate is a key component of the one-carbon metabolism cycle, necessary for DNA synthesis and methylation reactions. Insufficient folate or disruptions in folate metabolic pathways (e.g., MTHFR polymorphisms) can lead to aberrant gene silencing or activation, thereby predisposing to neural tube defects [28].

In summary, the embryogenesis of the neural tube represents a quintessential example of the complex interplay between cellular biomechanics, molecular signaling, genetic programming, and environmental modulation. Any perturbation in these pathways whether due to genetic mutations, nutritional deficiencies, or teratogenic exposures can derail the precise process of neurulation, leading to structural and functional malformations collectively known as neural tube defects (NTDs). Understanding this intricate developmental choreography is essential not only for elucidating the etiology of NTDs but also for developing effective prevention strategies.

2.2.2 Critical Window of Neural Tube Development

The development of the neural tube occurs within a tightly regulated and temporally critical window during embryogenesis, typically between days 19 and 29 post-fertilization, corresponding to the third and fourth weeks of gestation. This period is pivotal because it encompasses the entire process of neurulation from the formation of the neural plate to the closure of the anterior and posterior neuropores [21]. Failure of the neural tube to close properly during this developmental window results in neural tube defects (NTDs), a group of severe congenital malformations that can affect both cranial and spinal structures [22].

Closure of the neural tube is not a single event but rather a series of regionally distinct closures that occur at different sites along the rostrocaudal axis. In humans, the first closure is initiated at the level of the future cervical spine (termed Closure 1), and subsequent closures occur in a cranio-caudal fashion along the length of the neural plate [26]. The anterior (cranial) neuropore

typically closes around day 25/26 (approximately Carnegie Stage 11), and the posterior (caudal) neuropore follows suit by day 27/28 (Carnegie Stage 13) [19]. Each of these events is vulnerable to genetic mutations, epigenetic perturbations, and environmental insults, making this window exceptionally sensitive to developmental disruption.

This time frame is critical because the embryo is often still unrecognized by the mother, and organogenesis has not yet progressed far enough for most pregnancy detection methods to confirm gestation. As a result, preventative measures especially folic acid supplementation must be taken pre-conceptionally or immediately upon conception to be effective [27]. Maternal folate plays a pivotal role in regulating DNA synthesis and methylation during this period. Deficiency in folate or disruptions in one-carbon metabolismthrough genetic polymorphisms such as MTHFR C677Thave been linked to an increased risk of NTDs [28]. It is now well established that periconceptional folic acid supplementation can reduce the incidence of NTDs by up to 70%, underscoring the importance of nutritional and metabolic preparedness during this narrow developmental window [29].

Moreover, this window is sensitive to teratogenic influences such as maternal hyperthermia (e.g., high fever or hot tub use), certain pharmaceuticals (e.g., valproic acid, carbamazepine), environmental toxins (e.g., pesticides, heavy metals), poorly controlled maternal diabetes, and alcohol or tobacco exposureall of which have been independently associated with an increased risk of neural tube malformations [30, 31]. These exposures are particularly dangerous during neurulation because they can interfere with cellular processes like convergent extension, hinge point formation, or neural crest cell migrationeach of which is critical for proper closure.

In conclusion, the critical window of neural tube development spans a remarkably short but vitally important period in early gestation. The highly synchronized interplay between morphogenesis, gene expression, and environmental regulation during this time underlines the developmental precision required for successful neural tube closure. This narrow window also presents a compelling argument for proactive public health strategies, such as folic acid fortification and early maternal care, to prevent irreversible congenital anomalies that arise from disruptions during neurulation.

2.2.3 Neural Tube Defects (NTDs)

Neural tube defects (NTDs) are congenital malformations arising from failure of the neural tube to close properly during early embryonic development, typically within the first 28 days

post-fertilization. These anomalies occur when disruptions in the highly coordinated processes of primary and secondary neurulation lead to incomplete closure at one or more regions along the neural axis. The embryological basis of NTDs is multifactorial, involving intricate interactions among genetic, molecular, epigenetic, and environmental determinants that act during the critical window of neural tube development [22,28].

NTDs are broadly classified based on their embryonic origin into defects of primary neurulation and secondary neurulation. Primary neurulation defects, which involve the anterior portions of the neural tube, are responsible for conditions such as anencephaly, encephalocele, and open spina bifida (myelomeningocele). These result from improper folding, bending, or fusion of the neural plate. In contrast, secondary neurulation defects involve the caudal-most spinal cord and result in closed spinal dysraphisms, such as tethered cord syndrome or lipomyelomeningocele, where aberrant canalization and cavitation of the caudal cell mass are implicated [19, 20].

From a cellular perspective, the underlying embryopathology of NTDs involves failures in key morphogenetic movements and tissue interactions (figure 1). These include disrupted convergent extension movements driven by the planar cell polarity (PCP) pathway, improper apical constriction at the median and dorsolateral hinge points, impaired neural crest delamination, and defective cilia-based signal transduction [25, 26 ,35.). For instance, mutations in genes encoding PCP components (VANGL1, CELSR1) have been shown to cause wide, flattened neural plates and craniorachischisis, the most severe form of NTD [34].

Environmental teratogens can compound these genetic predispositions, creating a gene-environment interaction model for NTD etiology. A well-documented example is maternal folate deficiency, which disrupts nucleotide biosynthesis and DNA methylation, impairing the expression of closure-critical genes such as PAX3, ZIC2, and MTHFD1L [36]. Animal models lacking enzymes in folate metabolism pathways, such as MTHFR or Shmt1 knockout mice, exhibit a high frequency of NTDs, reinforcing the importance of metabolic competence during neural tube formation [37].

Another critical embryological insight comes from studies of apoptosis and cell proliferation during neurulation. Proper neural tube closure requires a balance between proliferation of neuroepithelial cells and programmed cell death to sculpt the folding tube. Inhibition of

apoptosis via caspase pathway disruption, or hyperproliferation due to upregulated WNT/β-catenin signaling, has been shown to result in open NTDs [35, 38]. Similarly, altered expression of adhesion molecules like N-cadherin interferes with the fusion of the neural folds, highlighting the importance of precise cell-cell interactions during closure.

The embryology of NTDs is also characterized by the concept of multisite closure, especially in humans, where the neural tube appears to close at several discrete initiation points rather than as a single zippering process. Failure at any of these sites can result in region-specific defects (figure 1). For example, closure failure at cranial sites results in anencephaly or encephalocele, while caudal failure results in spina bifida. This theory is supported by both human morphological studies and mouse models of NTDs [22, 29].

In conclusion, neural tube defects arise from complex disruptions in embryonic neurulation that may involve faulty morphogenetic mechanisms, gene mutations, epigenetic misregulation, and adverse environmental exposures. These embryological failures result in a spectrum of structural malformations that vary based on the site and timing of closure failure. A comprehensive understanding of the embryonic basis of NTDs not only elucidates the pathophysiology of these conditions but also underscores the importance of early maternal health, genetic screening, and preconceptional nutritional support in their prevention.

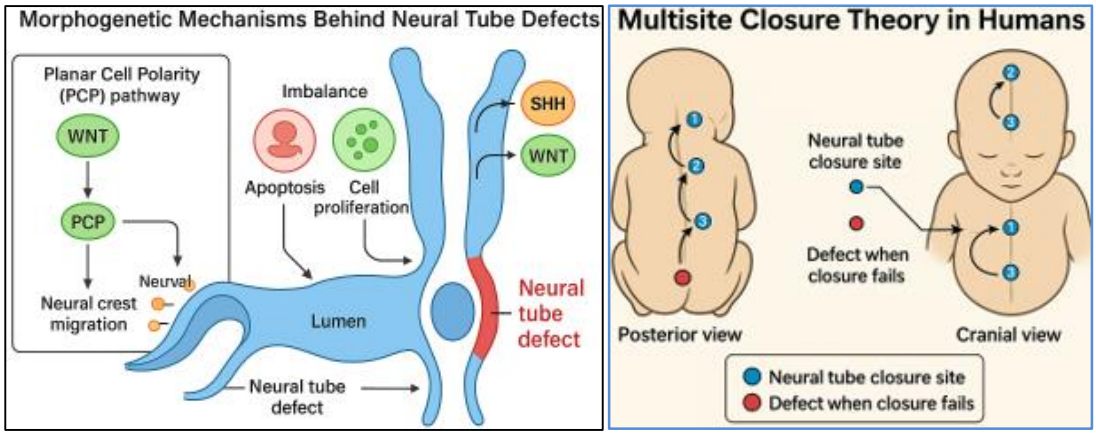

Figure 1: Mechanisms underlying the formation of neural tube defects (NTDs), illustrating how disturbances in morphogenetic processes and multisite closure events lead to defective neural tube formation.

2.2.4 Types of neural tube defects

From WHO classification perspective and public health relevance, NTDs are broadly categorized as either open or closed, depending on whether neural tissue is exposed or covered by skin [22, 27, 36-38].

Cranial neural tube defects

Among cranial NTDs, anencephaly is the most common and severe. It results from the failure of the anterior neuropore to close, leading to the absence of most of the brain, skull, and scalp. Anencephaly is universally fatal, with affected infants often stillborn or dying shortly after birth (Figure 2A). Risk factors include folate deficiency, maternal hyperglycemia, obesity, and elevated maternal temperature during early pregnancy [39, 40].

Encephalocele is another major cranial defect, involving the herniation of brain tissue and meninges through a skull defect, most frequently in the occipital region (Figure 2B). Although potentially compatible with life, encephalocele often results in neurological impairment and requires early surgical intervention [41, 42, 43].

Craniorachischisis, the most severe form of NTD, involves a combined failure of cranial and spinal neural tube closure, leaving both the brain and spinal cord exposed (Figure 2C). It is invariably lethal and often associated with mutations in genes such as VANGL2, CELSR1, and PAX3 [44, 45].

Figure 2: Cranial neural tube defects: A) Anencephaly, B) Encephalocele, C) Craniorachischisis (Images taken from public hospitals in Tigray Northern Ethiopia)

Spinal neural tube defects

Spinal NTDs are another major group, ranging in severity and clinical implications. Spina bifida occulta is the mildest form, characterized by incomplete fusion of the vertebral arches while the skin and spinal cord remain intact. Often asymptomatic, it may be discovered incidentally, though in some cases it presents with symptoms related to tethered cord syndrome or back pain [46, 47].

Meningocele involves herniation of the meninges through a vertebral defect, but the spinal cord itself remains in the vertebral canal. It usually has a favorable prognosis and can be surgically repaired [46, 47, 48-50].

Myelomeningocele is the most common and severe spinal NTD, occurring when both spinal cord and meninges protrude through a spinal defect. It is frequently associated with hydrocephalus and Arnold–Chiari II malformation and results in substantial lifelong neurological and urological impairments unless surgically addressed—ideally in the prenatal period [46, 51-55].

Figure 3: Spinal Neural Tube Defects(myelomemnigiocele/meningiocele) (Images taken from public hospitals in Tigray Northern Ethiopia)

Closed Spinal Dysraphism (Skin-Covered)

Closed spinal dysraphism form another important category and are often covered by skin, making them more difficult to detect. These include tethered cord syndrome, diastematomyelia (split cord malformation), and neurenteric cysts. These defects arise from abnormal development during secondary neurulation and often coexist with vertebral anomalies. While they can remain asymptomatic in early life, many patients present later with progressive neurological decline or orthopedic abnormalities, requiring MRI diagnosis and surgical intervention [56, 57].

Rare and Syndromic NTDs

Some NTDs are rare and syndromic. Iniencephaly, for instance, is a lethal defect characterized by retroflexion of the head, absence of the neck, and severe spinal deformities [58]. Syndromic NTDs are also seen in association with chromosomal abnormalities such as trisomy 13 and 18, often with multi-organ involvement, emphasizing the genetic and systemic nature of some NTD presentations.

Table 1: Types of Neural Tube Defects: Detailed Overview

NTD Type	Embryonic Defect	Closure Site / Timing	Clinical Features
Anencephaly	Failure reg. cranial neuropore closure	Around day 25	Absent cranial vault, brain malformed, lethal
Encephalocele	Cranial bone defect, brain protrusion	Variable cranial region	Brain tissue herniates; variable outcomes
Craniorachischisis	Complete CNS dorsally open	Central neuropore	Severe, often fatal
Spina bifida occulta	Vertebral arch non-fusion	Caudal neuropore (days ~27)	Usually asymptomatic; skin markers present
Meningocele/Myelomeningocele	Neural tissue protrusion through spina	Lumbar/sacral region	Varying neurological deficits; treatable
Diastematomyelia etc.	Split cord / abnormal cysts	Midline gastrulation issues	Tethered cord, scoliosis, neurological signs

2.2.5 Developmental Origins of Health and Disease (DOHaD) Perspective on Neural Tube Defects

The Developmental Origins of Health and Disease (DOHaD) hypothesis posits that environmental and nutritional exposures during critical windows of early development have long-lasting effects on an individual's health trajectory, including the risk of congenital anomalies such as neural tube defects (NTDs). While DOHaD originally focused on metabolic disorders like diabetes and cardiovascular disease, its relevance has since expanded to encompass structural birth defects, particularly those that occur during the earliest phases of embryogenesis. Neural tube closure, which takes place during the first month of gestation, represents one of the most sensitive periods for developmental programming under the DOHaD framework [59, 60].

The DOHaD paradigm emphasizes that maternal factorsranging from diet, metabolic health, stress, and inflammation to environmental exposurescan influence embryonic development via epigenetic modifications, gene-environment interactions, and altered placental function [61]. In the context of NTDs, maternal folate status serves as a classic example. Folate is essential not only for nucleotide biosynthesis but also for methylation reactions crucial to epigenetic gene regulation during neurulation. Suboptimal folate availability, whether due to dietary deficiency, malabsorption, or polymorphisms in one-carbon metabolism genes (e.g., MTHFR, MTRR), leads to impaired methylation of neural tube genes and disrupted cell differentiation [29, 62]

Epigenetic regulation, central to DOHaD theory, mediates how maternal nutritional and environmental exposures are recorded at the molecular level. DNA methylation, histone modifications, and microRNAs orchestrate the expression of genes critical for neural tube closure [63]. For instance, hypomethylation of neural tube regulators such as PAX3 and SHH has been associated with NTDs in both human cases and mouse models. These modifications may persist beyond gestation, influencing not only birth outcomes but long-term neurodevelopmental health, including cognitive impairment and motor deficits [64].

Emerging evidence also highlights the role of maternal metabolic disorders, particularly obesity and diabetes, in influencing neural tube development. Hyperglycemia during early pregnancy increases oxidative stress and disrupts intracellular signaling pathways (e.g., WNT, SHH, AMPK), leading to a higher risk of NTDs [65]. DOHaD research suggests that maternal glycemic and lipid profiles program early embryonic development in a way that predisposes

the neural tube to closure failure, possibly through mitochondrial dysfunction and redox imbalance [66].

Furthermore, intergenerational and transgenerational effects have been observed in DOHaD studies. For instance, maternal exposuressuch as famine, toxicants (e.g., BPA, phthalates), or deficient folate intakecan affect not only the immediate offspring but also subsequent generations through germline epigenetic modifications [67]. This raises the possibility that the risk of NTDs is not confined to a single pregnancy but can become embedded in family lineages due to altered developmental programming.

In addition, the placenta, as the interface between maternal and fetal environments, plays a critical role in modulating the embryonic risk landscape. Placental dysfunction can limit the delivery of micronutrients, including folate, zinc, and inositoleach of which has been implicated in neural tube development. Studies have shown altered placental folate receptor expression and transport dynamics in pregnancies affected by NTDs [68].In general, the DOHaD framework provides a compelling lens through which to understand the multifactorial origins of neural tube defects. It emphasizes that the risk for NTDs is shaped not only by immediate genetic and teratogenic factors but also by the broader maternal environment, which exerts its effects through epigenetic programming, placental mediation, and metabolic signaling. Recognizing this broader context enables more holistic approaches to NTD prevention—moving beyond isolated interventions like folic acid supplementation to encompass maternal health, nutrition, and intergenerational well-being.

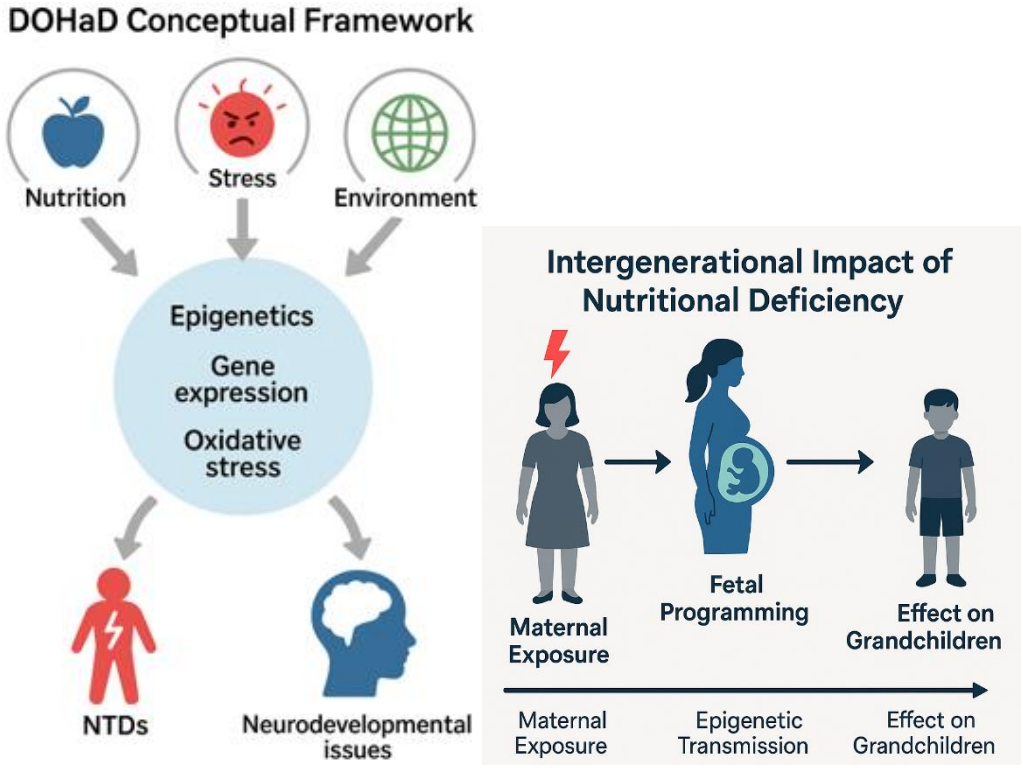

Figure 4: Infographics illustrations of Developmental Origins of Health and Disease (DOHaD)

2.2.6 Global Prevalence and Burden of NTDs

The global incidence and burden of NTDs remain a significant challenge for public health, particularly in regions with high fertility rates, inadequate folic acid intake, and weak health systems. While folic acid fortification and supplementation have been proven to be effective in reducing NTD incidence, sustained progress requires comprehensive strategies that integrate nutrition, education, surveillance, and health systems strengthening. Addressing the global burden of NTDs also necessitates political commitment, community engagement, and equitable access to preventive and curative services.

Neural tube defects (NTDs) represent one of the most common and serious congenital anomalies worldwide. Despite being largely preventable through folic acid supplementation, NTDs continue to pose a significant global health burden, particularly in low- and middle-income countries (LMICs) where preventive and surveillance strategies are often insufficient or inconsistently implemented [38].

Globally, the estimated prevalence of NTDs ranges between 0.5 and 2 per 1,000 live births, with significant regional disparities influenced by genetic, nutritional, environmental, and

socio-economic factors [38, 69]. For instance, regions such as sub-Saharan Africa, South Asia, and parts of Latin America report some of the highest incidence rates, often exceeding 30 per 10,000 births. The highest NTD record in the world is 199 [165.2-233.6] per 10,000 in China, Liliang prefecture, Shanxi province studied in 2004-2005 [70]. Developed countries such as the United States, Canada, and parts of Europe have experienced substantial reductions in NTD incidence following the mandatory fortification of staple foods with folic acid [71]. In the United States, for example, the implementation of folic acid fortification contributed to a 36% reduction of NTDs between 1996 and 2006 [72].

The burden of NTDs is multifaceted, encompassing direct health consequences, economic costs, and broader social impacts. From a health perspective, NTDs such as anencephaly are invariably fatal, while others like spina bifida are associated with lifelong disability, including paralysis, hydrocephalus, incontinence, and cognitive impairment [73]. The World Health Organization (WHO) estimates that over 300,000 babies are born each year with NTDs globally [38], with the highest rates of morbidity and mortality occurring in countries lacking access to prenatal diagnosis, surgical care, and rehabilitation services [36]. Mortality rates remain unacceptably high in many low-income countries due to limited neonatal and neurosurgical care [74].

The economic burden of NTDs is also substantial. Lifetime medical costs for individuals born with spina bifida in high-income countries can exceed $500,000 USD, encompassing surgical interventions, mobility aids, special education, and loss of productivity [75]. In low-resource settings, the absence of appropriate care contributes to economic hardship for families and communities, reinforcing cycles of poverty and disability. Moreover, many families experience psychosocial stress, stigmatization, and limited access to support systems [76]. From a public health perspective, the high burden of preventable NTDs underscores the importance of integrating maternal nutrition, preconception care, and birth defect surveillance into national health strategies.

International efforts to reduce the burden of NTDs have focused largely on folic acid supplementation and fortification. The WHO and the Food and Agriculture Organization (FAO) recommend daily folic acid supplementation of 400 μg for all women of reproductive age, particularly those planning pregnancy [72, 77]. Recently many countries have implemented national mandatory folic acid fortification programs, resulting in measurable declines in NTD prevalence [36]. However, coverage and compliance remain uneven, with

many countries especially in sub-Saharan Africa and Southeast Asia yet to adopt or effectively implement fortification policies. Barriers include lack of political will, limited industrial capacity, and challenges in reaching women before conception [78].

Robust epidemiological data are essential to quantify the global burden of NTDs and monitor progress. However, the quality and availability of data remain highly variable across countries. In high-income settings, national birth defect registries provide comprehensive and timely data. In contrast, many LMICs lack such surveillance infrastructure, resulting in underreporting and data gaps [79]. Recent global initiatives, such as the WHO's Birth Defects Surveillance Toolkit and the Global Birth Defects App, aim to improve data collection and capacity building in resource-limited contexts [80]. These tools facilitate harmonized case definitions, classification standards, and training for frontline health workers [36].

2.2.7 Prevalence and Burden of NTDs in Sub-Saharan Africa

Neural tube defects (NTDs), including anencephaly, spina bifida, and encephalocele, represent a significant public health challenge in Sub-Saharan Africa (SSA), where they are among the leading causes of neonatal morbidity and mortality. The burden in this region is considerably higher than the global average, largely due to multifactorial causes including folate deficiency, delayed healthcare access, and systemic health infrastructure gaps [37, 69]. In SSA, the reported prevalence of NTDs varies widely, ranging from 10 to over 110 per 10,000 births, depending on the region, study design, and diagnostic capacity [37]. This heterogeneity is further compounded by underreporting and limited surveillance infrastructure. While the burden is substantial in this region, it is also largely preventable through evidence-based interventions. A coordinated, multisectoral response is essential to reduce the incidence of NTDs and improve outcomes for affected children and families in the region.

The high prevalence of NTDs in SSA is attributed primarily to suboptimal maternal folate status. Folic acid deficiency remains prevalent among women of reproductive age in many countries across the region, partly due to low dietary intake of folate-rich foods and lack of national folic acid fortification programs [81]. Despite the strong evidence supporting the effectiveness of folic acid in preventing NTDs, only a handful of SSA countries have implemented mandatory food fortification with folic acid. For instance, South Africa initiated mandatory fortification of maize and wheat flour in 2003, which resulted in a significant reduction in NTD incidence from 14.1 to 9.8 per 10,000 births within a few years [82].

However, most other countries in the region including Ethiopia have yet to implement or enforce such policies, leaving a significant portion of the population at risk.

In Ethiopia, NTDs have emerged as a major public health concern, with multiple studies highlighting alarmingly high prevalence rates. A systematic review and meta-analysis by Bitew ZW et al estimated the pooled prevalence of NTDs in Ethiopia to be approximately 63.3 per 10,000 births, markedly higher than the global average [83]. Regional disparities within the country are also evident, with the highest prevalence rates reported in the Tigray, Amhara, and Oromia regions. Hospital-based studies from northern Ethiopia, such as those conducted in the Tigray regional state, have reported prevalence rates ranging from 131 to 215 per 1,000 births, indicating a possible geographic clustering of risk (10, 11). Similarly, Hospital-based studies from Addis Ababa and Amhara have reported prevalence estimates of NTDs to be between 32 and 128 per 10,000 births [84, 85, 86, 87]. These figures are likely underestimates, as they do not account for pregnancies terminated early due to severe fetal anomalies or cases occurring in communities without access to institutional delivery.

The health system response to NTDs in Ethiopia and much of SSA remains inadequate [81, 88]. There is a lack of routine screening, diagnostic, and surgical services, particularly in rural and conflict-affected regions. Consequently, many NTD-affected newborns die within days or weeks of birth due to complications such as infection, hydrocephalus, or respiratory failure [88, 43]. For survivors, the lack of rehabilitation and disability support services imposes lifelong burdens on families and caregivers. Furthermore, stigmatization and cultural misconceptions surrounding birth defects contribute to delayed care-seeking and social isolation of affected children [89].

The economic and social impact of NTDs in Ethiopia and SSA is profound. Families often face catastrophic healthcare expenses, loss of income, and psychosocial stress. At a national level, the economic cost associated with NTDs includes increased demand on the health system, loss of productivity, and reduced human capital development. The long-term disability associated with spina bifida, for instance, can prevent affected individuals from participating fully in education or the workforce, further perpetuating cycles of poverty and exclusion [89, 90, 91].

2.2.8 Evidence of birth defects, with emphasis on neural tube defects in conflict-Affected regions

Birth defects, particularly neural tube defects (NTDs), are among the most severe congenital anomalies affecting newborn survival and long-term neurodevelopment [87, 88]. While their prevalence varies globally, an increasing body of epidemiological literature highlights a disproportionately high burden in conflict-affected regions [88]. In such settings, health system disruption, maternal nutritional deficiencies, environmental contamination, and psychological stress converge to increase both the incidence and severity of congenital anomalies, especially NTDs [92, 93,94].

The epidemiology of neural tube defects in conflict-affected regions reveals critical insights into how war undermines maternal and child health [92, 93,94]. Retrospective hospital records and humanitarian surveys show consistent spikes in NTD prevalence during active conflict, fueled by poor nutrition, disrupted care, and environmental exposures. However, data collection remains highly challenging, with underreporting and diagnostic gaps. [95, 96]. Comparative studies across conflict timelines provide compelling evidence for both the impact of war on congenital outcomes and the potential for improvement through targeted interventions [92, 93,94]. Understanding these trends is essential for guiding maternal health policies in fragile and post-conflict settings.

Post-War Iraq: The Fallujah Case

A study conducted in Fallujah, Iraq, following the intense bombardments of 2004, reported a notable increase in congenital anomalies. An 11-month hospital-based investigation at Fallujah General Hospital (2009–2010) documented 291 congenital anomaly cases among 6,049 births, with central nervous system defects accounting for approximately 25% of these cases. The estimated congenital anomaly rate exceeded 400 per 10,000 births—substantially higher than national averages. Subsequent environmental analyses identified elevated concentrations of teratogenic metals, including uranium, lead, and vanadium, in hair and biological samples from affected families, implicating environmental contamination from wartime munitions as a potential etiological factor [97].

Birth Defects in Gaza During and After Armed Conflict

In the Gaza Strip, recurrent military assaults have also been associated with increased birth defects. A prospective registry study conducted at Al-Shifa Hospital between January 2011 and

January 2012 documented a major congenital anomaly prevalence of 140 per 10,000 live births, alongside elevated rates of stillbirth (70/10,000) and late miscarriage (230/10,000). Nervous system defects were among the most frequent anomalies. Case-control analyses indicated a correlation between birth defects and parental exposure to bombardment, especially white phosphorus attacks and the rebuilding of homes damaged by war, which may contribute to long-term toxic exposure [98].

Pakistan–Afghanistan Border Region

In 2023, a community-based survey in the post-conflict regions of the Federally Administered Tribal Areas (FATA) of Pakistan revealed a significant burden of congenital anomalies. Of 361 identified cases, 27.7% involved neurological anomalies including NTDs, hydrocephalus, and intellectual disability. Although formal prevalence rates were not established due to the convenience sampling approach, the study emphasized the predominance of sporadic (non-familial) cases, with low parental consanguinity, suggesting environmental rather than genetic etiologies in this war-affected and mine-contaminated region [99].

Syrian Refugees and Adverse Birth Outcomes

While direct surveillance in Syria remains unfeasible, studies on displaced Syrian populations provide insight into the impact of war on congenital anomalies. A hospital-based cohort study analyzing 3,638 Syrian refugee deliveries in Jordan (2019–2020) reported higher rates of stillbirth, perinatal death, and congenital anomalies compared to local Jordanian populations, even after controlling for maternal age and parity. Disruption of antenatal services, inadequate periconceptional folic acid supplementation, and psychosocial stress were identified as major contributing factors [100].

Systematic Evidence across Conflict Zones

A 2024 systematic review synthesizing data from A total of 50 papers treating 8 countries and 4 major and medically documented conflicts showed an increase in birth defects (BD) and perinatal death (PD) during and after the end of the conflicts was reported through all the conflicts analyzed, highlighted three recurrent causal pathways linking war to increased BD prevalence: (1) maternal folate deficiency due to food insecurity and lack of supplementation, (2) fetal exposure to teratogenic heavy metals from weapons and environmental degradation, and (3) collapse of reproductive health infrastructure. The review emphasized that every major conflict with credible surveillance data has reported a surge in congenital anomalies during and

following periods of conflict. The authors called for urgent global health interventions, including folic acid fortification and maternal health system recovery in conflict zones [101].

Across diverse geographic contexts, the epidemiological literature underscores a consistent association between armed conflict and increased rates of neural tube defects. The evidence supports both direct (e.g., toxic environmental exposures) and indirect (e.g., malnutrition, healthcare disruption) mechanisms of teratogenesis in these settings. These findings highlight the urgent need for targeted public health interventions, including emergency folic acid supplementation, restoration of antenatal care services, and environmental decontamination in conflict-affected areas.

2.2.9 The Case of Tigray

The war and siege in Tigray have led to the near-total collapse of maternal health services, with grave implications for antenatal care and the prevention of neural tube defects. The disruption of IFA supplementation, combined with widespread malnutrition and lack of health information, has created conditions highly conducive to an increased burden of NTDs. As the region begins to recover, targeted public health interventions focusing on maternal nutrition and ANC revitalization will be essential. Tigray's experience is a stark reminder of how conflict can obliterate decades of health progress, with multigenerational consequences for women and children. The armed conflict that erupted in November 2020 between the Ethiopian federal government and the Tigray People's Liberation Front (TPLF) has had devastating humanitarian and health consequences. The region of Tigray, home to over 7 million people, faced not only active warfare but a prolonged siege that severely limited access to basic services, including healthcare, food, fuel, communication, and banking [102, 103, 104].

During the siege, healthcare infrastructure in Tigray collapsed almost entirely. More than 70% of health facilities were rendered non-functional due to looting, destruction, or lack of supplies [105, 106]. Hospitals and clinics were stripped of medications and equipment, and many health workers fled or were unable to work due to insecurity or lack of pay [102, 106, 107. The blockade of humanitarian aid, including medical supplies and vaccines, exacerbated the situation, leading to what has been described as one of the most severe man-made healthcare crises in recent history [106, 107]. This collapse of healthcare delivery directly affected maternal, newborn, and child health (MNCH) services. Routine immunization, antenatal care (ANC), safe delivery services, and postnatal follow-up were disrupted for more than two years.

The lack of access to hospitals for delivery and emergency obstetric care contributed to a rise in maternal and neonatal mortality [102, 108, 109]. Moreover, health education programs and preventive services including those targeting nutrition and congenital anomaly prevention came to a halt. A regional survey in 2022 indicated that majority of pregnant women in rural Tigray had no access to ANC services during the peak of the siege, and most of them delivered at home without skilled attendants [110]. This erosion of maternal health services has had profound implications for the incidence and management of preventable birth defects, including NTDs.

2.3 Risk factors for developing a neural tube defect (NTDs)

Studying the risk factors for NTDs in such settings is vital not only to identify context-specific drivers of these preventable birth defects, but also to generate evidence for targeted interventions in fragile health systems. In the case of Tigray, Ethiopiaan area severely impacted by war and siege there is an urgent need to understand how the breakdown of healthcare infrastructure, famine-like conditions, and systemic barriers to maternal nutrition have contributed to the persistently high burden of NTDs. Such an investigation can inform policymakers, humanitarian actors, and public health professionals on the necessity of integrating reproductive health and nutrition services into emergency response and post-conflict recovery efforts. Moreover, it provides an empirical foundation for advocacy and international accountability regarding the indirect yet profound impacts of conflict on maternal and neonatal outcomes. As such, this study contributes to closing the research gap on congenital disorders in conflict settings and aligns with global efforts to reduce preventable birth defects through equity-focused and context-sensitive strategies.

Sociodemographic and Behavioral Risk Factors

Multiple studies have reported associations between sociodemographic characteristics particularly maternal education and maternal age and the risk of neural tube defects (NTDs). Evidence suggests that perinates born to older and uneducated women are at greater risk of being affected by NTDs compared to those born to younger or educated mothers [111, 112, 113]. Studies conducted in Colombia and Saudi Arabia corroborated this finding, indicating higher NTD risk among children born to older and less educated women [114, 115]. However, contrary findings were reported from Eritrea and Pakistan, where younger mothers were found to have a higher burden of NTDs [113, 116. One plausible explanation for the increased risk

among older women could be their reduced likelihood of taking folic acid supplements during the periconceptional period [117].

Regarding maternal education, the literature consistently highlights its protective effect against NTDs [111, 112, 113, 118]. Studies from Pakistan and India have demonstrated that uneducated women face a higher risk of delivering children with NTDs [113, 118]. A comprehensive meta-analysis of 45 studies further confirmed that lower educational attainment is significantly associated with an increased risk of NTDs [111]. In the Ethiopian context, awareness and use of periconceptional folic acid supplementation remain limited, especially in rural areas. Women residing in urban settings are more likely to be informed about and have access to folic acid supplementation [119-122]. Utilization of folic acid is significantly higher among women who are educated and those who attend antenatal care (ANC) and family planning services, owing to their better knowledge and access to healthcare [120, 121]. This aligns with findings from other reviews indicating that ANC attendance is positively associated with folic acid use and a subsequent reduction in NTD risk.

Beyond sociodemographic factors, personal behaviors such as cigarette smoking, alcohol consumption, and khat chewing have also been strongly linked to increased NTD risk. Studies conducted in various settings support this association [123, 124, 125]. These habits are believed to interfere with the regulatory mechanisms governing gene expression during neurulation, thereby heightening the fetus's vulnerability to neural tube malformations [126, 127]. In particular, excessive alcohol intake during pregnancy disrupts folic acid transport, further contributing to the risk [128]. In animal models, khat consumption during gestation has been shown to result in fetal growth retardation and developmental anomalies [129]. Moreover, khat cultivated with chemical pesticides often contains residue levels that exceed recommended limits, adding another layer of teratogenic risk for habitual users [130, 131]. These findings underscore the importance of addressing harmful personal behaviors during preconception care and integrating addiction treatment as part of broader reproductive health services [132].

A family history of NTDs also emerges as a notable risk factor. Studies from various contexts have documented increased NTD prevalence among women with first- or second-degree relatives affected by these defects [133, 134]. Although the precise inheritance pattern of NTDs remains unclear, evaluating family history during preconception care can serve as a critical step in identifying genetically susceptible individuals. Such assessments enable early interventions, including the timely initiation of folic acid supplementation, which could

mitigate the risk of recurrence in subsequent pregnancies [135]. Parity and the interpregnancy interval have been identified as significant maternal factors influencing the risk of neural tube defects (NTDs). Evidence suggests that short intervals between pregnancies may compromise maternal nutritional recovery, including folate replenishment, which is essential for neural tube development in early gestation. A classic case–control study conducted by Todoroff and Shaw [136] demonstrated that women with interpregnancy intervals of six months or less had a 1.5-fold increased risk of delivering a child with an NTD. Notably, the risk increased to two-fold when the preceding pregnancy resulted in a live birth, likely due to the greater nutritional depletion associated with full-term pregnancies. These findings highlight the importance of adequate birth spacing to reduce the risk of recurrence of birth defects. The World Health Organization (WHO) supports this recommendation, advocating for a minimum interpregnancy interval of 24 months to allow maternal physiological restoration, including the replenishment of folate and other micronutrients [137].

The association between parity and NTD risk is less consistent but remains a subject of investigation. Some studies have suggested that high parity may be associated with an increased risk of NTDs, potentially due to cumulative nutritional depletion over successive pregnancies or diminished health-seeking behavior in multiparous women. Conversely, other studies have reported no clear association, emphasizing the need for context-specific analysis that accounts for maternal nutritional status, interbirth intervals, and healthcare access.

Socioeconomic status (SES), both at the individual and community levels, also plays a critical role in shaping NTD risk. While low individual income has long been associated with adverse pregnancy outcomes, recent evidence underscores the independent influence of neighborhood-level deprivation on NTD occurrence. Analysis from the U.S. National Birth Defects Prevention Study, which included 1,829 NTD cases, found that women residing in more socioeconomically deprived neighborhoods had significantly higher adjusted odds of delivering infants with NTDs ranging from 1.2 to 1.3 across increasing quartiles of deprivation [138]. These findings suggest that contextual socioeconomic factors beyond individual income exert influence through multiple mechanisms, including reduced access to folate-rich and nutritious foods, increased exposure to environmental toxins, and chronic psychosocial stress. Such conditions may disrupt maternal metabolic and epigenetic processes critical to fetal development, including neural tube closure.

Collectively, these findings emphasize the multifactorial nature of NTD risk, where biological, behavioral, and structural determinants interact. Interventions aiming to reduce NTDs should therefore include strategies to promote optimal birth spacing, ensure nutritional support for women with high parity, and address the broader socioeconomic determinants of health through policies that improve food security, environmental safety, and equitable access to prenatal care.

War as catalyst for neural tube defects risk

War significantly increases the risk of neural tube defects (NTDs) and adverse maternal and child health outcomes due to a combination of destabilizing factors. In conflict zones, the collapse of healthcare infrastructure, displacement of healthcare workers, and shortages of essential medicines severely disrupt maternal health services. These disruptions limit access to antenatal care, emergency obstetric services, and crucial interventions like folic acid supplementation. For example, in Syria and Ethiopia's Tigray region, conflict has led to sharp rises in maternal mortality and the near-total breakdown of maternal health systems [92, 93, 94, 110]. War also contributes to widespread micronutrient deficiencies, exposure to teratogenic environmental hazards, and psychological trauma, all of which compound the risk of birth defects. Addressing these challenges requires a rights-based, multisectoral approach, prioritizing emergency nutrition programs, fortified food distribution, mobile health services, and robust surveillance systems. Reducing the preventable burden of NTDs in war-affected areas is not only a matter of effective public health strategy but also an ethical responsibility.

Disruption of Antenatal Care: Regular antenatal care (ANC) is a cornerstone of maternal health and a key entry point for preventing NTDs. The WHO recommends at least eight ANC contacts during pregnancy [139]. However, in conflict-affected regions, antenatal care (ANC) coverage is often severely compromised, with limited access to services, poor quality of care, and significant shortages of essential maternal health supplies, including micronutrient supplements. The disruption of health systems due to insecurity, displacement, and resource constraints contributes to these deficiencies [140, 141]. Displacement, insecurity, and health worker shortages contribute to low service utilization, which hampers early diagnosis of congenital anomalies and compromises pregnancy outcomes.

Famine and Food Insecurity: Famine and food insecurity, even in the absence of warfare, are potent contributors to NTD burden. During the Dutch Hunger Winter (1944–45), maternal starvation during early pregnancy was associated with a three-fold increase in male spina bifida

mortality, underscoring the critical role of adequate maternal nutrition during early neural development [142, 143]. A more recent study in the United States found that household food insecurity was associated with 1.7-fold increased odds of major birth defects, likely due to disrupted folate intake and maternal stress [144]. War has magnified these deficits in Tigray: a community-based study conducted nine months after the Pretoria peace agreement reported that 88.8 % of pregnant and lactating women were food-insecure and 50 % were experiencing household hunger [145]. Food shortages coincide with a collapse of health-service infrastructure, reducing opportunities for nutritional counselling and supplementation [108]. Together, chronic nutrient deprivation and acute stressors create a biologically plausible pathway to the very high NTD prevalence documented in northern Ethiopia.

Nutritional Deficiencies: Folate and other micronutrients are essential for fetal development, especially in the early gestational period. War and conflict-related food insecurity often forces affected populations to rely heavily on unfortified staple foods that are typically low in essential micronutrients such as folate. This dietary shift exacerbates existing nutritional deficiencies among vulnerable groups, particularly pregnant women, thereby increasing the risk of adverse pregnancy outcomes [146, 147]. A study conducted among Senegalese women aged 15–49 years revealed a high prevalence of both folate deficiency and anemia, highlighting significant gaps in micronutrient status and maternal nutrition in the region, directly increasing the risk of NTDs [148].

Preconceptional Supplementation and Barriers: WHO recommends 400 µg folic acid daily from at least one month pre-conception through 12 weeks' gestation [149], yet uptake across sub-Saharan Africa is strikingly low. A 2025 PLOS ONE meta-analysis of 28 studies (n = 12 562) estimated that only 14.1 % of women reported pre-conception supplementation, with the lowest proportion (1.9 %) in Ethiopia [81]. Stock-outs, out-of-pocket cost, late ANC booking and disrupted supply chains during conflict further constrain access in Tigray [108]. Ensuring an uninterrupted pipeline of low-cost multiple-micronutrient supplements through primary-care and humanitarian channels is therefore critical.

Environmental Exposures: War-related environmental degradation introduces additional teratogenic risks. The use of heavy metals, chemical weapons, and burn pits can contaminate air, water, and soil, exposing pregnant women to neurotoxic agents. Research conducted in Iraq has revealed significantly elevated rates of neural tube defects (NTDs) and other congenital anomalies in regions that experienced intense bombardment during the Gulf and Iraq wars.

These patterns have been suspected to be associated with environmental contaminants resulting from military activity [150].

Psychological Stress and Trauma: Maternal stress has been implicated in adverse pregnancy outcomes, including preterm birth, fetal growth restriction, and congenital anomalies. The neuroendocrine effects of chronic stress particularly elevated levels of cortisol can impair placental function and negatively affect embryonic development. Prolonged exposure to maternal stress hormones has been linked to alterations in fetal growth and developmental outcomes [151]. Case control studies conducted among low-income populations in the United States have demonstrated an association between maternal stress and the occurrence of neural tube defects (NTDs), particularly when compounded by poor nutritional status [152]. In conflict zones, the psychological toll of displacement, violence, and sexual trauma significantly increases such risks. Chronic activation of the maternal hypothalamic–pituitary–adrenal axis elevates cortisol and pro-inflammatory cytokines, alters uteroplacental perfusion and may disturb embryonic neurulation. A 2023 systematic review and meta-analysis of 13 studies found a 34 % increase in NTD risk among women reporting stressful life events during the periconceptional window [153]. Neuro-imaging evidence links prenatal distress with altered fetal hippocampal and cerebellar development, pathways that overlap with those implicated in NTD teratogenesis 154]. In war-torn Tigray, exposure to violence, displacement and loss of social support compound these biological stresses, while mental-health services remain severely curtailed [108].

Increased Unplanned and Adolescent Pregnancies: Armed conflicts frequently disrupt family planning services and are associated with increased rates of sexual violence, transactional sex, and early marriage. These factors collectively contribute to higher incidences of unplanned and adolescent pregnancies in affected populations [155]. These pregnancies often lack timely folate supplementation, further increasing NTD risk. Data from refugee populations, such as the Rohingya in Bangladesh, indicate a high prevalence of adolescent pregnancies alongside severely limited access to antenatal care services, reflecting the compounded vulnerabilities faced by displaced communities [156].

Reliable data on birth defects is scarce in conflict settings due to the collapse of health information systems. Armed conflicts are pervasive across sub-Saharan Africa and have been identified as a significant barrier to progress in reproductive, maternal, newborn, and child health (RMNCH). Assessing the impact of armed conflict on national-level RMNCH outcomes

presents methodological challenges, particularly due to disruptions in data collection and the variability of conflict intensity and duration [95]. This impedes efforts to identify trends, assess the impact of environmental exposures, and plan preventive interventions [96].

Other environmental Factors:

Environmental risk factors significantly contribute to the development of neural tube defects (NTDs), with maternal conditions and exposures during early pregnancy playing key roles. Hyperthermia, defined as an elevated maternal core temperature above 38.5 °C during the third to fourth weeks of gestation, is considered a "highly suggestive" risk factor for NTDs, with a pooled odds ratio (OR) of 2.2 (95% CI: 1.4–3.6), independent of folate status [112]. Case-control studies have implicated both febrile illnesses such as influenza and external heat exposures including sauna use, hot tubs, and electric blankets as contributing factors, with evidence of a dose-response relationship wherein combined exposures may increase the risk up to six-fold. A meta-analysis by Moretti et al. also estimated a two-fold increased risk [157]. While observational data suggest that early antipyretic treatment may partially reduce this risk, current public health guidelines emphasize the importance of fever control and avoiding high-temperature exposures during early pregnancy.

Another well-established risk factor is maternal diabetes. Pregestational diabetes mellitus, including both type 1 and type 2, has been shown to increase the risk of NTDs by two- to four-fold, depending on the level of glycemic control during the periconceptional period. Proposed mechanisms include oxidative stress, dysregulated apoptosis, and altered expression of neural-tube-related genes such as Pax3. Evidence indicates that achieving HbA1c levels below 6.5% before conception can reduce malformation rates to near baseline levels, underscoring the importance of integrated preconception care particularly in light of rising global diabetes rates [158, 159].

Certain medications are also known to be teratogenic, significantly increasing the risk of NTDs. Valproic acid, the prototypical example, is associated with NTD rates of up to 1.4% at standard doses and greater than 3% at doses exceeding 1,450 mg/day. Carbamazepine is associated with approximately a 1% absolute risk, while topiramate carries a smaller but statistically significant risk, especially at doses of 200 mg/day or higher. These findings support clinical guidelines recommending that valproate and topiramate be avoided in women of childbearing potential when alternatives exist, and that high-dose folic acid supplementation (4–5 mg daily) be used

when these antiseizure medications cannot be discontinued [160, 161]. Other teratogenic drugs include methotrexate (a dihydrofolate-reductase inhibitor) and isotretinoin (a retinoic-acid analogue), the latter of which has been linked to major malformations in up to one-third of exposed pregnancies, even under strict pregnancy-prevention protocols. Additional medications such as trimethoprim-sulfamethoxazole, high-dose selective serotonin reuptake inhibitors (SSRIs), and mycophenolate mofetilhave weaker but plausible associations with NTDs. For all of these agents, the greatest risk appears to occur between gestational weeks 3 and 6 [160, 161]. Many teratogenic drugs exert their effects through mechanisms involving folate antagonism, oxidative stress, or disruption of retinoid signaling pathways, all of which are crucial to the process of neurulation. These findings reinforce the rationale for careful pre-prescription counseling and folate optimization in women of reproductive age.

Genetic Factors

Genetic factors and family history contribute to NTD risk through a combination of specific gene mutations, genetic interactions, and inherited predispositions. These genetic factors can affect processes crucial for neural tube development, such as folate metabolism and cell growth. Understanding these mechanisms helps in identifying at-risk individuals and developing strategies for prevention and management of NTDs. Family history of NTDs or other genetic conditions can increase the risk. Certain genetic mutations can also make individuals more susceptible.

Certain mutations in genes that are crucial for neural tube development and closure can increase the risk of NTDs. These genes are involved in processes such as folate metabolism, DNA synthesis, and cell differentiation. Examples: Mutations in the MTHFR gene (which affects folate metabolism) and the SHH gene (which plays a role in embryonic development) have been associated with an increased risk of NTDs [162, 163].

The interaction between multiple genetic variants can also influence NTD risk. For example, having one or more genetic variants related to folate metabolism, combined with other genetic factors affecting cell growth and differentiation, can synergistically increase the risk of NTDs. Epigenetic modifications, which involve changes in gene expression without altering the DNA sequence, can be influenced by genetic factors and environmental conditions. Epigenetic changes can affect genes involved in neural tube development and contribute to NTD risk [162, 164].

Family history of NTDs also suggests a genetic predisposition. If a family has a history of NTDs, it may indicate the presence of inherited genetic factors that increase susceptibility. NTDs can follow different inheritance patterns, including: Autosomal Dominant in which some genetic mutations associated with NTDs may be inherited in an autosomal dominant manner, where only one copy of the mutated gene from either parent is sufficient to increase risk. Other genetic variants may follow an autosomal recessive pattern, where two copies of the mutated gene (one from each parent) are needed to increase risk [165].

Genetic susceptibility to NTDs can be modified by environmental factors such as diet, medication use, and exposure to toxins. For example, individuals with specific genetic variants related to folate metabolism may be more affected by folic acid deficiency, highlighting the interplay between genetics and environmental factors [166, 167].

2.4 Biomarker for NTDs risk assessment

2.4.1 Nutritional Sources of Folate

Folate, also known as vitamin B9, is a water-soluble B-complex vitamin that plays an indispensable role in a range of fundamental biochemical processes, most notably within the one-carbon metabolism pathway [168, 169, 170]. As a key donor and acceptor of one-carbon units, folate is central to reactions involving nucleotide biosynthesis, amino acid metabolism, and methylation reactions, which are vital for maintaining genomic stability, regulating gene expression, and supporting cellular differentiation. One of the most critical functions of folate is its involvement in the de novo synthesis of purines and thymidylate (dTMP), which are essential precursors for DNA and RNA production. Inadequate folate levels impair DNA synthesis and repair, leading to cell cycle arrest and chromosomal instability, particularly in rapidly dividing tissues.

Folate also plays a pivotal role in the remethylation of homocysteine to methionine, a reaction that occurs via the enzyme methionine synthase, in conjunction with vitamin B12 as a cofactor. The methionine produced in this reaction is then converted into S-adenosylmethionine (SAM), the universal methyl group donor involved in a multitude of methylation reactions including those regulating DNA, histones, neurotransmitters, and phospholipids [168, 169, 170]. Therefore, folate is not only critical for genetic information transfer and cellular replication but also for epigenetic regulation, neurological health, and immune function.

The physiological demand for folate increases significantly during periods of rapid growth and cellular proliferation, such as embryogenesis, fetal development, infancy, and adolescence, making adequate folate intake particularly crucial during pregnancy. Folate deficiency during early gestation is strongly associated with the occurrence of neural tube defects (NTDs), such as spina bifida and anencephaly, due to its role in neural tube closure during the first few weeks of fetal development. As such, many public health agencies worldwide have implemented folic acid supplementation or food fortification programsto reduce the incidence of NTDs. Beyond reproductive health, low folate status has been linked to elevated plasma homocysteine levels, a known risk factor for cardiovascular diseases, cognitive decline, and certain cancers, further highlighting its systemic importance [168].

Dietary Sources of Folate:

Folate is widely distributed in a variety of natural food sources, particularly those of plant origin, and its presence in the human diet is essential for supporting key metabolic functions such as DNA synthesis, cellular replication, and methylation. Among the richest natural sources of folate are dark green leafy vegetables, including spinach, kale, romaine lettuce, Swiss chard, and collard greens, which provide significant amounts of the vitamin in its biologically active polyglutamated form. **L**egumes, such as lentils, chickpeas, black beans, pinto beans, and kidney beans, are also notable for their high folate content and are especially important in plant-based diets. Fruits, including oranges, bananas, avocados, papayas, and strawberries, contribute moderate levels of folate and are commonly consumed as part of a varied diet. In addition to plant-based foods, animal liverparticularly beef liveris among the most concentrated sources of folate, offering extremely high levels per serving, although its intake may be limited in some populations due to dietary preferences or cholesterol concerns. Whole grains, especially when minimally processed, also contribute to dietary folate intake, although their content may be lower compared to other sources [171].

Despite the availability of folate-rich foods, dietary intake alone may be insufficient to meet physiological demands in certain populations, particularly women of reproductive age, individuals with increased metabolic needs, or those with limited access to diverse foods. To address this, many countries have adopted mandatory or voluntary food fortification policiesaimed at increasing population-wide folate intake. These policies commonly involve the addition of folic acid, the synthetic and more stable form of folate, to refined cereal grains, wheat flours, rice, cornmeal, and pasta products. Folic acid has higher bioavailability

(approximately 85%) compared to naturally occurring food folates (estimated at around 50%), particularly when consumed on an empty stomach, and is efficiently absorbed in the small intestine after conversion to 5-methyltetrahydrofolate (5-methyl-THF) in the liver [171, 172, 173].

The implementation of folic acid fortification has been one of the most impactful public health interventions of the past few decades. Since its adoption in countries such as the United States, Canada, Chile, and Australia, the prevalence of neural tube defects (NTDs)including spina bifida and anencephalyhas declined significantly. For example, post-fortification studies in North America have reported reductions in NTD incidence by as much as 20–50%, depending on baseline deficiency rates and population coverage [173]. Fortification has also contributed to improvements in folate status biomarkers, such as increased serum and red blood cell folate concentrations, especially among women of childbearing age.

2.4.2 Role of Folate in Neural Tube Development

Maternal folate deficiency is the most well-established modifiable risk factor for NTDs. Folate is essential for DNA synthesis, repair, and methylation, and its deficiency during early pregnancy significantly increases the risk of NTDs such as spina bifida and anencephaly [76]. Randomized controlled trials and epidemiological studies have demonstrated that periconceptional folic acid supplementation can reduce the incidence of NTDs by up to 70% [29, 173].

Folate (vitamin B9) is pivotal micronutrients required for proper embryonic development, particularly during the early stages of central nervous system formation. This nutrientparticipate in one-carbon metabolism, a tightly regulated biochemical network responsible for transferring one-carbon units necessary for the de novo synthesis of purines and thymidylate, as well as for homocysteine remethylation to methionine [62, 174]. These processes are indispensable for DNA replication, repair, cellular proliferation, and methylation reactionsfunctions that are especially critical during the rapid morphogenetic events of neurulation, occurring between post-conception days 21 to 28, corresponding to approximately four completed weeks of gestation [62, 173].

At the molecular level, 5-methyltetrahydrofolate (5-MTHF), the biologically active form of folate, donates a methyl group to homocysteine in a reaction catalyzed by methionine synthase. The resultant methionine is subsequently converted to S-adenosylmethionine (SAM), the

principal methyl donor in numerous epigenetic and metabolic pathways. Disruption at any point in this cascadedue to nutritional deficiency, genetic variants, or metabolic interferenceleads to elevated homocysteine levels, impaired SAM-dependent methylation, and defective DNA synthesis, all of which can compromise neural tube closure and increase the risk of neural tube defects (NTDs) [175, 176].

2.4.3 Thresholds for RBC folate level to minimize the risk of NTDs

In recognition of the importance of these pathways, the World Health Organization (WHO) has issued biochemical thresholds to guide population-level risk reduction strategies for NTDs. Based on epidemiologic and clinical trial data, WHO recommends that red blood cell (RBC) folate concentrations in women of reproductive age should be maintained at or above 906 nmol/L (approximately 400 ng/mL) to minimize the risk of NTDs [77, 149, 177, 178]. This value reflects a level beyond which further increases confer minimal additional risk reduction. RBC folate concentrations between 305–905 nmol/L are considered insufficient, and levels below 305 nmol/L indicate biochemical deficiency [77, 149, 177].

2.4.4 Critical exposure window and biomarker interpretation

The periconceptional period defined as the interval spanning several weeks before and after conception is the most critical window for ensuring adequate folate status. Because neural tube closure is complete by day 28 post-conception, maternal nutrient stores must be sufficient before pregnancy is recognized, reinforcing the need for preconceptional supplementation or fortification strategies [49, 77].

Importantly, RBC folate is considered a superior biomarker for assessing long-term folate status compared to serum folate, which fluctuates with recent dietary intake. RBC folate reflects folate intake over the lifespan of erythrocytes (~120 days) and is thus a reliable indicator of tissue stores during early gestation [49, 77, 149, 177]. Observational studies consistently show a stepwise reduction in NTD risk with increasing RBC folate, with the protective effect plateauing above the 906 nmol/L threshold [77, 178].

2.4.5 Maternal Micronutrient Status in Low-Resource Settings

2.4.5.1Global and National Trends of Folate Deficiencies

Micronutrient deficiencies, particularly those involving folate, remain major contributors to adverse maternal and neonatal outcomes across low- and middle-income countries (LMICs).

Folate deficiency, closely associated with impaired DNA synthesis and elevated homocysteine levels, significantly increases the risk of neural tube defects (NTDs) and other congenital anomalies [77, 177, 178]. According to systematic reviews of nationally representative data, over 20% of women of reproductive age (WRA) in low- and lower-middle-income countries are affected by folate deficiency, compared to less than 5% in high-income settings [179]. This striking disparity reflects profound inequities in diet quality, health infrastructure, and access to preventive interventions.

2.4.5.2 The Ethiopian Context: Magnitude of folate deficiency and Contributing Factors

In Ethiopia, micronutrient deficiencies persist as a significant public health challenge, particularly among WRA and pregnant women, who have increased metabolic demands. The prevalence of folate deficiency has been consistently linked to adverse pregnancy outcomes, most notably NTDs. A 2023 meta-analysis by Gebremichael et al. estimated that 20.8% of Ethiopian WRA are folate deficient, signaling a substantial nutritional vulnerability in the preconception and early gestational periods [180].

However, the burden intensifies during pregnancy. A cross-sectional study conducted in Haramaya District during antenatal care (ANC) visits in 2023 found that 49.3% of pregnant women were folate deficient, despite being enrolled in maternal health services [181]. This finding suggests that current interventions, such as iron-folic acid (IFA) supplementation, are insufficient to meet the increased nutritional needs of pregnancy or are inadequately implemented.

Despite a national policy endorsing IFA supplementation as a routine component of ANC, adherence remains below 50% in most Ethiopian regions, falling short of the Ministry of Health's 70% target [182]. Several structural and individual-level factors contribute to this gap, including: Inconsistent ANC attendance and limited early contact with healthcare services; Supply chain interruptions, leading to unavailability of supplements at health facilities; Side effects of IFA tablets, such as gastrointestinal discomfort; Low awareness of the importance of folate for fetal development; Sociocultural barriers, including food taboos and gender-related health decision-making constraints. These challenges highlight a critical disconnect between policy intentions and practical outcomes in Ethiopia's maternal nutrition strategy.

2.4.5.3Tigray Context War, Siege, Famine and Micronutrient Deficiencies

The Tigray region of northern Ethiopia has endured a catastrophic humanitarian crisis since the outbreak of armed conflict in November 2020. The war has resulted in the systematic destruction of infrastructure, displacement of millions, and a de facto siege that severely limited access to food, medicine, and humanitarian aid [105]. Health systems collapsed, markets ceased functioning, and public services including maternal and child health programs became non-operational in much of the region [139, 183, 184, 185]. These conditions have triggered a nutrition emergency, disproportionately affecting vulnerable groups such as pregnant women, lactating mothers, and children under five [183-190]. In such settings, micronutrient deficiencies become more severe and widespread, contributing to increased risks of maternal anemia, low birth weight, intrauterine growth restriction, and neural tube defects (NTDs) [146-148]. Understanding the impact of this conflict on maternal nutrition is critical for both humanitarian response and public health policy development.

Armed conflict disrupts all aspects of the food system from agricultural production and food distribution to household access and consumption. In Tigray, the closure of markets, blockade of supply corridors, destruction of farmlands, and interruptions to food assistance programs led to a dramatic reduction in dietary diversity [186-190]. Household food consumption patterns shifted toward survival-based diets dominated by staples such as sorghum and maize, with negligible intake of legumes, green leafy vegetables, and animal-source foods primary dietary sources of folate.

For pregnant and lactating women, whose micronutrient demands are heightened, these shifts have serious implications. In addition to inadequate food intake, the collapse of antenatal care (ANC) services further compounded the nutritional crisis. Clinics were looted or destroyed, health workers displaced, and the supply of iron-folic acid supplements was severely disrupted [102, 188, 189]. Nutritional counseling services, if available at all, were fragmented. Reports from healthcare professionals within the region emphasized that many women had no access to ANC for months, and others only received care during labor or delivery.

Globally, evidence from other conflict-affected settings such as Syria, Yemen, South Sudan, and Afghanistan demonstrates similar patterns: deteriorating maternal diet quality, collapse of public health systems, and subsequent increases in micronutrient deficiencies and adverse pregnancy outcomes [190-193]. These parallels strongly suggest that the Tigray crisis likely induced a comparable, if not more severe, burden of maternal undernutrition.

2.4.5.4 Anecdotal and published reports of severe food shortages in tigray

Humanitarian organizations and regional health actors have consistently reported alarming levels of food insecurity across Tigray. Anecdotal reports from midwives, physicians, and aid workers describe mothers forgoing meals so their children could eat, widespread consumption of wild plants, and community-wide hunger [105, 187]. Many families resorted to selling household items to purchase food when food was available at all.

The Integrated Food Security Phase Classification (IPC) report from June 2021 indicated that over 350,000 people were experiencing famine-like conditions, with more than 5.2 million (over 90% of the region's population) in urgent need of humanitarian food assistance. A follow-up rapid nutrition assessment conducted by the Emergency Food Security Assessment (EFSA) in 2022 found critical levels of acute malnutrition among pregnant and lactating women, exacerbated by the near-total collapse of primary healthcare and nutrition services [183].

Emerging academic work, though limited due to restricted access to the region, supports these findings. Retrospective hospital-based studies in Mekelle and other towns in Tigray have documented rising cases of maternal anemia, low birth weight, and preterm births indirect but compelling indicators of widespread micronutrient deficiency and maternal undernutrition [145]. Collectively, these findings underscore the severe nutritional consequences of prolonged siege and warfare in the region.

In crisis-affected settings such as Tigray, the assessment of nutritional status using conventional dietary survey tools such as food frequency questionnaires or 24-hour recalls poses fail to detect subclinical micronutrient deficiencies, which may be widespread and detrimental even in the absence of overt anthropometric failure. Therefore, the use of biochemical markers, particularly red blood cell (RBC) folate concentrations, becomes vital. These biomarkers provide objective, sensitive, and specific information on long-term micronutrient status. RBC folate, which reflects average folate intake over the past 2–3 months, is especially valuable for assessing preconception and early pregnancy folate status the critical period for neural tube closure [194]. The relevance of biochemical assessment in Tigray is heightened by the region's high pre-conflict rates of maternal anemia and undernutrition. Given that the blockade and conflict likely worsened pre-existing vulnerabilities, there is an urgent need to generate empirical data on micronutrient status to inform emergency nutrition programming and longer-term policy interventions. Furthermore, documenting the biochemical burden of deficiency contributes to global advocacy efforts and ensures that the

unique nutritional needs of conflict-affected populations are not overlooked in humanitarian responses. Generally, the siege and famine in Tigray have created a high-risk environment for widespread micronutrient deficiencies, especially among pregnant women. Incorporating biochemical assessments into ongoing and future nutrition surveys is essential for effective targeting, intervention planning, and long-term recovery strategies.

2.5 Conceptual framework

The conceptual framework presented is designed to illustrate the expected relationships between independent variables and outcome variables related to NTDs (Figure 5). This framework is grounded in findings from the literature review, which provides a comprehensive understanding of the problem under investigation and guides the analysis and interpretation of results. The framework outlines the following key components:

1. Prevalence of NTDs: It provides an overview of how the war and regional conflict has impacted the incidence of NTDs
2. Risk Factors: It identifies and categorizes the sociodemographic, nutritional, and environmental factors that are believed to influence the occurrence of NTDs.
3. Integration and Analysis: It outlines how the different elements of the study are interconnected. By linking the prevalence, and risk factors, the framework supports a comprehensive analysis that draws meaningful conclusions about the impact of the identified variables on NTDs.
4. Dissertation Structure: The framework also indicates how each section of the dissertation is interrelated. It shows how the study's findings on prevalence and risk factors will be integrated to address the research questions and objectives, leading to a cohesive set of conclusions.

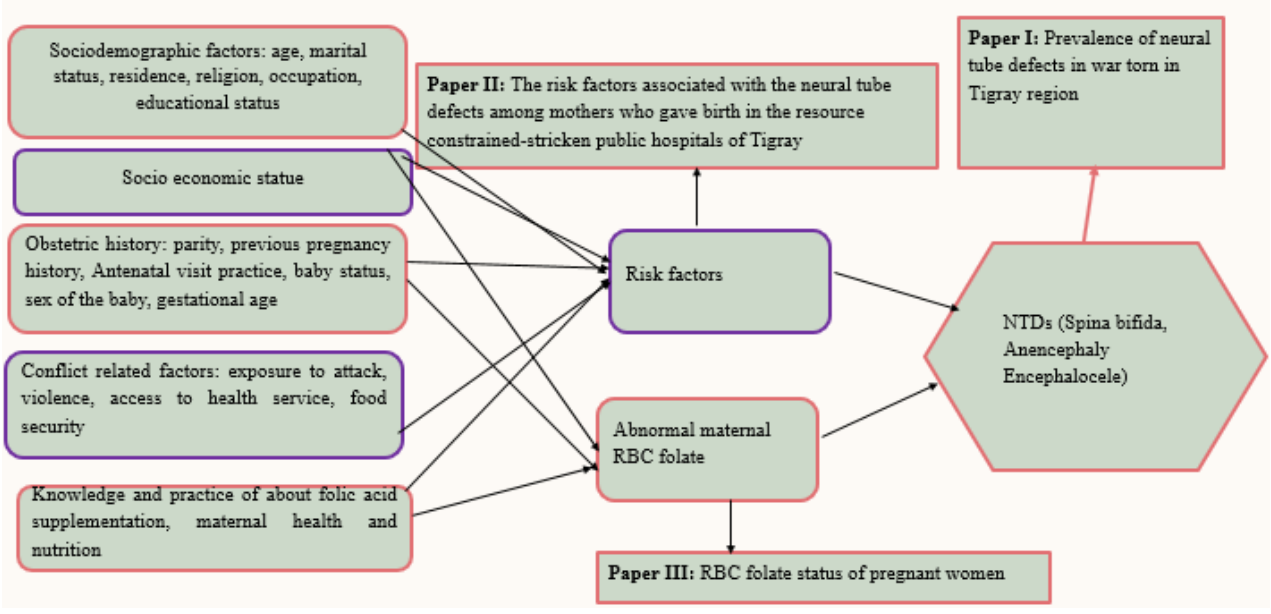

Figure 5: Conceptual frame**w**ork of neural tube defects and its possible risk factors (self developed).

Chapter 3: General Methodology

3.1 Study Setting and Population

The research was conducted across 11 government hospitals within the Tigray Region (Figure 6). A simple random sampling method was used to select participating hospitals from the pool of relatively functioning public hospitals in Tigray. These hospitals serve as key healthcare providers for the region and include a mix of urban and rural facilities, ensuring a comprehensive representation of the healthcare landscape in Tigray. Tigray Region is the northernmost regional state in Ethiopia, sharing borders with Eritrea to the north and Sudan to the west. As of 2019, the estimated population of Tigray is approximately 9 million [18, 195].

The region is diverse, home to the Tigrayan (Tegaru), Irob, and Kunama peoples. Mekelle, the capital and largest city of Tigray, serves as a central hub for administrative, economic, and healthcare activities. Tigray is the fifth-largest regional state in Ethiopia. It is the fourth-most populous and the fifth-most densely populated among Ethiopia's 11 regional states. The total fertility rate in the region is 4.6 children per woman (UNICEF). Approximately 80% of the population are engaged in farming, which contributes 46% to the regional gross domestic product [18, 195].

In early November 2020, a war erupted between the Tigray Regional Government, led by the Tigray People's Liberation Front (TPLF), and the Ethiopian Federal Government. The conflict quickly expanded to involve Eritrean forces, Amhara paramilitary groups, and Amhara Fano militia, rapidly escalated into the regional war, destabilizing the region and exposing a well-organized campaign to weaken and subdued the region government as indicated by European union officials (reference). The conflict has led to significant loss of life, with estimates indicating that more than 600,000 people have been killed [12, 13, 14]. The conflict has severely disrupted healthcare services in the region. As a result of theconflict in the region, an estimated 70-80% of health facilities were either looted, destroyed, or severely damaged, leading to a critical shortage of medical supplies and a significant disruption in health services [184, 196].

The study population encompasses women who delivered in these hospitals during the specified periods, as well as pregnant women attending antenatal care (ANC) services in designated high-risk zones.

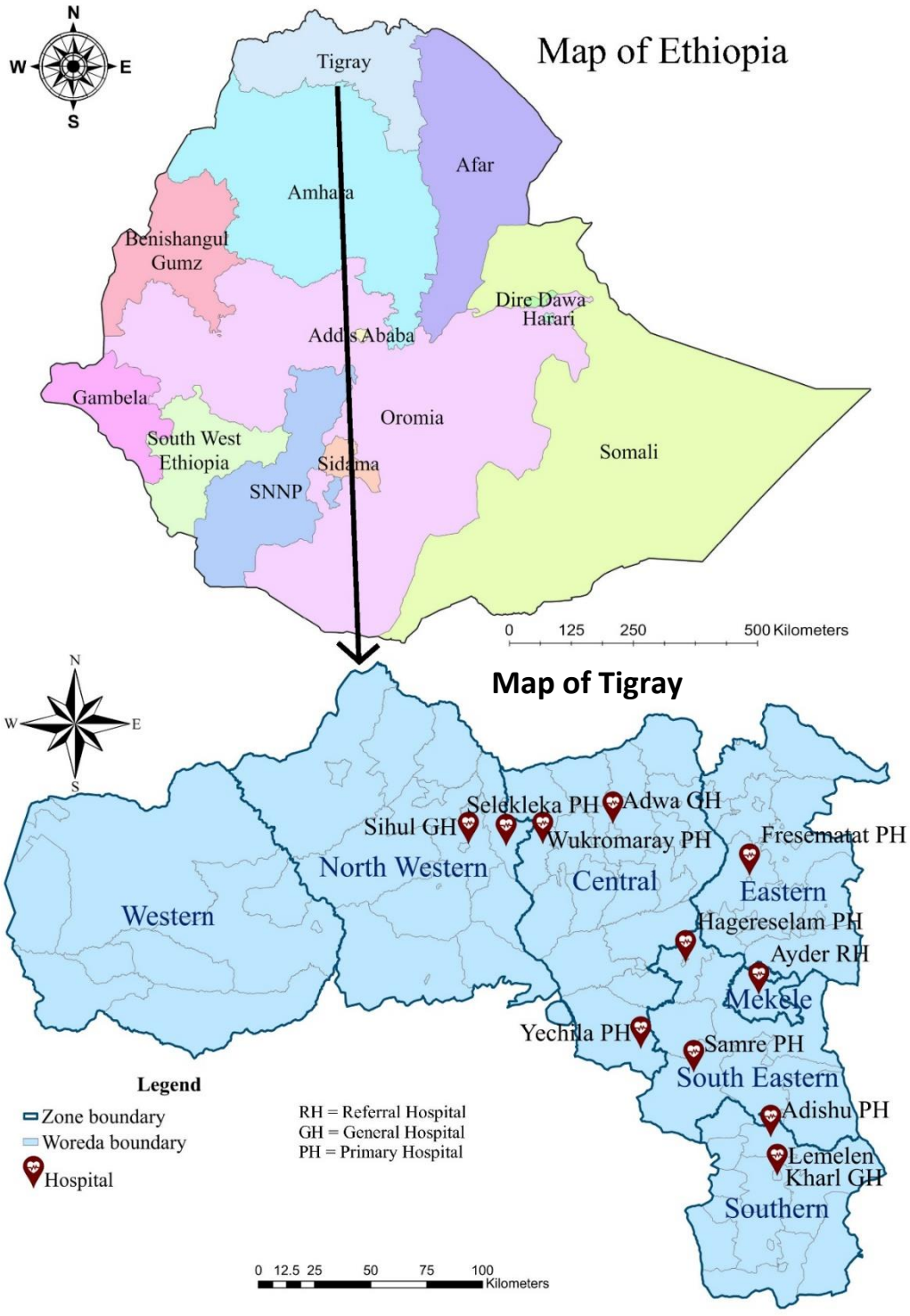

Figure 6: Administrative map of Ethiopia, Tigray and the location of the health facilities included in the study (for objective 1 and2).

3.2 Study Design and period

This epidemiological investigation employed a mixed-method, multi-phased approach comprising retrospective, prospective, and biochemical assessments to comprehensively assess the burden, risk factors, and biochemical correlates of Neural Tube Defects (NTDs) in the war- and siege-affected Tigray Region of Ethiopia. The methodology is guided by the overarching goal of understanding the burden and determinant of NTDs in an extreme humanitarian context where health systems have been severely disrupted by conflict. The chapter presents the detailed methodologies aligned with the three core objectives of the study:

Objective 1: retrospective record analysis: Estimating the prevalence of NTDs based on existing hospital delivery records over nearly three years (October 2020 – December 2023).

Objective 2: Case-Control Study: Identifying maternal and fetal risk factors associated with NTDs, involving active enrollment of cases and controls during a defined period (December 2023 – January 2024).

Objective 3: Cross-Sectional biomarker Study: Measuring RBC folate level among first-trimester pregnant women in high-risk areas, conducted from March to May 2025.

Each component complements the other, providing a comprehensive picture of NTD burden, associated risk factors, and biological markers in a conflict-affected setting.

3.3 Study Population and Sampling Strategy

Retrospective Component (Objective 1)

Study Population and Sample size

The study focused on all delivery registries from 11 government hospitals in the Tigray Region. The period under investigation was from late October 2020 to December 2023. A total of 54,626 delivery registries were included in the study. These records were retrieved from the 11 participating hospitals and were used to determine the prevalence neural tube defects (NTDs) in the Tigray region. This sample size provides a robust dataset for analyzing the incidence of NTDs.

Sampling Techniques and procedure

A simple random sampling method was used to select participating hospitals from the pool of relatively functioning public hospitals in Tigray. This ensures that every hospital has an equal chance of being included in the study, thus reducing bias and enhancing the representativeness

of the sample. Consecutive sampling was used to retrieve all delivery registries from the randomly selected hospitals. All delivery registries with complete information from the selected hospitals was retrieved. These registries include data on live births, stillbirths, and elective terminations. To determine the number of delivery registries to study from each selected health facility, data on the current one-year client flow (i.e., the number of deliveries) was obtained from the Health Management Information System (HMIS) reports of each selected hospital. Based on the one-year client flow data, the calculated sample size was distributed proportionally across the selected health facilities. This approach ensures that the sample size from each hospital reflects its contribution to the overall client flow, maintaining representativeness and statistical power (Figure 7)

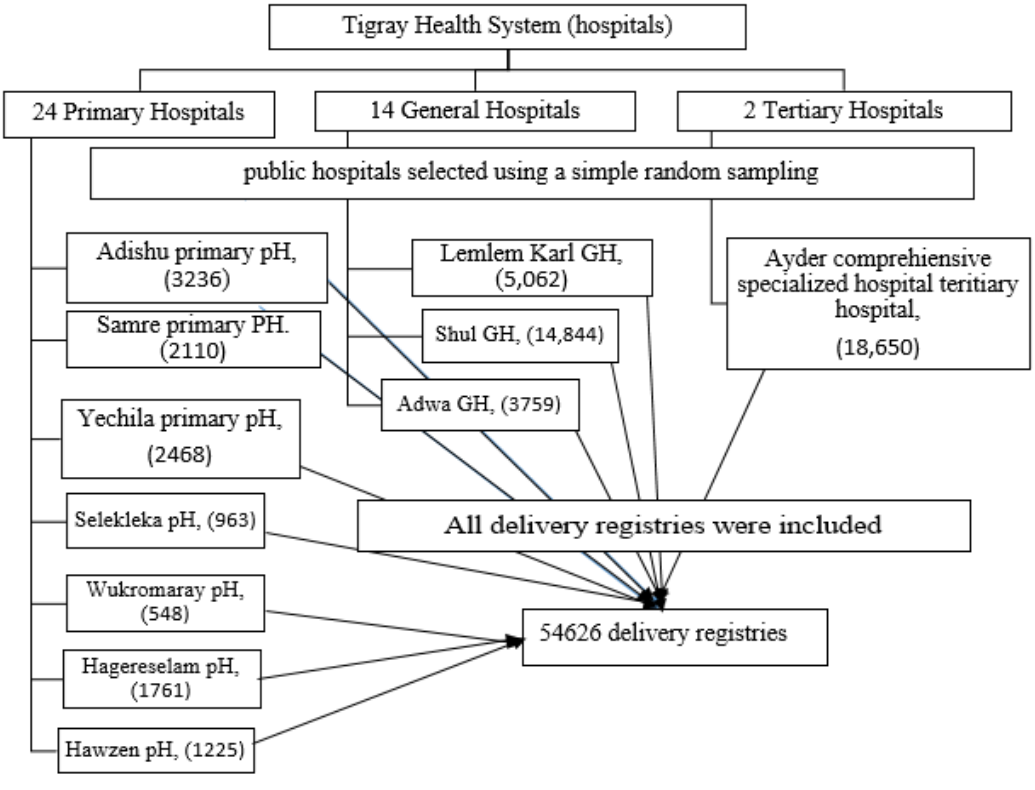

Figure 7: Pictorial representation of the sampling technique (objective 1).

Prospective Component (Objective 2):

Target Population and Sample Size

The study population consisted of patients diagnosed with neural tube defects (NTDs), including both live-born and stillborn cases. The conditions under investigation included spina bifida, anencephaly, encephalocele, and other forms of NTDs. The control group was

composed of women who gave birth to children without congenital anomalies at the same hospitals.

The sample size for this unmatched case-control study was determined using OpenEpi info, Version 3, an open-source calculator (SSCC). The following assumptions were used in the calculation:

Two-sided confidence level (1-alpha): 95%

Power (probability of detecting the effect): 80%

Ratio of Controls to Cases: 2:1

Hypothetical proportion of controls with exposure: 40%

Hypothetical proportion of cases with exposure: 57.57%

Least extreme Odds Ratio to be detected: 2.04

Based on these assumptions, the calculated sample sizes were as follows:

Sample Size for Cases: 103

Sample Size for Controls: 205

Total Sample Size: 308

For the purposes of this study, we employed an unmatched concurrent case-control analysis, in which all cases were compared to a randomly selected group of controls from the same hospitals. The final sample comprised 103 mothers of infants with NTDs (cases) and 205 mothers with healthy pregnancy outcomes (controls), resulting in a case-to-control ratio of 1:2.

Sampling Method: All eligible NTD cases within the study period were consecutively recruited as cases, while controls were randomly sampled from the same hospitals (figure 8).

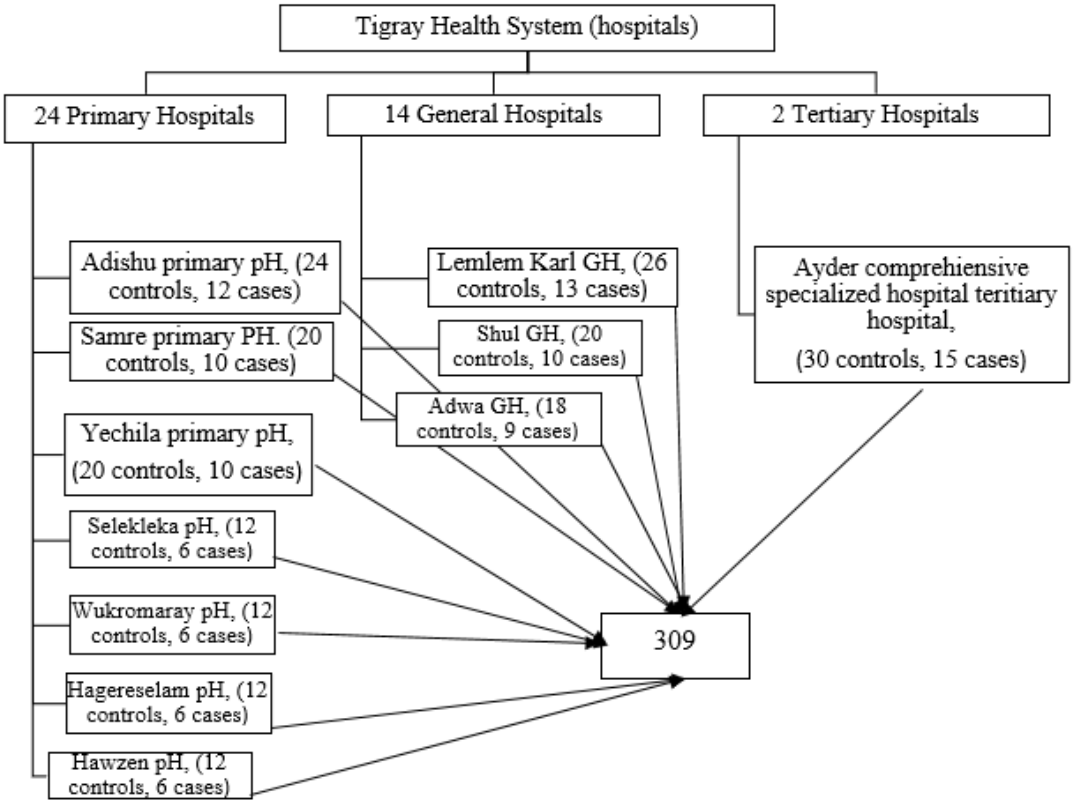

Figure 8: Pictorial representation of the sampling technique (objective 2).

Laboratory Component (Objective 3):

Study setting and subjects

This study was conducted between March and May 2025 across four zonal administrations in Tigray, northern Ethiopia (figure9). First-trimester pregnant women attending antenatal care (ANC) were recruited from five strategically selected hospitals to ensure geographic and demographic representation. Ayder Comprehensive Specialized Hospital, located in Mekelle the capital city of Tigray serves as a major referral center with an extensive catchment area that includes Mekelle and surrounding towns and districts. In the Southern Zone, two hospitals were included: Lemlem Karl Hospital, serving the town of Maichew and its surrounding rural communities, and Adishu Hospital, which covers Adishu town and its surrounding rural communities. From the Southeastern Zone, Samre Hospital was selected, serving Samre town and neighboring areas. Lastly, Yechila Hospital, located in the Central Zone, was included to represent women from Yechila town and its surrounding rural populations.

Figure 9: Administrative map of Ethiopia, Tigray and the location of the health facilities included in the study (for objective 3)

Study Population and Sample size

The required sample size was determined using a single-population proportion formula, assuming a 27% prevalence of folate deficiency among pregnant women based on RBC folate levels in the first trimester, as reported in a previous study from Addis Ababa [197]. Using this estimate, and considering a 95% confidence level and 5% margin of error, the minimum required sample size was calculated to be 303 participants. However, a total of 400 pregnant women were included in the study.

This larger sample size was intentionally selected to increase the statistical power of the study and improve the precision of the prevalence estimates. It also provides a buffer against potential issues such as non-response, missing data, or loss to follow-up. Moreover, the increased sample size enhances the ability to conduct meaningful subgroup analyses thereby strengthening the robustness and generalizability of the findings.

Sampling Techniques and Procedure

To determine the number of patients to be included from each selected health facility, data on the current one-year client flow of each selected health facility is obtained from hospitals of health management information system report of each selected health. Accordingly, the calculated sample size was distributed proportionally (figure 10)

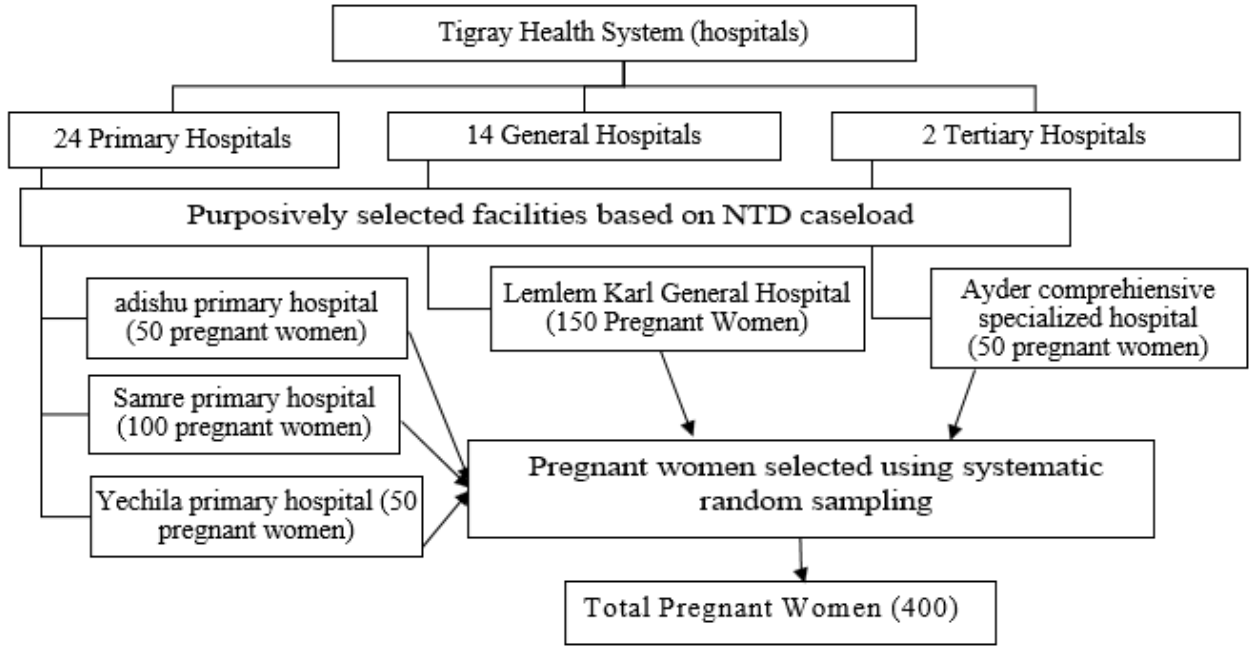

Figure 10: Pictorial representation of the sampling technique (objective 3).

3.4 Eligibility criteria

Table 2 Inclusion and Exclusion Criteria

Study component		Inclusion Criteria	Exclusion Criteria
Retrospective (**Objective 1**)		Delivery records with complete data within the period	Records lacking key outcome data or outside the study period
Prospective (**Objective 2**)	Cases	Mothers of infants diagnosed with NTDs	Mothers unable or unwilling to provide informed consent
	Controls	Mothers with uncomplicated deliveries, no congenital anomalies	Mothers with incomplete data or who decline participation
Laboratory (**Objective 3**)		confirmed first-trimester pregnancy, permanent residency in the study area, and provision of informed oral consent.	Hematological disorders or chronic diseases (e.g., HIV/AIDS, diabetes), Current or recent malaria infection or use of antimalarial drugs, History of folic acid supplementation prior to conception, Inability to give informed consent, Visitor or non-permanent residency status.

3.5 Data Collection Procedures

Retrospective Data Extraction (Objective 1):

Data was collected using a structured questionnaire adapted from the WHO birth defect surveillance tool [36, 198, 199]. This tool is designed to capture comprehensive information on NTDs and associated factors. The data collection tool/checklist was digitized and utilized with ODK (Open Data Kit) or KOBO Collect software for electronic data gathering. The prepared tool includes variables representing key maternal and fetal characteristics, including: socio-demographic Characteristics: Information on age, education, socioeconomic status, and residence; obstetric History: Details on previous pregnancies, prenatal care, and other obstetric-related information; neural tube defects: any type of neural tube defects as recorded in medical charts and patient history records.

Medical records from the selected hospitals were reviewed to extract relevant information on delivery outcomes, including live births, stillbirths, and elective terminations. The checklist within the ODK/KOBO Collect software were used to systematically capture data on all NTDs noted in patient charts and medical history records. Study cases with more than one congenital defect were counted only once for each specific defect to avoid duplication. Each case was categorized based on the primary diagnosis or the most significant defect as per the case definition. Cases were included if they meet the established case definitions for the congenital defects being studied. This ensures that the data collected is relevant and specific to the research

objectives. Cases not meeting the case definitions or with incomplete information was excluded from the analysis.

Prospective Interviews and Clinical Data (Objective 2):

Data was collected through face-to-face interviews with mothers, by using a structured questionnaire adapted from the WHO birth defect surveillance tool [36, 198]. Data was gathered electronically using ODK/KOBO collect. The prepared data collection tool/checklist contains required variables representing the maternal and fetal characteristics. Data collected include maternal age, residence, education, occupation, obstetric history, lifestyle factors (e.g., folic acid intake, dietary habits), conflict exposure, and pregnancy intentions. Medical records were reviewed for clinical confirmation of NTD diagnosis, gestational age, sex of the fetus, and birth weight. Study cases with more than one birth defect was counted once. Cases were included if they met the case definition of required disease to be studied.

Laboratory Sample Collection and Analysis (Objective 3):

Sociodemographic and obstetric Data

Trained data collectors were involved and they used a structured and pretested questionnaire to collect information on: Sociodemographic characteristics such as age, marital status, residence, education, occupation, perceived economic status; Obstetric history such as parity, pregnancy planning, previous outcomes; Health-related behaviors such as health care service utilization, family support.

Dietary Diversity and Food Frequency

Dietary data were collected through a 24 hours food group recall and a standardized food frequency questionnaire (FFQ) administered during face-to-face interviews at the first ANC visit. Seven-day food frequency questions were grouped into the FAO 13 food-group minimum dietary diversity for women (MDD-W) tool; scores were categorized as Low DDS (≤3 food groups), Medium DDS (4–6 food groups), or High DDS (≥7 food groups) based on FAO guidelines [200].

Anthropometric Measurements

Mid-Upper Arm Circumference (MUAC): was measured to assess maternal nutritional status, categorized as: Low MUAC: <23 cm and Normal MUAC: ≥23 cm

Folate status measures

Folate status measures (RBC folate) in blood was conducted using established immunoassays, enzyme-linked immunosorbent assays (ELISA) (methods of quantification or measurement) in collaboration with Armauer Hansen Research Institute, Addis Ababa, Ethiopia.

Specimen collection and management:

Four milliliters of venous blood was collected by venipuncture after an overnight fast of at least 8 h from the arm using a syringe and was transferred into serum separator tubes, was allowed to form a clot by letting it stand in an upright position for at least 30 min, subjected to centrifugation (15 min, 4,000 rpm) at room temperature, divided into aliquots in labeled cryotubes, and was transported from the site of collection on an ice box containing frozen ice packs to the core laboratory of the CHS, MU and stored at −80◦C until biochemical analyses. Whole blood specimens was protected from light and from elevated temperatures to avoid folate losses and was stored in a refrigerator or a cold box with ice packs. 100 µL of carefully mixed EDTA whole blood (blood collection tube inverted 8–10 times) is added to a vial containing 1 mL of ascorbic acid solution (1% weight/volume). The vial with the haemolysatewas mixed well and stored immediately in a -20°C freezer, for a maximum of one month, and shipped on dry ice to a laboratory for analysis. For storage beyond one month, the haemolysatewas stored at -70 to -80˚C.

Biochemical Analysis of RBC Folate level

Each blood sample was stored for a maximum of 2 months before analyses. A competitive enzyme-linked immunosorbent assay (ELISA) (Cloud-Clone Corp. Katy, TX, USA) was used to determine the RBC levels of folate. The analyses were performed according to the manufacturer's instructions. Each analysis was performed in duplicates and the average of the duplicate measurements was taken. The analyses were done at the immunology laboratory in Ethiopian public health Institute, Addis Ababa, Ethiopia.

Study Variables

Dependent variables:

- Presence or absence of NTDs (Objective 1 and 2).
- RBC folatelevels (Objective 3).

Independent variables:

Objective 1:

maternal and fetal factors that can be expressed as sociodemographic characters, Age (years), Residence, Parity, Previous pregnancy history, Chronic medical illness, Antenatal visit, consumption of multivitamin and foliate during pregnancy, status, sex of baby, Gestational age, Birthweight in kg.

Objective 2: Sociodemographic and Obstetric Factors: Maternal age, marital status, residence, religion, education, occupation, parity, history of pregnancy outcomes, sex of baby in last pregnancy, family history of birth defects, history of children with birth defects, practice of periconceptional folic acid supplementation, and history of X-ray imaging.

Conflict-related Factors: Type of conflict event, use of healthcare services during wartime, pregnancy intention during wartime, family food source during wartime, and meal frequency during wartime.

Objective 3: Maternal factors: Age, Residence, Parity, Previous pregnancy history, Chronic medical conditions, Antenatal visit history, Knowledge of pre-conception planning and folic acid use, Dietary intake.
Fetal factors: Sex of the baby, Gestational age, Birth weight.
Laboratory measures: RBC folate.

3.6 Operational Definitions

Neural Tube Defects (NTDs):

- Neural tube defects (NTDs) are a group of congenital anomalies that result from the incomplete closure of the neural tube during early embryonic development [198, 199]. The neural tube is the precursor to the brain and spinal column, and its proper closure is crucial for normal development.

Key Definitions:

- **Neural Tube:** The neural tube is an embryonic structure that forms early in pregnancy and eventually develops into the brain and spinal cord. Closure of the neural tube is expected to occur within the first month of conception.

- **Anencephaly:** A severe neural tube defect where the majority of the brain and skull fail to develop. This condition is incompatible with life, and affected infants are typically stillborn or die shortly after birth. Absence of a major portion of the brain and skull, resulting in a non-survivable condition.
- **Spina Bifida:** A condition in which the spinal column does not close completely, leading to defects in the spinal cord and nerves. This often results in varying degrees of paralysis and other neurological impairments. Exposure of the spinal cord through the defect in the vertebrae, leading to possible paralysis of the legs and other complications.
- **Encephalocele:** A rare type of NTD where a portion of the brain protrudes through a defect in the skull. This condition may vary in severity depending on the extent of the protrusion and associated brain damage. Visible bulging of brain tissue through an opening in the skull, potentially associated with neurological deficits.
- **Hydrocephalus:** A condition characterized by an abnormal accumulation of cerebrospinal fluid (CSF) within the ventricles of the brain, leading to increased intracranial pressure. Enlargement of the head, potentially accompanied by developmental delays, motor dysfunction, and other neurological issues. Hydrocephalus can occur alone or in conjunction with other NTDs.

3.7 Data Quality Assurance (Objective 1-3)

Training: Data collectors and laboratory personnel undergo intensive training on data collection tools, ethical considerations, and specimen handling. Data collection instruments were pretested in a similar setting to ensure clarity and appropriateness. Regular supervision visits was conducted to monitor data collection quality, adherence to protocols, and troubleshoot challenges. Daily checks for completeness and consistency were performed. For the Laboratory quality assurance Calibration of ELISA equipment, use of control samples, and duplicate testing were implemented for reliability.

3.8 Ethical Considerations (Objective 1-3)

Ethical Approval was obtained from the Mekelle University Institutional Review Board and relevant regional health authorities. Informed consent in writing was obtained from prospective participants after explaining the study objectives, procedures, risks, and benefits. For retrospective data, a waiver of consent may be obtained, provided data wereanonymized.

Confidentiality: Data was anonymized by removing personal identifiers, stored securely with restricted access, and used solely for research purposes.

Risks and Benefits: Participants were informed about minimal risks related to blood sampling and potential benefits of contributing to public health knowledge.

3.9 Data Management and Analysis (Objective 1-3)

Data Entry and Cleaning: Data from electronic forms was exported to SPSS version 27. Data cleaning procedures includes checking for missing values, outliers, and inconsistencies. Descriptive analyses generate frequency distributions, means, medians, and proportions.

Statistical Analysis

Prevalence Estimation: NTD prevalence was calculated as the number of cases per 10,000 total births. Bivariate analysis (Chi-square tests, t-tests, or Mann-Whitney U tests) wasused to assess associations between independent variables and NTD occurrence. Variables with $p < 0.25$ in bivariate analysis was included in multivariate logistic regression to identify independent predictors, adjusting for confounders.

Biomarker Analysis: RBC folate status ware compared between cases and controls using non-parametric tests if distributions are skewed. Penalized regression models were used to evaluate the predictors associated with RBC folate status.

3.10 Limitations and Mitigation Strategies

Potential underreporting due to disrupted services: Disrupted health records may lead to incomplete data; cross-verification with multiple sources (e.g., hospital logs, reports) were performed. To minimize bias, all eligible cases during the study period were enrolled consecutively. Security risks may impact data collection; safety protocols and coordination with local authorities was followed.

Summary

This comprehensive methodology integrates epidemiological, clinical, and biochemical approaches to elucidate the burden, determinants, and biological markers associated with NTDs in the Tigray region affected by armed conflict and prolonged siege. The use of diverse study designs, rigorous data collection, and ethical standards help to ensure the validity, reliability, and relevance of findings to inform public health interventions.

Chapter four

Neural tube defects in a war-torn Tigray regional state of Ethiopia: a retrospective study of 54,626 deliveries

Berihu *et al. BMC Pregnancy and Childbirth* (2025) 25:108
https://doi.org/10.1186/s12884-025-07254-3

BMC Pregnancy and Childbirth

RESEARCH Open Access

Neural tube defects in a war-torn Tigray regional state of Ethiopia: a retrospective study of 54,626 deliveries

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Abstract

Background: The Tigray region of Ethiopia has a significantly high prevalence of neural tube defects (NTDs), ranging from 1.31% to 2.15% of total births. The prevalence has worsened due to ongoing regional war and conflict since October 2020. This study aims to assess NTD prevalence in these challenging conditions.

Methods: This institution-based, retrospective cross-sectional study was conducted across 11 public hospitals in the Tigray region. The study reviewed all delivery records from October 2020 to December 2023. Data were collected from hospital records, focusing on cases of neural tube defects (NTDs) and relevant maternal and neonatal characteristics. This retrospective analysis aimed to identify the prevalence of NTDs, as well as factors contributing to their occurrence. The data analysis involved using SPSS version 27 for comprehensive data management and statistical evaluation. Descriptive statistics provided an overview of the data, while binary logistic regression offered insights into the factors associated with neural tube defects. The results were systematically presented in both textual, tabular and graph formats to facilitate understanding and interpretation.

Results: Out of 54,626 delivery records, 1,612 cases of NTDs were identified (1,434 NTD cases and 178 isolated hydrocephalus cases). The specific birth prevalence of NTDs was 262.5 per 10,000 (95% CI, 249.1-276.5 per 10,000), with NTDs being the predominant cause of stillbirths. Anencephaly (136.6 per 10,000), spina bifida (110.6 per 10,000) and encephalocele (15.4 per 10,000) were the most common defects. Risk factors for NTDs include maternal age (20-29 years), rural residency, first pregnancies, a history of early neonatal death, lack of folic acid and multivitamin use, as well as neonatal factors like stillbirth, male sex, and preterm birth.

Conclusion: This study reveals the alarmingly high prevalence of neural tube defects (NTDs) in the Tigray region, with a birth prevalence of 262.5 per 10,000 births. Anencephaly, spina bifida, and encephalocele were common, contributing to stillbirths. Risk factors include maternal age (20-29), rural residency, first pregnancies, lack of folic acid and multivitamins, and neonatal factors like male sex and preterm birth. The findings stress the need for public health interventions, including folic acid awareness, better prenatal care, maternal nutrition research, stronger health systems, and a national surveillance system to prevent birth defects.

Keywords: Neural Tube Defects, Spina Bifida, Anencephaly, Encephalocele, Stillbirths, Live Births

4.1 Introduction

Maternal and infant health is pivotal to societal well-being [1,2]. Birth defects (BDs) are a major cause of infant and childhood mortality, with neural tube defects (NTDs) being the most common and severe [1]. These congenital anomalies arise from incomplete closure of the neural tube during early embryonic development in between 21 and 28 days after conception—often before a woman knows she is pregnant. Globally, the prevalence of NTDs averages two cases per 1,000 births, with significantly higher rates in developing countries [201]. Although the incidence of NTDs has decreased in the developed world, global infant mortality due to congenital nervous system malformations still exceeds 10%. In developing countries, the estimated incidence ranges from 10 to 110 per 10,000 births [3, 7]. Despite the high burden of NTDs, they often remain a low priority on maternal and infant healthcare agendas in many sub-Saharan African countries [5].

In Ethiopia, systematic reviews suggest a prevalence of 71.48 to 86.58 per 10,000 births [8], nearly double the estimated prevalence in East Africa [9]. The Tigray region, in particular, bears a significant burden of birth defects [10, 11]. A prospective hospital-based study by Mekonen HK et al. at nine government hospitals in the region found that out of 12,225 admissions, 383 (3.13%) neonates had a birth defect, with 68.7% of these being neural tube defects [10]. The reported prevalence of NTDs in Tigray ranges from 1.31% to 2.15% of total births [10, 11]. NTDs are influenced by a variety of factors, including maternal nutritional status, particularly folic acid deficiency, and environmental factors such as exposure to teratogens [178, 202). Additionally, maternal age, a history of NTDs, and inadequate prenatal care are known to increase the risk of NTDs. Other factors, such as genetic predispositions and maternal health conditions, can also contribute to the occurrence of these defects [178, 202). In the context of the Tigray region, the war has further exacerbated these risk factors, limiting access to healthcare and essential resources, which in turn may increase the likelihood of adverse pregnancy outcomes, including NTDs.

Recently, war has broken in the region that impacted the population in many problems including A large number (~2million) of internally displaced people, hunger and famine, sex and gender-based violence (SGBV), long-lasting military combats, cross-border infiltration, and poor law-and order situation gravely impacted the health systems [13-15, 203]. The risk of pregnancies is common in Tigray region due to war and a systematic sexual violence. This in

turn render to lack accessibility for health services and antenatal care, food and other basic need shortages. Most of the health facility in the region were looted and destroyed by allied forces (Ethiopian, Eritrean, and Amhara militants) [102, 106, 185, 204]. Hence, the risk of adverse pregnancy outcome is believed to be increased at alarming rate due to the war and the total siege and blockage of the region by the allied forces which impacted availability of medicines and supplies for more than two years. The Tigray region, severely affected by conflict, presents a unique opportunity to assess how war conditions impact NTD prevalence. This study aims to investigate this issue to inform prevention and treatment strategies.

4.2 Methodology

Study Area and Setting

The research was conducted across 11 government hospitals within the Tigray Region, which is located in the northernmost part of Ethiopia. These hospitals serve as key healthcare providers for the region and include a mix of urban and rural facilities, ensuring a comprehensive representation of the healthcare landscape in Tigray.Tigray Region comprises the Tigrayan (Tegaru), Irob, and Kunama ethnic groups. Mekelle is the capital and largest city of Tigray. It is the fifth-largest region by area, the fourth-most populous, and the fifth-most densely populated of Ethiopia's 11 regional states. As of 2019, the estimated population of Tigray is approximately 9 million [18, 195], with a total fertility rate of 4.6 children per woman [18]. The majority of the population (80%) are farmers, contributing 46% to the regional gross domestic product. In early November 2020, a conflict erupted between the Tigray regional government, led by the Tigray People's Liberation Front, and the Ethiopian federal government, with support from Eritrea and Amhara forces. This conflict escalated into a regional war, resulting in widespread devastation, including the death of more than 600,000 people [102, 106, 185, 204]. The war severely impacted the healthcare system, with 89% of health facilities being looted and destroyed by allied forces.

Selection of study hospitals

The study focused on 11 public hospitals in the Tigray Region, which were selected for their relatively functional status during the recent severe conflict and blockade. A simple random sampling method was used to select participating hospitals from the pool of relatively functioning public hospitals in Tigray. In our initial study design, we opted for a simple random sampling approach to select the participating hospitals. This decision was made to minimize potential selection bias and to ensure that all relatively functional public hospitals in the Tigray

Region had an equal chance of being included in the study, given the constraints of the prolonged war and blockade in the region. Given the relatively small pool of hospitals available for selection, we believed that simple random sampling would still provide a robust representation of the available healthcare facilities in the region. The Selected Hospitals are namely Yechila Hospital: Serves approximately 69,318 people, Wukromaray Hospital: Serves approximately 55,000 people, Adwa Hospital: Serves approximately 113,438 people, Shul Shire Hospital: Serves approximately 230,000 people, Selekleka Hospital: Serves approximately 115,967 people, Samre Hospital: Serves approximately 150,450 people, Hagereselam Hospital: Serves approximately 105,770 people, Lemlem Karl Hospital: Serves approximately 100,570 people, Adishu Hospital: Serves approximately 99,995 people, Fresemaetat Hospital: Serves approximately 110,833 people, and Ayder Comprehensive Specialized Hospital: Serves approximately 1.8 million people.

Overall, these hospitals cater to a combined population of about 2.9 million people. This includes not only the population directly served by the hospitals but also those in the surrounding woredas that rely on these facilities for healthcare services. Ayder Comprehensive Specialized Hospital has an extensive catchment area that encompasses Mekelle and surrounding towns and districts. This hospital provides specialized healthcare services and has a significant impact on a large portion of the region's population. The selected hospitals offer comprehensive healthcare services, including Pediatrics Gynecology and Obstetrics, Midwifery, and Nursing Services. By focusing on these hospitals, the study aims to gather crucial data on the prevalence of NTDs and assess whether the conflict and resulting conditions have exacerbated the risk of such defects.

Design of the study

A hospital based, retrospective analysis of all delivery records was conducted.

Study Population and Sample Size

The study focused on all delivery registries from 11 government hospitals in the Tigray Region. The period under investigation was from late October 2020 to December 2023. A total of 54,626 delivery registries were included in the study. These records were retrieved from the 11 participating hospitals between September and November 2023. While it is recognized that the war may have affected case registration and reporting completeness, every effort was made to include all available and verifiable data from hospital records to estimate the prevalence of

neural tube defects (NTDs) in the Tigray region. This sample size provides a robust dataset for analyzing the incidence of NTDs.

Eligibility criteria

Inclusion Criteria: all delivery registries from the specified time frame (October 2020 - December 2023). And Complete records with relevant information about each delivery were included.

Exclusion Criteria: Delivery registries with incomplete information or missing data that could not be used for analysis were excluded.

Study variables

Dependent variable

The outcome of interest of this study was presence or absence of NTDs were defined as cases of anencephaly, spina bifida, and encephalocele (ICD-10) [199] among infants of any gestational age and medically terminated NTDs incompatible with life.

Independent variable

The independent variables are maternal characteristics such asAge, residence, parity, Previous pregnancy history, Antenatal care, use of multivitamins and folic acid, chronic medical conditions. Neonatal characteristics such asSex of baby, birthweight, gestational age, baby status.

Operational Definitions

Neural Tube Defects (NTDs): are a group of congenital anomalies that result from the incomplete closure of the neural tube during early embryonic development.

Anencephaly: A severe neural tube defect where the majority of the brain and skull fail to develop.

Spina Bifida: A condition in which the spinal column does not close completely, leading to defects in the spinal cord and nerves.

Encephalocele: A rare type of NTD where a portion of the brain protrudes through a defect in the skull.

Data collection tool and technique:

Data was collected using a structured questionnaire adapted from the WHO birth defect surveillance tool [36, 198]. This tool is designed to capture comprehensive information on

NTDs and associated factors. The data collection tool/checklist was digitized and utilized with ODK (Open Data Kit) Collect software for electronic data gathering. All NTDs recorded in patient charts and medical history record books were collected. Medical records from the selected hospitals were reviewed to extract relevant information on delivery outcomes, including live births, stillbirths, and elective terminations. The checklist within the ODK Collect software were used to systematically capture data on all NTDs noted in patient charts and medical history records. Study cases with more than one congenital defect were counted only once for each specific defect to avoid duplication. Each case was categorized based on the primary diagnosis or the most significant defect as per the case definition. Cases were included if they meet the established case definitions for the congenital defects being studied. This ensures that the data collected is relevant and specific to the research objectives. Cases not meeting the case definitions or with incomplete information was excluded from the analysis.

Quality Control Measures

The data collection process was designed to ensure accuracy and consistency, with a focus on quality control through comprehensive training, pretesting, and ongoing supervision. The use of electronic data collection via ODK Collect facilitated efficient data management and real-time monitoring. Data were collected by trained midwives or nurses from each of the selected hospitals. A pretest was conducted to assess the clarity, understandability, and flow of each question, as well as the time required to complete the questionnaire. Adjustments were made based on the pretest findings to ensure the tool was effective and efficient. Cases with more than one defect were counted only once to avoid duplication. Only cases meeting the specific case definitions for NTDs were included. Cases with ambiguity or multiple congenital anomalies were excluded to prevent misclassification. Supervisors reviewed all filled questionnaires daily for completeness and accuracy through the ODK Collect server. The supervisor used ODK data collect server to monitor data quality in real-time, ensuring any issues were promptly addressed.

Data Analysis

Data were entered into SPSS from the ODK Collect dataset. The data analysis involved using SPSS version 27 for comprehensive data management and statistical evaluation. Data entry, cleaning, error checking, and analysis were performed. The dataset was cleaned to identify and correct any inaccuracies or inconsistencies. This included checking for missing values, outliers,

and logical errors. Descriptive statistics provided an overview of the data. The prevalence of neural tube defects (NTDs) was determined by calculating the proportion of births with NTDs (numerator) among the total number of deliveries (denominator) over the study period of three years. This was expressed as a rate per 10,000 births. Binary logistic regression offered insights into the factors associated with neural tube defects. A p-value <0.05 was considered statistically significant for all tests. The results were systematically presented in both textual, tabular, and graph formats to facilitate understanding and interpretation.

Ethical clearance

Ethical clearance for the study was obtained from Mekelle University, College of Health Sciences, institutional review bord (MU, CHS-IRB). An official ethical approval letter and supporting documentation were provided by these institutions, ensuring compliance with ethical standards. Confidentiality of the data was maintained throughout the study. Information collected from medical records was stored securely and used exclusively for research purposes. Measures were implemented to ensure that all data handling and storage were conducted in a manner that protected participant privacy. All study procedures adhered to the Declaration of Helsinki [205], which outlines ethical principles for medical research involving human subjects. This includes ensuring that the study was conducted with respect for the rights, dignity, and well-being of participants.

4.3 Result

Maternal and neonatal characteristics

The majority of neural tube defect (NTD) cases were observed in mothers were aged 20–29 years (57.2%), followed by those aged 30–39 years (33.9%). Fewer mothers were under 20 years (5.8%) or aged 40 years or older (3.1%). Regarding residence, a slight majority (53.7%) of mothers lived in rural areas, while 46.3% resided in urban settings. In terms of parity, most mothers had 1–3 children (70.9%), with smaller proportions having 3–5 children (16.2%) or more than 5 children (7.7%). When examining pregnancy history, 64.4% of mothers reported having healthy previous pregnancies, while 16.9% had an abortion, 15% had a stillbirth, and 7.5% experienced early neonatal death. Concerning chronic illness, 92.2% of mothers did not have chronic medical conditions, while 7.8% did. A concerning finding was that 60% of mothers did not receive any antenatal visits, with only 40% attending antenatal care during

pregnancy. Additionally, 84.9% of mothers did not take periconceptional folate or multi-vitamins during the first trimester, which could represent a missed opportunity for the prevention of neural tube defects (NTDs). Regarding neonatal characteristics, 73% of babies were stillborn, while 27% were live births. The sex distribution showed 45.5% male, 40.6% female, and 14% with an unidentified sex. A significant portion of babies were born preterm (52.2%), with 45.9% born at term and 1.9% post-term. Birth weight data revealed that more than half of the babies (55.3%) had a birth weight under 2.5 kg, indicating a high incidence of low birth weight within this group (Table 3).

Table 3: Maternal and neonatal characteristics n=1612(1434 NTDs and 178 hydocephalous)

		Frequency	
Observed characteristics		**Number**	**Percent**
Maternal characteristic			
Age category (years)	<20	94	5.8%
	20-29	922	57.2%
	30-39	547	33.9%
	40+	49	3.1%
Residence	Rural	866	53.7%
	Urban	746	46.3%
Parity	prime	90	5.6%
	1-3	1138	70.9%
	3-5	260	16.2%
	>5	124	7.7%
Previous pregnancy history	Abortion	260	16.9%
	Early neonatal death	115	7.5%
	Healthy	993	64.4%
	Stillbirth	244	15%
Chronic medical illness	No	1486	92.2%
	Yes	126	7.8
Antenatal visit	No	969	60%
	Yes	643	40%
Took periconceptional folate supplementation	No	1368	84.9%
	Yes	244	15.1%
Took multivitamin during first trimester of pregnancy period	No	1368	84.9%
	Yes	244	15.1%
Neonatal characteristics			
Baby status	Live birth	435	27%
	Stillbirth	1177	73%
sex of baby	Female	654	40.6%
	Male	733	45.5%
	unidentified	225	14.0%
Gestational age	Preterm	842	52.2%
	Term	740	45.9%
	Post term	30	1.9%
Birth weight	<2.5	892	55.3%
	>=2.5	720	44.7

Prevalence of neural tube defects

During the study period, a total of 54,626 deliveries were retrieved from the hospital registries in the study area of which 1612 (1434 NTDs and 178 Isolated hydrocephalous) cases were identified. This showed that neural tube defects were the highest defect with a birth prevalence of (1434/54626) 262.5per 10, 000 (95% CI, 249.1-276.5 per 10000) and that represented the majority of stillbirths. When the data is further disaggregated by health facility, 296.7, 250.1, 233.2, 130.6, 238.5, 442.5, 469.2, 280.4, 223, 127.7, 255.3 per10,000 births were observed in AshuPh, AdwGp, ACSH, FrePh, HagPh, LKPh, SamPh, SlkPh, ShlGh, WkmPh, YchPh, respectively (Table 4, figure 11).

Table 4: Characteristics of neural tube defects; 95% confidence interval (CI) for Poisson distribution data (n= 54626)

Hospital	Tigray zone	Total delivery	Total NTD	NTDs per 10,000	95% (CI), Poisson		Characteristics (rate per 10, 000)					
							Livebirths			Stillbirths		
					LL	UL	Ane	SB	Ence	Ane	SB	Ence
AdiShu	South	3236	96	296.7	240.3	362.3	61.8	40.2	37.1	80.3	71.1	6.2
Adwa	Central	3759	94	250.1	202.1	306	0.0	77.1	34.6	85.1	45.2	8.0
Ayder	Capital	18650	435	233.2	211.8	256.2	18.2	22.5	7.5	94.4	82.0	8.6
Firesemaetat	Eastern	1225	16	130.6	74.7	212.1	0.0	0.0	0.0	98	32.7	0.0
Hagereselam	S. east	1761	42	238.5	171.9	322.4	0.0	51.1	0.0	119.3	68.1	0.0
Lemelen Karl	South	5062	224	442.5	386.5	504.4	4.0	77.0	0.0	308.2	53.3	0.0
Samre	S. east	2110	99	469.2	381.3	571.2	9.5	109	4.7	218	47.4	80.6
Selekleka	N. West	963	27	280.4	184.8	407.9	0.0	62.3	0.0	197.3	20.8	0.0
Suhul	N. West	14844	331	223.0	199.6	248.3	11.5	17.5	0.0	103.7	90.3	0.0
wukromaray	Central	548	7	127.7	51.4	263.2	91.2	0.0	36.5	0.0	0.0	0.0
Yechla	Central	2468	63	255.3	196.2	326.6	12.2	36.5	0.0	125.6	81.0	0.0
Total		54626	1434	262.5	249.1	276.5	15.4	35.9	7.7	123.2	73.6	7.0

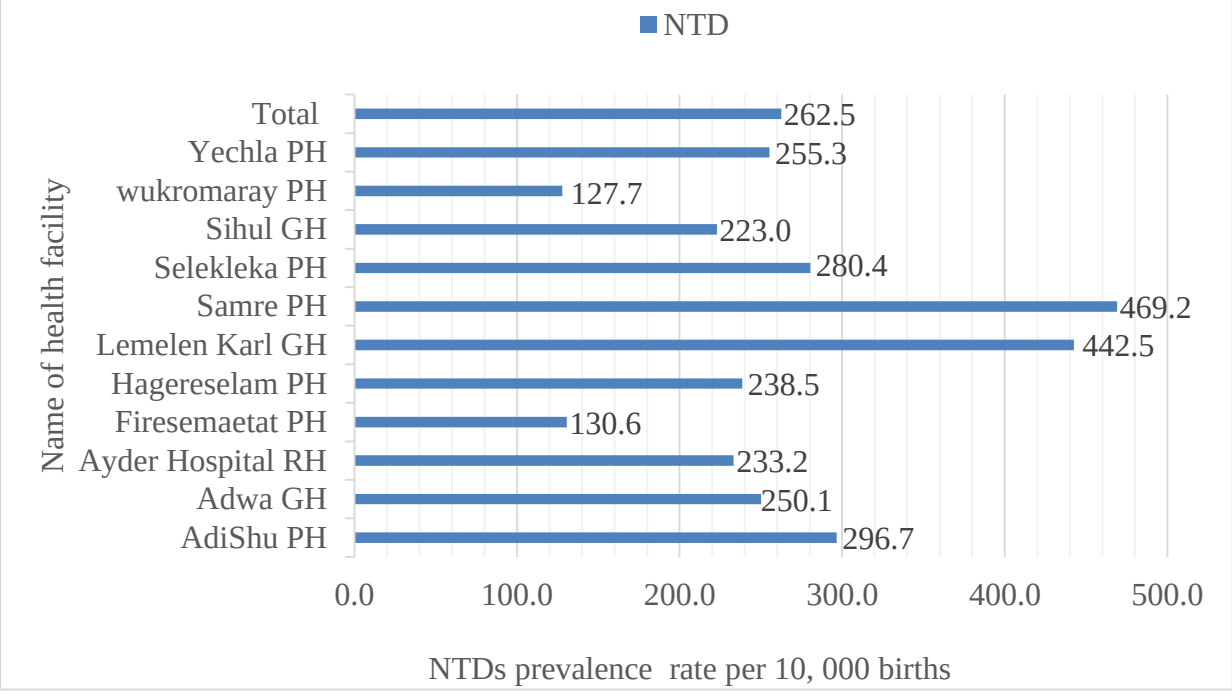

Figure 11: Prevalence neural tube defects per 10,000 births in each health facility (total birth outcome assessed (n= 54626)

Type and characteristics of the observed neural tube defects

Anencephaly (Ane) was the most common type of neural tube defect (NTD) with a prevalence rate of 136.6 per 10,000 births, followed by spina bifida (SB) at 110.6 per 10,000 births, and encephalocele (Ence) at 15.4 per 10,000 births (Figure 12). Similarly, among stillbirths affected by NTDs, anencephaly was the most prevalent, occurring at a rate of 123.2 per 10,000 births, followed by spina bifida at 73.6 per 10,000 births, and encephalocele at 7 per 10,000 births (Table 2). Anencephaly was the predominant NTD, observed in 894 cases (55%), followed by spina bifida in 487 cases (30.2%), with hydrocephalus and encephalocele also noted. The most commonly associated anomalies with spina bifida were hydrocephalus, followed by clubfoot and omphalocele (Table5).

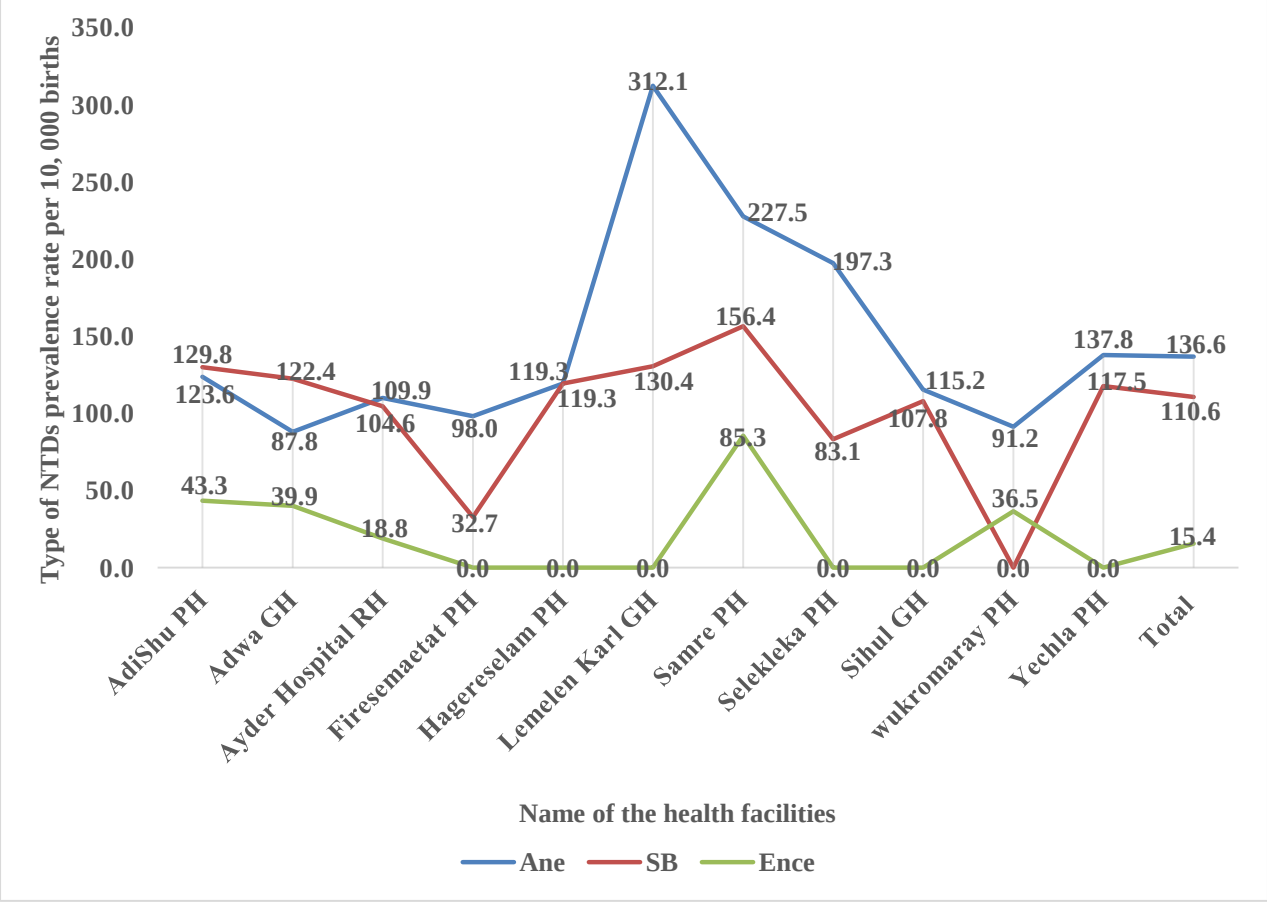

Figure 12: Typology of neural tube defects observed in public health facilities in Tigray (prevalence rate per 10, 000). Ane: Anencephaly; SB: Spina bifida; Ence: Encephalocele.

Table5: Classification of neural tube defects based on clinical presentation in Tigray province, Northern Ethiopia, 2020-2023

Type of birth defects based on ICD 10	Type of birth defects		Female N (%)	Male N (%)	Unidentified sex N (%)	Total N (%)
Central nervous system, (Q00–Q07) n(%)=1612(78.4%)	Anencephaly		325(20.2)	380(23.6)	189(11.2)	894(55.5)
	Encephalocele		28(1.7)	25(1.6)	0	53(3.3)
	Hydrocephalous		92(5.7)	80(5)	6	178(11)
	Spina bifida		209(13)	248(15.4)	30(1.9)	487(30.2)
	Associated anomalies with Spina bifida	Club foot	11(0.7)	29(1.8)		40(2.5)
		Hydrocephalous	27(1.7)	56(3.5)		83(5.1)
		Omphalocele	17(1.1)	5(0.3)		22(1.4)

Association neural tube defects in relation to maternal and fetal characteristics

Binary logistic regression has shown prime gravid (OR=2.495, 95% CI=1.143-5.446) and parity 1-3 (OR = 1.720, 95% CI = 1.249–2.370, p < 0.001) were more likely associated with an increased risk of babies with neural tube defects. Mothers who have no history of taking periconceptional folate supplementation shown to have high risk of NTDs as compared to the supplemented mothers (OR=1.509, 95% CI = 1.198–1.900, p < 0.0001).

Comparable finding was also found among mothers having no practice of multivitamin during first trimester of pregnancy period (OR=1.509, 95% CI = 1.198–1.900, p < 0.0001) was strongly associated with an increased risk of babies with neural tube defects as compared to the mothers who consume multivitamin during first trimester of pregnancy period. Neonatal characteristics such as still birth (OR = 1.470, 95% CI =1.184–1.826, p < 0.0001), sex of neonate female (OR=1.649, 95%CI= 1.274-2.134, p<0.0001), male (OR=1.704, 95% CI=1.311-2.216, p<0.0001) were more likely associated with an increased risk of babies with neural tube defects (table 6)

Table 6: Binary logistic regression: Association of neural tube defects in relation to maternal and fetal characteristics, Tigray, Ethiopia

		Frequency			95% (CI)		P-value
Maternal and neonatal characteristics		**Number**	**Percent**	**Odds ratio**	**Lower**	**Upper**	
Maternal characteristic							
Age category (years)	<20	94	5.8%	0.595	0.317	1.117	0.106
	20-29	922	57.2%	0.542	0.327	0.899	0.018
	30-39	547	33.9%	0.618	0.379	1.009	0.054
	40+						
Residence	Rural	866	53.7%	1.227	1.050	1.434	0.010
	Urban	746	46.3%				
Parity	prime	90	5.6%	2.495	1.143	5.446	0.022
	1-3	1138	70.9%	1.720	1.249	2.370	0.001
	3-5	260	16.2%	1.314	0.944	1.829	0.105
	>5						
Previous pregnancy history	Abortion	260	16.9%	0.959	0.677	1.359	0.816
	Early neonatal death	115	7.5%	0.719	0.578	0.896	0.003
	Healthy	993	64.4%	0.818	0.605	1.105	0.191
	Stillbirth						
Chronic medical illness	No	1486	92.2%	1.229	0.919	1.644	0.165
	Yes						
Antenatal visit	No	969	60.1%	0.882	0.732	1.062	0.184
	Yes						
Took periconceptional folate	No	1368	84.9%	1.509	1.198	1.900	0.0001
supplementation	Yes	244	15.1%				
Took multivitamin during first	No	1368	84.9%	1.509	1.198	1.900	0.0001

trimester of pregnancy period	Yes	244	15.1%				
Neonatal characteristics							
Baby status	Livebirth						
	Still birth	1177	73%	1.321	1.153	1.634	0.0001
Sex of baby	Female	654	40.6%	1.649	1.274	2.134	0.0001
	Male	733	45.5%	1.704	1.311	2.216	0.0001
	unidentified	225	14.0%				
Gestational age	Preterm	842	52.2%	0.561	0.450	0.700	0.0001
	Term	740	45.9%	0.793	0.444	1.418	0.434
	Post term	30	1.9%				
Birth weight	<2.5	892	55.3%	1.033	0.838	1.273	0.760
	>=2.5						

Variable(s) entered: Age (years), Residence, Parity, Previous pregnancy history, Chronic medical illness, Antenatal visit, took multivitamin and foliate during pregnancy, Baby status, sex of baby, Gestational age, Birthweight in kg.

4.4 Discussion

Our study revealed an alarming birth prevalence of neural tube defects (NTDs) at 262.5 per 10,000 births (95% Poisson confidence interval: 249.1 - 276.5 per 10,000 births). Notably, two hospitals in Tigray reported extraordinarily high rates: SamPh Hospital had a prevalence of 469.2 per 10,000 births, while LKGh Hospital reported 442.5 per 10,000 births. These figures highlight an urgent public health crisis in the region. Despite Ethiopia's advancements in reducing undernutrition, significant disparities remain [206], especially in areas like Samre and Endamohoni, which have long faced severe poverty, limited economic opportunities, low agricultural productivity and inadequate infrastructure. The recent conflict has exacerbated these challenges, likely contributing to the elevated NTD rates observed.

The prevalence of NTDs reported in our study marks a substantial increase compared to previous figures of 215 per 10,000 and 131 per 10,000 births in Tigray prior to the war [10, 11]. This sharp rise can likely be attributed to the adverse effects of the war, which may have severely disrupted access to essential health services, including antenatal care, and contributed to significant shortages of food and other basic necessities [102, 106, 185, 204]. These disruptions could have exacerbated the situation, leading to the observed increase in NTD prevalence.

Our findings reveal the highest reported prevalence of neural tube defects (NTDs) globally, significantly surpassing those observed in other regions of Ethiopia, including eastern Ethiopia (211.89 per 10,000 at Adama Medical College Hospital, 244.05 per 10,000 at Hiwot Fana Specialized Teaching Hospital, and 75.9 per 10,000 at Dil Chora Hospital) and Addis Ababa (126 per 10,000) [87, 207]. Additionally, our prevalence far exceeds the World Health

Organization's (WHO) estimate for Ethiopia of 22 per 10,000 births [208] and the reported rate of 11.7 per 10,000 births across eight African countries [38]. This significant increase highlights the urgent need for immediate and effective public health interventions.

We believe that the unexpectedly high prevalence of neural tube defects (NTDs) in our study is linked to the prolonged war and siege, which have likely devastated healthcare systems, causing a three-year disruption in services [102, 106, 185, 204]. This, in turn, has exacerbated poverty, malnutrition, and limited access to antenatal carecritical factors in preventing NTDs. The deficiency of folate, essential for neural tube closure, may have been worsened by these disruptions, contributing to the rise in NTDs.

Anencephaly was the most common NTD (55% of cases), followed by spina bifida (30.2%). Anencephaly was the most common NTD (55% of cases), followed by spina bifida (30.2%). This predominance of anencephaly over spina bifida may suggest differences in the timing or type of neural tube closure failure, as anencephaly results from defects in cranial closure occurring earlier in embryonic development. The relatively higher frequency of anencephaly could also reflect greater in-utero lethality, underreporting of less severe spina bifida cases, or differences in environmental and nutritional exposures particularly folate deficiency during early pregnancy. This pattern aligns with previous reports from the Tigray region [10, 11], suggesting that both biological and contextual factors may influence the observed distribution of NTD subtypes. It is unfortunate that the region is stricken by repeated war and famine in the last three decades. This may have its own impact on new born that though investigations should be conducted in the future. Environmental contaminants from the ongoing war, along with poor maternal nutrition, particularly folic acid deficiency, may contribute to these trends.

Additionally, the high incidence of stillbirths among affected newborns, consistent with other studies [209, 210], reflects the severe nature of NTDs. The types of NTDs observed in our study are also consistent with those reported in studies from eastern Ethiopia [207], Addis Ababa [87], the Amhara region [211], the Bale zone in Oromia [212]. Similar trends have been observed in studies from South West Iran [213], Morocco [214], India [215]. Similar trends have been noted in studies from Yaoundé, Cameroon, and Texas, USA, where the prevalence of anencephaly was found to be higher than that of spina bifida [216, 217].

This study offers valuable insights into the demographic and reproductive patterns of mothers affected by neural tube defects (NTDs). A significant proportion of cases were observed in

mothers aged 20-29 years (57.2%), followed by those aged 30-39 years (33.9%), likely reflecting higher fertility rates in these age groups. The lower prevalence in mothers under 20 (5.8%) and over 40 (3.1%) may be attributed to both biological and societal factors [218-221]. Advanced maternal age is generally associated with reduced fertility and a lower likelihood of conception, which could partly explain the smaller proportion of NTD cases among women over 40 years. However, this pattern may also reflect age-related differences in pregnancy intentions, healthcare-seeking behavior, and prenatal detection or reporting. Therefore, the lower incidence of NTDs in this age group should be interpreted cautiously, as it may be influenced by both biological and sociocultural factors rather than fertility alone [218–221]. Conversely, urban mothers generally benefit from better healthcare access, which may explain the lower NTD rates in these regions.

Our findings indicate that primigravid women and those with 1–3 previous pregnancies had a higher likelihood of having babies with NTDs. While this pattern may partly reflect differences in nutritional status and access to prenatal care, it could also be influenced by other interrelated factors such as maternal age and the spacing between pregnancies. Short inter-pregnancy intervals have been associated with depleted maternal folate stores and increased risk of adverse pregnancy outcomes, including NTDs [225, 226]. Similarly, younger maternal age groups common among primigravid women may be less informed about preconception folate supplementation and antenatal care services. Recognizing these multifactorial influences is important before designing targeted interventions. Strategies should therefore emphasize improving maternal nutrition, promoting adequate birth spacing, and enhancing awareness of prenatal care among all reproductive-age women, particularly those at earlier parity levels.

The failure of many mothers to seek antenatal care (ANC) in this study highlights gaps in maternal health practices, contributing to high neural tube defect (NTD) rates. ANC is vital for monitoring health, providing nutrition education, and folic acid supplementation to reduce NTD risks. In rural and war areas, limited access, financial barriers, and lack of awareness prevent timely prenatal care, missing key opportunities for early detection and management of risk factors like folate deficiency. This emphasizes the need for public health interventions to improve ANC access and reduce NTD prevalence.

This study found that mothers who did not take periconceptional folate or multivitamins during the first trimester had a significantly higher risk of NTDs. Folic acid is critical in the prevention

of NTDs, as it plays an essential role in the closure of the neural tube during the early stages of pregnancy [178, 202). Lack of supplementation increases the risk of NTDs, highlighting a critical gap in maternal healthcare practices. Women who did not take folic acid before conception or during the early stages of pregnancy had a higher likelihood of delivering babies with severe birth defects such as anencephaly and spina bifida [178, 202). The absence of folate supplementation is a modifiable risk factor. The strong association between folate deficiency and the incidence of NTDs suggests that public health efforts should prioritize the dissemination of information about the importance of periconceptional folate supplementation. Public health campaigns should be tailored to reach women of reproductive age, especially in rural areas where access to healthcare and education on prenatal nutrition is limited.

This study reveals the association between stillbirth with an increased risk of NTDs is clinically significant because it underscores the severe outcomes of NTDs, particularly in regions where access to timely medical care is limited. The increased likelihood of stillbirth in cases of NTDs points to the severe and often fatal nature of these birth defects, with high rates of perinatal mortality [227, 228]. Furthermore, the observed differences in NTD prevalence between male and female neonates (higher prevalence in male infants) may warrant further investigation into potential gender-based differences in the etiology or outcomes of NTDs. The finding that stillbirths are strongly associated with NTDs provides a compelling argument for early detection and intervention. It may also inform healthcare practices that aim to identify pregnancies at risk of NTDs, thus potentially reducing the high rate of stillbirths associated with these defects. Further research into sex-based differences could elucidate whether gender-specific factors—biological or environmental—play a role in the development of NTDs.

The high prevalence of neural tube defects (NTDs) in Ethiopia, particularly in the Tigray region, can largely be attributed to the lack of primary prevention measures such as preconception care and mandatory folic acid fortification of staple foods. Evidence shows that mass fortification can significantly reduce NTD burden and lower associated healthcare costs [178, 202, 229]. The higher risk in Tigray may be linked to low folate levels among women of reproductive age, exacerbated by the war and siege since 2020, which disrupted healthcare systems, damaged infrastructure, and led to the loss of healthcare professionals [102, 106, 185, 204]. The long-term impacts of war and malnutrition could have lasting effects, potentially influencing future generations [230].

In countries like the United States, China, and Canada, where folic acid levels are adequate, the expected prevalence of spina bifida and anencephaly is about 5 per 10,000 births [178, 202). In contrast, our study found a prevalence of 262 per 10,000 births, 52 times higher than expected. This highlights the urgent need for effective programs ensuring that all women of reproductive age have adequate folic acid levels, which could prevent 96% of spina bifida and anencephaly cases in the Tigray region.

4.5 Conclusion and Recommendations

Conclusion

Our study reveals an alarmingly high prevalence of neural tube defects (NTDs) in the Tigray region, with a birth prevalence rate of 262.5 per 10,000 births, significantly higher than expected. Anencephaly emerged as the most common NTD, followed by spina bifida. Key factors associated with an increased likelihood of NTDs include maternal age (20-29 years), rural residency, prime gravida, and a history of early neonatal death. Furthermore, the absence of periconceptional folate supplementation and the non-use of multivitamins during the first trimester were significant risk factors. Neonatal characteristics such as stillbirth, male sex, and preterm birth, were strongly associated with higher rates of NTDs. These findings underscore the critical role of maternal nutrition, prenatal care, and specific pregnancy histories in the development of NTDs, providing clear areas for focused intervention.

Recommendations

Post-War Health System Recovery and Expansion: Rebuild and strengthen the healthcare infrastructure in Tigray, with particular emphasis on expanding preconception services to identify high-risk women. Public health campaigns should focus on the importance of periconceptional folate intake and multivitamin use, especially in rural areas where access to supplements may be limited.

Targeted Interventions for Young and High-Risk Mothers: young mothers, prime gravida, and those with a history of early neonatal death or stillbirth should be prioritized for tailored healthcare interventions. This includes more frequent prenatal monitoring, counseling, and education on preventing NTDs to reduce the incidence among these high-risk groups.

National Surveillance and Research: Establish a national surveillance system to monitor the prevalence of NTDs and evaluate the effectiveness of folic acid supplementation programs.

Furthermore, research should be conducted to investigate genetic and environmental factors contributing to NTDs, facilitating the development of more precise prevention and treatment strategies.

4.6 Limitation of the study

The following limitations are acknowledged in this study. Because the study was conducted in health facilities, it may not represent the true prevalence of NTDs in the general population, only include individuals who had access to healthcare, potentially excluding those who did not seek care or were not diagnosed. Determinants of NTDs have not been investigated or attempted. Because this is a retrospective study, there are significant limitations to the recorded data. In some cases, the necessary investigation and complete history were not properly documented. As the study was conducted in war-stricken region the data may not be extensive enough and thorough, as the premises of the health facilities are affected.

Chapter Five

Risk Factors for Neural Tube Defects in the War and Siege-Affected Tigray Regional State of Ethiopia: A Case-Control Study

Berihu *et al. BMC Pregnancy and Childbirth* (2025) 25:638
https://doi.org/10.1186/s12884-025-07724-8

BMC Pregnancy and Childbirth

RESEARCH Open Access

Risk factors for neural tube defects in the war and siege-affected tigray regional state of ethiopia: a case-control study

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Abstract

Background Neural tube defects (NTDs) remain a significant global health concern, with varying prevalence and risk factors across regions. In Tigray, the war and siege have severely disrupted healthcare services, exacerbating pregnancy complications and increasing NTD prevalence. This study investigates the sociodemographic, obstetric, and conflict-related risk factors contributing to NTDs in Tigray during the crisis.

Methods A case-control study was conducted during 01 December 2023 to 30 January 2024, including 103 NTD cases and 205 controls. Data were collected using a WHO-adapted birth defect survey and ODK/KOBO software. Statistical analysis was conducted using SPSS version 27, with descriptive statistics used for participant characteristics and logistic regression models applied to identify risk factors for NTDs, using a significance threshold of $p < 0.05$.

Result A total of 308 participants were included (103 NTD cases and 205 controls). Stillbirths accounted for 79.7% of NTD cases, with anencephaly as the most common defect, followed by spina bifida and encephalocele. Among NTD cases, 23.3% were male, 18.4% female, and 58.3% had unidentified sex. Associated congenital anomalies were less common, including hydrocephalus (2.9%), clubfoot, omphalocele, and cleft palate (1-1.9%). Key risk factors for NTDs included younger maternal age, low education, history of abortion or stillbirth, and lack of prenatal care. Conflict-related factors, such as violence, unintended pregnancies, and limited healthcare, worsened NTD prevalence. Food insecurity was found a significant issue, with many women relying on aid or subsistence farming and consuming fewer than two meals per day.

Conclusion The study demonstrates that neural tube defects, particularly anencephaly the most frequently observed subtype are strongly associated with increased stillbirth rates. Diagnostic challenges, including high rates of unidentified fetal sex and low detection of associated anomalies, coupled with limited prenatal care and maternal sociodemographic factors, contribute to poor prognoses. The ongoing conflict in Tigray has further worsened maternal health risks through healthcare disruptions, increased violence, unintended pregnancies, and food insecurity, potentially contributing to the higher prevalence of neural tube defects. These findings underscore the urgent need for strengthened maternal healthcare systems, targeted prevention strategies, and improved access to care, particularly in conflict-affected regions.

Keywords Neural tube defects, Spina bifida, Anencephaly, Encephalocele, Sociodemographic factors, Obstetric factors, Conflict related factors, Tigray

5.1 Background

Neural tube defects (NTDs), including spina bifida, anencephaly, and encephalocele, present a significant public health challenge worldwide. These congenital abnormalities result from the incomplete closure of the neural tube during early embryonic development and are associated with considerable morbidity and mortality, especially in low-resource settings [231]. On a global scale, the average prevalence of NTDs is approximately two cases per 1,000 births, translating to an estimated 214,000–322,000 affected pregnancies annually [201]. The incidence and related complications of NTDs are notably higher in developing countries, where access to healthcare and nutritional resources is often limited [201]. Research consistently shows that NTDs are more prevalent in regions with inadequate prenatal care and insufficient folic acid supplementation [232, 233, 234]. Sub-Saharan Africa and parts of Asia, for example, continue to report high rates of NTDs [5]. In Ethiopia, the prevalence of NTDs is significantly higher than in Western countries seven times greater, in fact with marked regional variation. In Addis Ababa, the rate is 126 per 10,000 births, while Tigray reports a considerably higher rate ranging from 131 to 215 per 10,000 births [8, 10, 11].

The prevalence and risk factors for NTDs differ across regions, and these factors are particularly exacerbated in populations affected by war and siege. Armed conflict is a major disruptor of public health systems, with maternal and reproductive health being particularly vulnerable [235]. War zones present numerous barriers to healthcare, directly and indirectly affecting maternal and birth outcomes. Limited access to healthcare services, particularly prenatal care, is a well-established risk factor for NTDs [8, 201, 236]. In the case of Tigray, the war and prolonged siege have severely impacted healthcare infrastructure, leading to the closure of health facilities, displacement of healthcare workers, and obstructing pregnant women's access to essential care [102, 106, 185, 204]. The destruction of health infrastructure has also disrupted access to folic acid supplementation, which is crucial for the prevention of NTDs.

Given these circumstances, it is essential to examine how sociodemographic, obstetric, and conflict-related factors intersect to influence the prevalence of NTDs. Understanding the clinical and epidemiological implications of these factors can guide future interventions and health strategies, particularly for women in war-affected areas. In Tigray, the war and prolonged siege have been intensified instability, limiting access to vital health services and family planning, which in turn jeopardizes maternal and child health [102, 106, 185, 204]. Our

recent retrospective study conducted during the war in Tigray showed a significant increase in the prevalence of NTDs, with a rate of 262.5 per 10,000 births [237], compared to pre-war rates in Tigray of 215 and 131 per 10,000 births [10, 11]. This increase is likely a direct consequence of the war and siege, which have disrupted essential health services like antenatal care and contributed to shortages of food and basic necessities [102, 106, 185, 204]. These factors have likely compounded the situation, leading to the observed rise in NTDs. Therefore, this study aims to identify the key risk factors for NTDs in this context, improving our understanding of the role the war and prolonged siege have played in their increased prevalence. The study also seeks to inform more effective prevention and treatment strategies for Tigray's healthcare system moving forward.

5.2 Methodology

Study area and setting

This study was conducted across 11 public hospitals within the Tigray Region, situated in the northernmost part of Ethiopia. These hospitals encompass both urban and rural settings, providing a representative overview of the healthcare landscape in the region. The Tigray Region is inhabited predominantly by the Tigrayan (Tegaru), Irob, and Kunama ethnic groups. Mekelle, the regional capital and largest city, serves as a major administrative and economic hub. Tigray is Ethiopia's fifth-largest region by area, fourth in population size, and fifth in population density among the country's 11 regional states. As of 2019, the estimated population of Tigray is approximately 9 million [18, 195], with a total fertility rate of 4.6 children per woman [18]. Agriculture is the primary livelihood, with approximately 80% of the population engaged in farming, contributing 46% to the regional gross domestic product. In November 2020, an armed conflict erupted between the Tigray regional government, led by the Tigray People's Liberation Front (TPLF), and the Ethiopian federal government, supported by Eritrean and Amhara regional forces. This conflict rapidly escalated into a regional war, leading to widespread humanitarian and infrastructural devastation, including the reported deaths of over 600,000 individuals [102, 106, 185, 204]. The healthcare system was severely affected, with an estimated 89% of health facilities looted and destroyed during the conflict.

Selection of study hospitals

The study targeted 11 public hospitals within the Tigray Region that remained relatively functional during the conflict and subsequent blockade. Hospitals were selected using a simple random sampling technique from the available pool of operational public hospitals. This

approach was employed to minimize selection bias and ensure equal probability of inclusion for all eligible facilities, despite the challenges posed by the conflict and limited accessibility. Although the number of functioning hospitals was relatively small, random sampling was considered methodologically sound to ensure a representative sample. The selected hospitals included: Yechila Hospital, Wukro Maray Hospital, Adwa Hospital, Shul Shire Hospital, Selekleka Hospital, Samre Hospital, Hagereselam Hospital, Lemlem Karl Hospital, Adishu Hospital, Fresemaetat Hospital, and Ayder Comprehensive Specialized Hospital. These facilities provide a broad spectrum of healthcare services, including pediatric care, gynecology and obstetrics, midwifery, and general nursing services.

Study design and period

A case-control study design was used to identify and analyze the risk factors associated with neural tube defects (NTDs) among mothers who gave birth in health care facilities in the Tigray Regional State of Ethiopia. The study was conducted in selected hospitals that provide maternal and child health services within the region, which has been affected by the recent war and prolonged siege. The collection of data was from participants at a single point in time, providing insights into the risk factors for NTDs in this context. Data collection took place from 01 December 2023 to 30 January 2024.

Study population and sample size

The study population consisted of cases of mothers who gave births affected with neural tube defects (NTDs), including both live-born and stillborn cases. The conditions under investigation included spina bifida, anencephaly, encephalocele, and other forms of NTDs. The control group was composed of mothers of infants without any congenital anomalies, who gave birth in the same hospitals concurrently. The sample size for this unmatched case-control study was determined using OpenEpi, Version 3, an open-source calculator. The following assumptions were used in the calculation: Two-sided confidence level (1-alpha): 95%, Power (probability of detecting the effect): 80%, Ratio of Controls to Cases: 2:1. Hypothetical proportion of controls with exposure: 40%, Hypothetical proportion of cases with exposure: 57.57%, Least extreme Odds Ratio to be detected: 2.04, Based on these assumptions, the calculated sample sizes were as follows: Sample Size for Cases: 103, Sample Size for Controls: 205.

Total Sample Size: 308, Based on these parameters, the required sample size was calculated to be 103 cases and 205 controls, resulting in a total of 308 participants. All cases were compared

with a randomly selected group of controls drawn from the same facilities, using an unmatched concurrent case-control approach.

Sampling technique and procedure

To ensure proportional representation across participating health facilities, the calculated sample size was allocated according to the annual number of deliveries at each site. Facility-specific delivery volumes over the preceding year were obtained from Health Management Information System (HMIS) reports. This proportional allocation was intended to enhance the representativeness of the sample and reduce potential selection bias.

Eligibility criteria

Inclusion criteria: Cases: All mothers who gave birth affected with any form of neural tube defects (live and stillbirths) and who consented to provide adequate information were included. Control: Mothers of infants without any congenital anomalies during the same period were included.

Exclusion criteria: Mothers who were seriously ill and unable to provide information. Live births, stillbirths, and elective terminations involving non-permanent -resident women of the study districts (mothers residing outside the study districts for < 6 months prior to delivery).

Study variables

Dependent variable

The dependent variable in this study is the presence or absence of neural tube defects (NTDs), which includes conditions such as anencephaly, spina bifida, and encephalocele (as defined by International Classification of Diseases, Tenth Revision (ICD-10) [199]. This variable also accounts for medically terminated NTDs that are incompatible with life, across infants of any gestational age.

Independent variables

Sociodemographic and Obstetric Factors: Maternal age, marital status, residence, religion, education, occupation, parity, history of pregnancy outcomes, sex of baby in last pregnancy, family history of birth defects, history of children with birth defects, practice of periconceptional folic acid supplementation, and history of X-ray imaging. Conflict-related Factors: Type of conflict event, use of healthcare services during wartime, pregnancy intention during wartime, family food source during wartime, and meal frequency during wartime.

Data collection tool and technique

The NTDs survey tool was adapted from the WHO birth defect surveillance tool [198]. Data were gathered electronically using the ODK/KOBO Collect software. The data collection

tool/checklist contained variables representing maternal and fetal characteristics, including socio-demographic and clinical data, obstetric history, and conflict-related factors. If a study case had more than one birth defect, it was counted only once. Cases were included when they met the case definition for the specific condition being studied. The study employed prospective data collection, with interviews and questionnaires administered to mothers of both NTD cases and controls to collect information on potential risk factors (sociodemographic, obstetric, and conflict-related).

Daily data collection was carried out by midwives who had received structured training in the pathophysiology of neural tube defects (NTDs) and standardized newborn assessment protocols. Throughout the study period, these trained midwives systematically screened all deliveries for potential NTD cases. Data were recorded during the postpartum or post-abortal period, prior to maternal discharge from the health facility. For the purposes of this study, NTDs were defined as cases of anencephaly or spina bifida in infants of any gestational age. Suspected cases were verified through physical examination by senior physicians, including pediatricians, obstetricians, gynecologists, or general practitioners, based on the availability of medical personnel at the participating hospitals.

Quality assurance

Eleven well-trained BSc midwives were involved in data collection. Both data collectors and supervisors underwent five days of training on the data collection procedure and the use of the ODK data collection software before the actual data collection. Supervisors checked all filled questionnaires daily for completeness using the ODK data collection server. Both supervised and unsupervised visits to study sites were regularly conducted by the core study team to ensure data quality.

Data analysis

Data were analyzed using SPSS version 27. Descriptive statistics were used to summarize the demographic and clinical characteristics of the study population. Binary logistic regression analysis was conducted to determine the strength of association between various risk factors and the occurrence of NTDs. Variables with $p < 0.25$ were considered for further multivariate analysis. Multivariate logistic regression analysis was used to identify independent risk factors for NTDs, adjusting for potential confounders. A significance level of $p < 0.05$ was used to determine statistically significant associations. Results were interpreted in the context of existing literature and public health implications, and risk factors identified were compared

with findings from other studies to validate results and understand their relevance to the local context.

Ethical considerations

This study was part of a PhD program within Mekelle University's large-scale research initiative focused on using periconceptional folic acid supplementation to prevent neural tube defects in Tigray, Northern Ethiopia. Ethical approval was obtained from the Mekelle University Institutional Review Board (MU-IRB) under protocol number MU-IRB 1800/2020, with a subsequent renewal (ref No. MU-IRB 2101/2023). Orally informed consent was obtained from all participants before data collection. All personal and medical information was kept confidential and used only for research purposes. All study procedures adhered to the Declaration of Helsinki [205], which outlines ethical principles for medical research involving human subjects. This includes ensuring the study was conducted with respect for the rights, dignity, and well-being of participants.

5.3 Result

A total of 308 participants were recruited (103 NTD cases and 205 controls) in the selected study hospitals with two-month period.

Case series

Characteristic of the observed neural tube defects (NTDs) cases, n = 103

The study examined 103 cases of mothers who gave birth affected with any form of neural tube defects, including anencephaly, spina bifida, and encephalocele (Table 7). Among these, anencephaly was the most common (54 cases, 52.4%), followed by spina bifida (43 cases,41.75%) and encephalocele (6 cases, 5.8%). Stillbirths occurred in 82 cases (79.7%), with anencephaly accounting for 54cases (52.4%), spina bifida for 22 cases (21.35%), and encephalocele for 6 cases (5.8%). Live births were reported in 21 cases of spina bifida, representing 20.4% of the total.

Regarding sex distribution, 24 cases (23.3%) were male, which included 10 cases of anencephaly (9.7%), 13 cases of spina bifida (12.6%), and 1 case of encephalocele(1%). Female cases totaled 19 (18.4%), including 7 cases of anencephaly (6.8%), 9 cases of spina bifida (8.7%), and 3 cases of encephalocele (2.9%). Additionally, 60 cases (58.3%) were unidentified sex, which consisted of 37 cases of anencephaly (36%), 21 cases of spina bifida (20.4%), and 2 cases of encephalocele (1.9%). Associated congenital malformations were

observed in several affected pregnancies, including hydrocephalus (3 cases, 2.9%), clubfoot (1 case, 1%), omphalocele (2 cases, 1.9%), and cleft palate (2 cases, 1.9%).

The study also assessed the recurrence risk of NTDs, focusing on both blood relatives with a history of birth defects (BD) and prior births affected. Five cases reported a family history of birth defects, including a grandmother with clubfoot, a cousin with clubfoot, an aunt with congenital heart defects, and a grandfather with polydactyly. Additionally, eight cases had a history of birth defects, including two with spina bifida, three with clubfoot, one with anencephaly, one with an abdominal defect, and one with ventriculomegaly, all recurring in the current pregnancy.

Table 7: Characteristics of the pregnancy outcome affected with the neural tube defects (NTDs), n=103

			Type of neural tube defects (n=103)			
			Anencephaly	Spina bifida	Encephalocele	
Characteristics			N (%)	N (%)	N (%)	Total N (%)
Baby status	Live birth			21(20.4)		21(20.4)
	Stillbirth		54(52.42)	22(21.35)	6(5.83)	82(79.7)
	Total		54(52.42)	43(41.75)	6(5.83)	103(100)
Sex	Male		10(9.7)	13(12.6)	1	24(23.3)
	Female		7(6.8)	9(8.7)	3(2.9)	19(18.4)
	Unidentified		37(36)	21(20.4)	2(1.9)	60(58.3)
	Total		54(52.42)	43(41.75)	6(5.83)	103(100)
Associated congenital malformations	Hydrocephalous			3(2.9)		3(2.9)
	Club foot			1		1
	Omphalocele			2(1.9)		2(1.9)
	Cleft palate			2(1.9)		2(1.9)
Recurrence risk associated with neural tube defect						
Blood relative with birth defects (BD)	No					
	Yes	Grandmother (club foot)	1			1
		Cousin (clubfoot)	1			1
		Aunt (Congenital heart defect)	1	1		2(1.9)
		Grandfather (polydactyl)	1			1
Previous child with birth defects (BD)	No					
	Yes	Spinal bifda	1	1		2(1.9)
		Club foot	3(2.9)			3(2.9)
		Anencephaly		1		1
		Abdominal defect		1		1
		Ventromegally		1		1

Case control analysis

Sociodemographic and Obstetric Characteristics of Study Participants (n = 308: 103 NTDs cases and 205 controls)

The findings offer valuable insights into how various factors, including maternal age, marital status, education, occupation, obstetric history, and family history, contribute to the risk of giving birth affected with the neural tube defects (NTDs) (Table 8).

In the NTDs cases, 14.9% of mothers were 25 years or younger, 14.9% were between 26 and 35 years, and 3.6% were older than 35. In the control group, 32.1% were 25 or younger, 30.5% were between 26 and 35, and 3.9% were older than 35. Most mothers in both cases and controls were married, comprising 27.6% of NTD cases and 55.2% of controls. Unmarried individuals accounted for 5.2% of NTD cases and 10.1% of controls, while divorced individuals made up 0.6% and 1.3%, of the cases and controls respectively. Regarding residence, a larger percentage of NTD cases (18.5%) lived in rural areas, while 14.9% of cases resided in urban areas. In comparison, 35.7% of the control group lived in rural areas, and 30.8% lived in urban areas. Religious affiliation was predominantly Orthodox in both cases and controls, with 31.8% of NTD cases and 65.3% of controls identifying as Orthodox Christians. Educational attainment varied between the cases and controls. Among NTD cases, 6.5% had no formal education, 11.7% had completed elementary school, 10.7% had finished high school, and 4.5% had attained a college diploma or higher. In the control group, 15.3% had no formal education, 22.7% had completed elementary school, 19.5% had finished high school, and 9.1% had a college degree or higher. Occupational patterns also differed between the groups. Among NTD cases, 13.3% were housewives, 12.7% were laborers, 8.1% were merchants, and 7.5% were farmers. In the control group, the most common occupation was housewife (21.1%), followed by farmer (19.5%) and laborer (8.8%).

Regarding obstetric history, 15.6% of NTD cases had 1–3 children, 6.5% had more than 3 children, and 11.4% were primiparous. Among controls, 32.1% had 1–3 children, and 19.5% were primiparous. regarding the history of the pregnancy outcomes, 19.2% of NTD cases had previously given birth to full-term live babies, while 2.9% had experienced an abortion or stillbirth. Among controls, 34.1% had full-term live births, and 13.0% had experienced an abortion or stillbirth. The sex distribution of the last pregnancy revealed that 11.7% of NTD cases had a female child, 8.8% had a male child, and 1.6% had an unidentified sex. In the control group, 21.1% had a female child, and 21.1% had a male child. Concerning family

history of birth defects, 1.6% of NTD cases and 2.3% of controls reported a family history of conditions such as clubfoot and congenital heart defects. Notably, 2.6% of NTD cases had a previous child with birth defects, compared to 1.3% of controls, with conditions including anencephaly, clubfoot, hydrocephalus, and spina bifida. Exposure to X-ray imaging during pregnancy was reported by 0.6% of NTD cases and 1.0% of controls. Lastly, the study revealed that none of the cases and controls, had consumed periconceptional folic acid supplementation.

Table 8: Sociodemographic and obstetric characteristics of study participants, n=308 (103 NTDs cases, 205 controls

		Neural tube defects (NTDs)		
		Yes	**No**	**Total**
Characteristics		**N (%)**	**N %**	**N %**
Sociodemographic history				
Maternal age	<=25	46(14.9)	99(32.1)	145(47.1)
	26-35	4614.9)	94(30.5)	140(45.5)
	>35	11(3.6)	12(3.9)	23(7.5)
Marital status	Not married	16(5.2)	31(10.1)	47(15.3)
	Divorced	2(0.6)	4(1.3)	6(1.9)
	Married	85(27.6)	170(55.2)	255(82.8)
Residence	Rural	57(18.5)	110(35.7)	167(54.2)
	Urban	46(14.9)	95(30.8)	141(45.8)
Religion	Orthodox	98(31.8)	201(65.3)	299(97.1)
	Muslim	5(1.6)	4(1.3)	9(2.9)
Education	No formal education	20(6.5)	47(15.3)	67(21.8)
	Elementary school	36(11.7)	70(22.7)	106(34.4)
	High school	33(10.7)	60(19.5)	93(30.2)
	College diploma and above	14(4.5)	28(9.1)	42(13.6)
Occupation	House wife	41(13.3)	65(21.1)	106(34.4)
	Farmer	23(7.5)	60(19.5)	83(26.9)
	Labor	39(12.7)	27(8.8)	66(21.4)
	Merchant		25(8.1)	25(8.1)
	Collage educated professional		28(9.1)	28(9.1)
Obstetric history				
Parity	Prime	35(11.4)	60(19.5)	95(30.8)
	parity 1-3	48(15.6)	99(32.1)	147(47.7)
	parity >3	20(6.5)	46(14.9)	66(21.4)
History of Pregnancy outcome	No prior pregnancy	35(11.4)	60(19.5)	95(30.8)
	Abortion/stillbirth	9(2.9)	40(13.0)	49(15.9)
	Full-term live baby	59(19.2)	105(34.1)	164(53.2)
Sex of baby of last pregnancy	No prior pregnancy	35(11.4)	60(19.5)	95(30.8)
	Unidentified	5(1.6)	15(4.9)	20(6.5)
	Female	36(11.7)	65(21.1)	101(32.8)
	Male	27(8.8)	65(21.1)	92(29.9)
Family history of birth defects	Yes	5(1.6)	7(2.3)	12(3.9)9
	No	98(31.8)	198(64.3)	296(96.1)
If yes relationship with you relative and	Club foot, uncle	2(0.6)	7(2.3)	9(2.9)
Type of birth defect.	Congenital heart defect, aunt	1(0.3)		1(0.3)
	Congenital heart defect, aunt	1(0.3)		1(0.3)
	Maternal cousin, club foot and hydrocephalous	1(0.3)	1(0.3)	2(0.6)
	maternal, club foot		1(0.3)	1(0.3)
Previous child with birth defects	Yes	8(2.6)	4(1.3)	12(3.9)
	No	95(30.8)	201(65.3)	296(96.1)

If yes, type of the birth defects	Anencephaly	2(0.6)		2(0.6)
	Club foot	3(1)	2(0.6)	5(1.6)
	Hydrocephalous		1(0.3)	1(0.3)
	Spina bifida	2(0.6)	2(0.6)	4(1.3)
History of X-ray imaging	No	101(32.8)	202(65.6)	303(98.4)
	Yes	2(0.6)	3(1)	5(1.6)
Consumption of Periconceptional folic acid supplementation	Yes			
	No	103(33.4)	205(66.6)	308(100)

Effects of adverse conditions on Pre-pregnancy safe motherhood practices and neural tube defects in war and siege-affected Tigray (n = 308: 103 NTDs cases and 205 controls).

This study highlights the multifaceted challenges faced by both study groups due to the war and siege, including exposure to various form of attack, limited access to healthcare, unintended pregnancies, and food insecurity (Table 9). These conditions have had a significant impact on maternal health and are likely contributors to the increased prevalence of neural tube defects (NTDs) in the region. The findings provide critical insights into the complex factors affecting the health of pregnant women in Tigray.

A substantial proportion of the study participants experienced various war-related events, with attacks on populations being the most common (50.6%). Other reported events included acts of war (28.3%) and property damage (21.1%), all of which disrupted access to essential services and resources, exacerbating maternal health challenges. Healthcare access was particularly affected, with 91.9% of women not receiving any health services during their pregnancy. Only 8.1% of participants were able to access medical care, underscoring the significant barriers created by the destruction of healthcare facilities and war. The absence of prenatal care emerged as a critical factor contributing to the elevated risk of NTDs in the region.

The study also revealed a high prevalence of unintended pregnancies, accounting for 64.3% of all pregnancies. This lack of control over reproductive health was attributed to several factors: 7.8% of women cited the destruction of health facilities as a barrier to accessing family planning services, 40.6% reported the unavailability of family planning options, and 21.4% indicated a lack of support from their families. Additionally, 2.3% of pregnancies resulted from rape or unknown circumstances.

Food insecurity emerged as another significant challenge in Tigray, with 24.7% of families relying on food aid and 30.2% purchasing food, while only 36.4% depended on their own cultivation. Nutritional inadequacy was widespread, as 58.4% of women reported consuming

two or fewer meals per day. This food insecurity likely contributed to poor maternal nutrition, which is a well-established risk factor for NTDs.

Table 9: Pre-pregnancy Safe Motherhood Among study participants amidst challenging conditions in War-Torn Tigray

		Neural tube defects (NTDs)		
		Yes	No	Total
Characteristics		N (%)	N (%)	N (%)
Type of conflict event?	Act of war	35(11.4)	52(16.9)	87(28.3)
	Attack on populations	46(14.9)	110(35.6)	156(50.6)
	Damage to property	22(7.1)	43(14)	65(21.1)
Use health service	No	94(30.5)	189(61.4)	292(91.9)
	Yes	9(2.9)	16(5.2)	25(8.1)
Pregnancy intended/wanted	No	79(25.7)	119(38.6)	222(64.3)
	Yes	24(7.8)	86(27.9)	110(35.7)
If No, Reason for unwanted/intended pregnancy	Health facility damaged	4(1.3)	20(6.5)	24(7.8)
	lack of family planning	51(16.6)	74(24)	125(40.6)
	Not supported by family	48(15.6)	18(5.8)	66(21.4)
	pregnancy from unknown person/rape		7(2.3)	7(2.3)
Family food Source	Cultivation/production	36(11.7)	76(24.7)	112(36.4)
	Food aid	25(8.1)	51(16.6)	76(24.7)
	Purchasing	29(9.4)	64(20.8)	93(30.2)
	support from relative	13(4.2)	14(4.5)	27(8.8)
Meal frequency	<=2 times per day	84(27.3)	96(31.2)	180(58.4)
	>=3 times per day	19(6.2)	109(35.4)	128(41.6)

Association Between Sociodemographic Factors and Neural Tube Defects in Tigray: A Binary Logistic Regression Study

The findings highlight several significant associations suggesting that maternal characteristics play a critical role in the occurrence of neural tube defects (NTDs) (Table 10). Women aged ≤ 25 years were significantly more likely to have a child with NTDs, with an adjusted odds ratio (AOR) of 6.585 (95% CI: 1.799–24.101, p = 0.004), compared to older age groups. Similarly, women aged 26–35 years also exhibited an increased risk, with an AOR of 3.530 (95% CI: 1.123–11.091, p = 0.031), compared to those over 35 years. Maternal education level was strongly associated with NTD occurrence. Women with no formal education had significantly higher odds of having a child with NTDs (AOR = 20.846, 95% CI: 2.265–191.856, p = 0.007). Likewise, those with elementary school education (AOR = 14.365, 95% CI: 1.703–121.177, p = 0.014) and high school education (AOR = 13.801, 95% CI: 1.672– 113.911, p = 0.015) were also at significantly increased risk compared to women with a college diploma or higher. Additionally, a history of abortion or stillbirth was associated with an elevated risk of NTDs, with an AOR of 2.516 (95% CI: 0.990–6.397, p = 0.053). Although this association was borderline significant, it indicates that women with a history of adverse pregnancy outcomes might face a heightened risk of having children with NTDs.

Table 10: Association of sociodemographic and obstetric factors with the occurrence of Neural tube defects (NTDs) in Tigray

		95% C.I. for EXP(B)		
Characteristics		**COR**	**AOR**	**P value**
Sociodemographic and obstetric history				
Maternal age	<=25	1.973(0.810-4.803)	6.585 (1.799-24.101)	0.004
	26-35	1.873(0.769-4.566)	3.530 (1.123-11.091)	0.031
	>35			
Marital status	Not married	0.969(0.502-1.869)	1.917 (.713-5.151)	0.197
	Divorced	1(0.180-5.569)	1.32(.136-12.822)	0.811
	Married			
Residence	Rural	1.070(.665-1.722)	0.808(.390-1.673)	0.566
	Urban			
Education	No formal education	1.175(0.513-2.689)	20.846(2.265-191.856)	0.007
	Elementary school	0.972(0.456-2.073)	14.365(1.703-121.177)	0.014
	High school	0.909(0.421-1.962)	13.801(1.672-113.911)	0.015
	College diploma and above			
Parity	Prime	0.745(0.381-1.457)	0.677(.220-2.084)	0.497
	parity 1-3	0.897(0.479-1.680)	0.714(.293-1.742)	0.459
	parity >3			
History of Pregnancy	No prior pregnancy	0.963(0.570-1.628)		
outcome	Abortion/stillbirth	2.497(1.133-5.504)	2.516 (0.990-6.397)	0.053
	Full-term baby			

5.4 Discussion

Characteristics of pregnancy outcomes affected by neural tube defects (NTDs)

Our findings provide important insights into the characteristics of pregnancy outcomes affected by neural tube defects (NTDs), focusing on types of NTDs, the associated outcomes, and additional factors like sex, associated congenital malformations, and recurrence risks.

This study reveals that anencephaly is the most common type of neural tube defect (NTD), followed by spina bifida and encephalocele, respectively. A significant trend observed is the high rate of stillbirths among pregnancies affected by NTDs. The majority of pregnancies with anencephaly and spina bifida ended in stillbirths. This highlights the severe prognosis typically associated with these NTDs, particularly anencephaly, where the absence of a major portion of the brain and skull contributes to in utero death. These findings align with those of other studies, which similarly report a poor prognosis for pregnancies affected by NTDs, especially anencephaly, which is often fatal either in utero or shortly after birth [40, 238]. Spina bifida, although often survivable with surgical intervention, still has a high rate of fetal loss, especially in settings with limited access to prenatal care or surgical treatments [239].

In this study, the majority of cases had an unidentified sex, which is unusually higher than in most studies, where NTDs, particularly spina bifida, show a slight male predominance [240]. Research generally indicates that males are more commonly affected by NTDs, particularly spina bifida (22, 241, 242). The high proportion of unidentified sex in the present study may reflect limitations in the data collection process, particularly if many stillbirths occurred at early gestational stages where sex may not have been recorded or confirmed and lack of ANC reported in this study may also attribute for this. This also underscores the challenges of documenting sex in stillbirth cases, which are often less systematically recorded than live births.

This study highlights the incidence of associated congenital malformations, such as hydrocephalus, club foot, and cleft palate, which aligns with findings from other studies reporting that NTDs, particularly spina bifida, often occur alongside other anomalies (242, 243). For instance, a study by Blount JP et al. (244) found that hydrocephalus is frequently observed in spina bifida cases due to the obstruction of cerebrospinal fluid flow, which increases the risk of developmental delays and other neurological issues. However, the incidence of other malformations, such as club foot and omphalocele, is somewhat lower than expected when compared to other large cohort studies.

For example, club foot is commonly reported in association with spina bifida and other NTDs (245, 246). This discrepancy may be attributed to the relatively small sample size in the current study or differences in the diagnostic criteria used to identify co-occurring defects.

This study found that some cases had a family history of birth defects, including congenital heart defects and clubfoot, with a recurrence of NTDs such as spina bifida and anencephaly. These findings are consistent with established research suggesting an increased risk of recurrence for NTDs among individuals with a family history of birth defects [134]. Studies have shown that having a sibling with an NTD or a family history of congenital anomalies can increase the risk of recurrence in subsequent pregnancies (134, 227, 240]. Specifically, the recurrence risk of spina bifida in siblings can range from 2-5%, depending on the severity and nature of the family history (247, 248).

The recurrence of neural tube defects (NTDs) is a crucial topic for understanding the genetic and environmental factors that influence these conditions. Numerous studies have examined the familial patterns associated with NTDs, highlighting the importance of family history in assessing the risk of recurrence [227, 347, 249]. The presence of NTDs in a family can led to increased awareness and concern about the potential for recurrence in future offspring. Specific studies suggest that the recurrence rate of NTDs can be significantly higher in families with a history of these defects [227, 247, 249]. The genetics of NTDs plays a key role in their development [227, 247, 249]. These defects are believed to result from a combination of genetic and environmental factors [134, 227, 247]. Genetic studies indicate that certain hereditary factors may predispose individuals to NTDs, suggesting that families with a history of these defects may share common genetic susceptibilities [250]. These hereditary factors can vary, and identifying specific genetic markers could help predict the recurrence risk for affected families. This emphasizes the importance of genetic counseling and monitoring for women with a history of NTDs, particularly in settings with limited access to healthcare. However, the small number of affected families in this study limits the strength of the conclusions that can be drawn regarding genetic predispositions. Despite this, the findings reinforce the need for genetic counseling and careful monitoring for women with a history of NTDs, especially in environments with severely restricted healthcare access.

Key determinants and risk factors

Sociodemographic and obstetric disparities

This study highlights significant associations between maternal age, educational level, and prior pregnancy outcomes with the occurrence of NTDs.

The analysis reveals a significant association between maternal age and the occurrence of NTDs. Maternal age was identified as a key risk factor, with women aged ≤25 years and 26-35 years showing a notably higher likelihood of having children with these defects. This finding is consistent with previous studies, which have consistently linked younger maternal age to an increased risk of NTDs, often due to inadequate prenatal care, nutritional deficiencies, and limited access to health services during pregnancy [236, 251]. The increased risk can be attributed to various socio-economic factors, including limited access to prenatal care and a higher prevalence of adverse pregnancy outcomes, such as low birth weight and preterm delivery, among young mothers [252]. In conflict zones, these challenges are further exacerbated by limited resources and ongoing instability, which hinder young mothers from accessing essential healthcare services.

This study also found a strong association between lower maternal education and an increased risk of NTDs. Multiple studies have consistently shown that mothers with lower maternal education attainment are more likely to have children with NTDs [7, 253, 254,255]. For example, women with less than a high school education face a 1.7-fold higher risk of delivering infants with NTDs compared to those with at least a high school diploma [7, 253-255]. This elevated risk persists even when accounting for other socioeconomic factors [7, 253- 255].

Mothers from lower socioeconomic status (SES) often encounter barriers such as limited healthcare access, inadequate nutrition, and lower health literacy, all of which contribute to poor pregnancy outcomes. Education plays a crucial role in promoting awareness of health-related behaviors, including preconception care and folic acid supplementation, which are essential for preventing NTDs [102, 235, 253]. These findings underscore the importance of targeted health education initiatives to improve maternal health and encourage folic acid use, particularly among women with lower levels of education.

A history of abortion or stillbirth showed a borderline association with the occurrence of NTDs (AOR=2.516, 95% CI: 0.990-6.397, p=0.053). While not statistically significant, this finding suggests that women with previous adverse pregnancy outcomes may have an increased risk of

NTD-affected pregnancies. Research indicates that such outcomes can be predictive of future birth defects [8, 227, 256]. Family history is a crucial factor, as maternal genetics and a prior occurrence of birth defects, such as spina bifida, can elevate the risk of NTD recurrence in future pregnancies [8, 201, 227]. The association identified in this study underscores the need for further research with a larger sample size to better assess its strength and significance. Moreover, women with a history of pregnancy complications may have underlying health conditions or environmental exposures that could contribute to the recurrence of birth defects, highlighting the importance of targeted prenatal care and risk assessment.

A striking finding in this study is that neither woman in the NTD group nor those in the control group reported consuming peri-conceptional folic acid supplements, despite its well-established role in preventing NTDs [201, 229, 232]. This lack of supplementation in Tigray may stem from limited access to preconception care within the healthcare system. The war and siege have further worsened these challenges [102, 106, 185, 204, 237], emphasizing the urgent need to improve access to folic acid supplements and strengthen maternal health education to help reduce the prevalence of NTDs in the region.

Conflict-Related Factors

This study highlights the severe impact of war on maternal health in Tigray, where many women with pregnancies affected by neural tube defects faced significant barriers such as exposure to violence, unintended pregnancies, limited access to health services, inadequate family planning, and food insecurity. These factors emphasize the public health challenges in conflict zones, where the collapse of healthcare services worsens pregnancy outcomes, increasing vulnerability to neural tube defects and other birth defects [257]. Armed conflicts often destroy healthcare facilities and displace medical personnel, significantly limiting women's access to maternal health services [102, 106, 185, 204]. This disruption increases the risk of adverse pregnancy outcomes, including neural tube defects and maternal mortality, as essential care becomes inaccessible (237, 258). These gaps in maternal healthcare contribute to congenital malformations, such as neural tube defects. Mlambo C et al [257]. also highlight that living in conflict zones increases women's vulnerability to sexual violence, poor reproductive health, unintended pregnancies, and negative maternal outcomes.

Armed conflict disrupts vital infrastructure such as food, water, and sanitation systems, further limiting healthcare access and worsening maternal health risks. Our study found that 50.6% of participants faced attacks on civilians, 28.3% experienced acts of war, and 21.1% suffered property damage, underscoring the severe impact of the war in Tigray on maternal well-being [102, 106, 185, 204, 237]. Research shows that violence, displacement, and trauma in conflict zones significantly increase the risk of adverse maternal outcomes, including birth defects [257, 259, 260]. The high rates of violence in this study indicate that conflict impedes access to prenatal care and safe delivery services, crucial for reducing the risk of neural tube defects. Additionally, psychosocial stress from such instability is linked to poor pregnancy outcomes like preterm births and low birth weights [261, 262,263].

Only 8.1% of women in Tigray, both with and without NTD-affected pregnancies, reported accessing health services, while 91.9% were unable to seek care due to the war and siege. This highlights the collapse of the region's healthcare system, with attacks on medical facilities, shortages of healthcare professionals, and resource depletion severely limiting access to essential prenatal care [102, 106, 185, 204, 237]. Prenatal care, especially folic acid supplementation, is crucial for preventing NTDs [201, 229, 232]. The lack of these services emphasizes the urgent need for humanitarian intervention and healthcare reconstruction to improve maternal health and reduce the high prevalence of NTDs and other birth defects in the region.

Limited healthcare access during armed conflict is a well-documented issue, with many women unable to receive basic maternal care due to the destruction of medical infrastructure, displacement of healthcare workers, and insecurity [257, 264, 265]. This study's findings align with other conflict settings, where disruptions in healthcare contribute to adverse pregnancy outcomes, increased birth defects, and higher maternal and infant mortality [266, 267. This study also shows many women with NTD-affected pregnancies reported unintended pregnancies, often due to limited family planning services and lack of family support, which is linked to delayed prenatal care, higher stress, poor nutrition, and increased NTD risks [257, 262, 263, 265]. Additionally, many women faced gender-based violence, such as rape, which further worsened their health and the risk of adverse pregnancy outcomes [257, 262, 263, 265].

Food insecurity emerged as a major challenge in this study, with many women depending on food aid or subsistence farming as their primary sources of nutrition. A significant proportion consumed

fewer than two meals per day, highlighting severe nutritional deficiencies a critical risk factor for NTDs. Research has consistently shown that inadequate maternal nutrition, particularly deficiencies in folic acid and other essential nutrients, plays a key role in the development of neural tube defects [201, 229, 232]. The combination of food scarcity and limited access to nutritional supplements, such as folic acid, may contribute to the heightened risk of NTDs in this population. This finding aligns with research by Carmichael et al [268], which associates food insecurity with an increased risk of congenital anomalies, including cleft palate, tetralogy of Fallot, spina bifida, and anencephaly.

5.5 Conclusion

This study highlights the impact of neural tube defects (NTDs) on pregnancy outcomes, with anencephaly as the most common type, leading to a high rate of stillbirths. The high prevalence of unidentified sex and fewer associated congenital anomalies reflect diagnostic challenges and the complexity of NTDs. Limited prenatal care worsens prognosis, while maternal age and education were key risk factors. The war in Tigray has further intensified maternal health risks, with violence, healthcare disruptions, unintended pregnancies, and food insecurity collectively increasing NTD prevalence. These findings underscore the urgent need for improved maternal healthcare, particularly in conflict-affected regions. This study highlights the urgent need to strengthen maternal healthcare and public health infrastructure in Tigray. Targeted prevention strategies are essential to address key NTD risk factors. However, the small sample size and data limitations, including unidentified sex, underscore the need for further research on genetic and environmental determinants.

5.6 Recommendations

Enhance Prenatal Care Access: Prioritize the restoration and expansion of prenatal healthcare services in war-affected areas, particularly in Tigray, to prevent NTDs and improve pregnancy outcomes for women with limited healthcare access.

Expand Folic Acid Supplementation: Strengthen folic acid distribution programs in conflict-affected regions and launch public health campaigns emphasizing its critical role in preventing NTDs, especially in areas facing food insecurity.

Strengthen Maternal Health Education: Develop targeted health education initiatives to improve maternal health literacy, focusing on NTD risks, prenatal care, and the importance of proper nutrition, including folic acid intake.

Support Women in Conflict Zones: Provide access to family planning, reproductive health services, and psychosocial support for women affected by war. Addressing gender-based violence and unintended pregnancies is vital for improving maternal health.

Implement Genetic Counseling & Family Planning: Offer genetic counseling to families with a history of congenital anomalies to inform reproductive decisions. Expanding family planning services can help reduce unintended pregnancies and promote maternal health planning.

Conduct Further Research: Larger studies are needed to validate findings on environmental pollution or genetic factors, detailed study on the long-term outcomes of surviving infants with NTDs, NTDs recurrence, sex distribution, and associated anomalies. Future research should also examine the specific effects of conflict on maternal health and NTD outcomes.

5.7 Limitation of the study

The study provides important insights into the risk factors associated with neural tube defects (NTDs) in Tigray, Ethiopia, a region affected by devastating war and siege. However, several limitations must be considered when interpreting the findings and directing future research. First, as a cross-sectional study, it cannot establish causal relationships. Longitudinal research would provide a better understanding of the temporal effects of war on maternal and fetal health. Second, the sample was limited to 11 hospitals, which may not accurately represent the broader population, especially women from remote areas or those with severe complications. Third, the war and siege may have compromised data accuracy, and recall bias could have affected self-reported risk factors. The small sample size (308 participants) limits statistical power, particularly for rare risk factors. Additionally, unmeasured confounders, such as environmental pollution or genetic factors, were not explored and could have influenced the relationship between the studied variables and NTDs. The study also lacked detailed data on the long-term outcomes of surviving infants with NTDs, which limits understanding of the broader impacts on affected families. Finally, while the findings are valuable for the Tigray context, their applicability to other war-affected regions in Ethiopia or sub-Saharan Africa may be limited due to differences in healthcare access, and the nature of war and siege. Further studies in similar settings are needed to confirm and expand upon these results.

Chapter six

Near-universal folate deficiency in first trimester pregnant women from Tigray, Ethiopia: implications for neural tube defect prevention. BMC Pregnancy Childbirth (Under review).

Abstract

Background: Folate deficiency in early pregnancy is a major, preventable cause of neural tube defects (NTDs). Despite Tigray's high NTDs prevalence, data on red blood cell (RBC) folate, the gold-standard marker of long-term folate status, are limited. This study assessed the prevalence and determinants of folate deficiency among first-trimester pregnant women in Tigray.

Methods: A cross-sectional study was conducted in March–April 2025 in five public hospitals in Tigray, involving 400 first-trimester pregnant women selected through systematic sampling. Data included interviews and RBC folate levels measured using the Elecsys® assay. Data analysis using SPSS v27 included descriptive statistics and normality testing, revealing a non-normal distribution of RBC folate levels. Due to the substantial class imbalance (395 folate-deficient vs. 5 folate-sufficient cases), a penalized logistic regression model with L2 regularization (Ridge) was used to identify factors associated with folate sufficiency, with model performance assessed on both original and balanced datasets.

Results: Among 400 first-trimester pregnant women, RBC folate testing, an indicator of long-term folate status revealed a strikingly high prevalence of deficiency. An alarming 98.8% had levels below the WHO-recommended threshold of 400 ng/mL for NTD prevention. The median RBC folate concentration was 204.9 ng/mL (range: 120.0–439.3), with a mean of 221.6 ± 68.6 ng/mL. Penalized logistic regression (Ridge) identified low dietary diversity ($\beta = +1.39$), medium dietary diversity ($\beta = +0.98$), and older maternal age ($\beta = +0.85$) as key risk factors. Notably, 84.5% of participants reported low dietary diversity, highlighting a critical gap in maternal nutrition.

Conclusions: This study presents one of the most extreme cases of folate deficiency ever documented among pregnant populations globally, signaling a hidden but potentially devastating nutritional emergency in the region. The low median folate concentration and strong association with poor dietary diversity underline a severe nutritional gap in this high-risk population, amounting to a folate emergency demanding immediate public health action. Supplementation of all women of reproductive age capable of pregnancy before conceiving (pre-conception care), early registration of pregnant women into the antenatal care follow-up, and social and behavior change initiatives to improve dietary diversity are recommended.

Keywords: Folate deficiency, neural tube defects, pregnant women, Red blood cell folate, Tigray, Ethiopia

6.1 Background

Folate, a B-vitamin essential for DNA synthesis, repair, and methylation, plays a critical role in cellular division and growth, particularly during periods of rapid fetal development in early pregnancy [62, 247, 269]. Inadequate maternal folate levels during the periconceptional period have been strongly associated with neural tube defects (NTDs) a group of severe congenital malformations of the brain and spinal cord, including spina bifida and anencephaly [247, 270]. NTDs are among the most common congenital anomalies worldwide and contribute substantially to neonatal mortality, accounting for approximately 300,000 neonatal deaths annually [38, 69].

Red blood cell (RBC) folate is considered the most reliable biomarker for assessing long-term folate status and correlates strongly with NTD risk [62, 269, 270]. Unlike serum folate, which reflects short-term status, RBC folate indicates longer-term folate stores. The World Health Organization (WHO) recommends RBC folate concentrations ≥400 ng/mL to minimize the risk of NTDs [77, 271]. Despite global efforts to promote folic acid supplementation and food fortification, folate deficiency remains a public health challenge, especially in low-resource settings [272- 274].

In many sub-Saharan African countries, particularly in rural and underserved regions, folate deficiency is widespread due to poor dietary diversity, limited access to fortified foods, and low awareness of maternal nutrition [275-278]. Persistent structural and systemic barriers continue to undermine the effectiveness of global folate supplementation efforts, leaving many women of reproductive age in Africa at elevated risk of folate deficiency and consequently, of giving birth to infants with NTDs. Ethiopia has one of the highest reported NTD burdens globally estimated at 71.48 per 10,000 births—ten times higher than the 2030 Sustainable Development Goal (SDG) target [8]. Among all the regions in Ethiopia, Tigray presents the most alarming figures, with NTD prevalence ranging from 130 to 262 per 10,000 births [10, 11, 237]. This high burden indicates a critical gap in maternal nutrition, particularly concerning folate status, during the early stages of pregnancy.

Maternal folate status during the first 28 days post-conception is especially vital, as this is the window when neural tube closure occurs. However, there is a paucity of data on RBC folate levels among pregnant women in high-risk areas such as Tigray, Ethiopia. Therefore, this study aimed to address this gap by evaluating RBC folate concentrations among first-trimester pregnant women

in the Tigray region of Ethiopia. It also assessed their nutritional and demographic profiles. The findings are expected to inform evidence-based public health policies and interventions aimed at preventing NTDs through high impact maternal nutrition interventions.

6.2 Methodology

Study setting and period

The study was conducted in Tigray, identified as a high-risk setting for NTDs, located in northern Ethiopia. Moreover, Tigray is one of the twelve administrative regions and two administrative cities in Ethiopia (Figure 1). Administratively, Tigray is divided into seven zones, namely Central, Eastern, Mekelle, North Western, South Eastern, Southern, and Western Zones. The region is further divided into 94 districts (locally called woredas). In the Tigray region, there are approximately 992,635 households, with an average household size of 3.4 persons in urban areas and 4.6 in rural areas [279]. Socioeconomic indicators reveal that 31.6% of the population falls within the lowest wealth quintile. Adult literacy rates show a significant gender gap, with 67.5% of men and only 33.7% of women being literate [280].

According to the 2016 Ethiopian Demographic and Health Survey (EDHS), antenatal care (ANC) coverage for at least one visit (ANC1) was 90%, while coverage for four or more visits (ANC4+) stood at 57%. Additionally, 56.9% of births in the region were reported to have occurred in health facilities, reflecting a moderate level of institutional delivery service utilization [281]. Apart from the private health facilities, the health system in Tigray includes 741 village health posts, 230 health centers, 24 primary hospitals, 14 general hospitals, and two referral (tertiary) hospitals. Data were collected from one tertiary hospital, one general hospital, and three primary hospitals (Figure 1). These were Ayder Comprehensive Specialized Hospital (tertiary level), Lemlem Karl General Hospital, Adishu Primary Hospital, Samre Primary Hospital, and Yechila Primary Hospital from March to April 2025. These hospitals provide 24-hour obstetrics and gynecology care and different type of health care services for their representative population.

Figure 13: Administrative map of Ethiopia, Tigray and the location of the hospitals included in the study

Study design

Cross-sectional laboratory-based study was conducted in selected health institutions across Tigray, northern Ethiopia.

Sample size

The required sample size was determined using a single-population proportion formula, assuming a 49% prevalence of folate deficiency among pregnant women in Haramaya District, Eastern Ethiopia [30]. With a 95% confidence level and a 5% margin of error, the initial calculated sample

size was 384. To account for a potential 10% non-response rate, the final required sample size was increased to 422 participants.

Sampling technique

The study hospitals (one tertiary, one general, and three primary hospitals) were randomly selected from pool of government hospitals. The sampling frame consisted of all first-trimester pregnant women attending ANC visits at the five hospitals during the study period (March–April 2025). Based on ANC registration logs, the total number of eligible women across the five hospitals was 1,240: Ayder Comprehensive Specialized Hospital (n = 155), Lemlem Karl General Hospital (n = 465), Adishu Primary Hospital (n = 155), Samre Primary Hospital (n = 310), and Yechila Primary Hospital (n = 155).

The final required sample size (n=422) was proportionally allocated to each hospital according to its share of eligible women using the largest-remainder method: Ayder n=53, Lemlem Karl n=158, Adishu n=53, Samre n=105, and Yechila n=53 (summing to 422). Within each hospital, participants were selected using systematic random sampling. The sampling interval (k) was calculated separately for each hospital by dividing the total eligible women by the allocated sample (e.g., for Ayder: $155/53 \approx 3$). A random starting point between 1 and 3 was selected by lottery at each hospital, after which every 3rd eligible woman listed on the ANC register was invited until that hospital's allocation was attained. If the end of a clinic day's register was reached before meeting the allocation, recruitment continued on subsequent clinic days using the same interval and procedure.

Eligibility was verified from ANC logs at the point of approach; duplicate entries (e.g., repeat visits) were removed using unique ANC identifiers to avoid resampling the same individual. Non-responders were not replaced; reasons for non-participation were recorded. In total, 422 women were approached; 400 consented and completed the study (response rate: 94.8%), with non-participation due to refusal (n=22).

Study population

The study population included pregnant women in their first trimester (≤13 weeks gestation as confirmed by ultrasound and last menstrual period) during their antenatal care follow up.

Eligibility criteria

Inclusion criteria: Women with confirmed first-trimester pregnancy and permanent residents (more than six months) in the study area were included in the study.

Exclusion criteria

Women were excluded if they had Hematological disorders or chronic diseases (e.g., HIV/AIDS, diabetes), current or recent malaria infection or use of antimalarial drugs, history of folic acid supplementation before conception, alcohol or substance abuse, as it impairs folate metabolism, twin pregnancies alter folic acid metabolism differently than singleton pregnancies, MTHFR gene mutations (if known or reported by the mother).

Study variables

Dependent variable: RBC folate concentration (ng/mL)

Independent variables: socio-demographic and economic status, obstetric history, health-related behaviors, and nutritional status.

Data collection procedures

Sociodemographic

Trained data collectors used a structured and pretested questionnaire to collect information on sociodemographic characteristics such as age, marital status, residence, education, occupation, perceived economic status; Obstetric history such as parity, pregnancy planning, previous outcomes; health-related behaviors such as health care service utilization, and family support.

Dietary Diversity and Food Frequency

Dietary data were collected through a 24 hours food group recall and a standardized food frequency questionnaire (FFQ) administered during face-to-face interviews at the first ANC visit. 24 hours recall period food frequency questions were grouped into the FAO 10 food-group minimum dietary diversity for women (MDD-W) tool; scores were categorized as Low DDS (≤5 food groups), Medium DDS (5-7 food groups), or High DDS (8-10 food groups) based on FAO guidelines [200]. As an anthropometric measurementwas were madefollowing standard techniques, the measurement of arm circumference (to the nearest 1.0 cm) was made on the mid-upper left arm using a non-stretch tape with the pregnant women in the comfort sitting position. MUAC

measurement wascategorized according to WHO classifications, women with MUAC: <23 cm was categorized as malnourished and with MUAC: ≥23 cm as normal.

Laboratory investigation

Sample collection and handling

Approximately 4 mL of venous blood was collected aseptically into EDTA vacutainer tubes from each participant during their antenatal care visit. The samples were labeled and immediately stored at 2–8°C in portable cold boxes at the collection sites in Tigray, Ethiopia.

Sample transportation and storage

The collected samples were stored at –20°C in deep freezer at each collection sites until transported to EPHI for analysis. Blood samples were transported in cold-chain conditions (2–8°C) from the health facilities in Tigray to the Ethiopian Public Health Institute (EPHI) in Addis Ababa. Upon arrival at EPHI, all samples were stored at –20°C until analysis. Samples were processed within three weeks from the date of collection, in accordance with manufacturer and WHO recommendations for RBC folate stability.

Preparation of the hemolysate sample

RBC folate was measured using the Elecsys® Folate RBC assay (Test No. 1210) on the Cobas e 411 analyzer (Roche Diagnostics GmbH, Mannheim, Germany), based on electrochemiluminescence immunoassay (ECLIA) technology. Sample preparation followed Roche's standard protocol as described below. 100 µL of whole blood was mixed with 3.0 mL of Folate RBC hemolyzing reagent (0.2% ascorbic acid solution). The hemolysate was incubated at 20–25°C for 90 ± 15 minutes with caps closed. The samples were stored at –20°C (±5°C) and analyzed within one month allowing only one freeze-thaw cycle.

Laboratory analysis of RBC folate

Red blood cell (RBC) folate concentrations were measured using the Elecsys® Folate RBC assay (Test No. 1210) on the Cobas e 411 analyzer (Roche Diagnostics GmbH, Mannheim, Germany), a fully automated platform based on electrochemiluminescence immunoassay (ECLIA) technology.

Assay Principle

The Cobas e 411 system uses a competitive binding principle based on electrochemiluminescence immunoassay (ECLIA). In the first step, the folate in the sample competes with a ruthenium-

labeled folate derivative for binding sites on the folate-binding protein (FBP) immobilized on the solid phase (streptavidin-coated microparticles). After washing to remove unbound substances, a voltage is applied to the reaction mixture, triggering a light-emitting reaction. The chemiluminescent signal produced by the bound ruthenium complex is inversely proportional to the amount of folate in the sample.

Calibration and quality control

The Cobas e 411 analyzer was calibrated using Elecsys Folate RBC CalSet according to the manufacturer's instructions. Two levels of quality control (QC) materials Folate RBC Control 1 and 2 were run with each batch of patient samples to ensure accuracy and precision. Assay performance was monitored by evaluating QC results against expected target ranges.

Calculation of RBC folate concentration

RBC folate concentration was calculated using the following formula.

$$\text{RBC Folate (ng/mL)} = \frac{\text{Total Folate in Hemolysate (ng/mL)} \times \text{Dilution Factor}}{\text{Hematocrit}}$$

Hematocrit values were obtained from complete blood count (CBC) analysis conducted in parallel, and the final results were expressed in ng/mL. Folate status was determined based on the WHO-recommended cutoff value: < 400 ng/mL: folate deficiency (associated with increased NTD risk) or ≥ 400 ng/mL: folate sufficiency.

Biosafety

All laboratory activities were conducted at the Ethiopian Public Health Institute under strict biosafety protocols in accordance with standard operating procedures (SOPs) and Good Laboratory Practice (GLP) guidelines. Biosafety procedures followed the EPHI laboratory biosafety manual. All personnel were trained in sample handling, and equipment maintenance was performed regularly.

Statistical Analysis

Data were analyzed using SPSS version 27. Descriptive statistics summarized participant characteristics. The Shapiro-Wilk test assessed normality of red blood cell (RBC) folate levels, revealing a non-normal distribution. Due to the substantial class imbalance (395 folate-deficient vs. 5 folate-sufficient cases), a penalized logistic regression model with L2 regularization (Ridge) was employed. Model performance was assessed using both the original dataset and a resampled

dataset with balanced folate status categories, with the goal of identifying potential risk factors associated with folate sufficiency. Standardized penalized regression coefficients (β) were used to assess the strength and direction of associations. Based on established interpretive guidance, variables with $|\beta| \geq 0.10$ were considered to have meaningful effects, with positive values indicating risk factors and negative values indicating protective effects [282]. Visualizations including tables, histograms, and bar graphs were used to support data interpretation.

Ethical Considerations

Ethical approval was obtained from the Institutional Review Board (IRB) of the College of Health Sciences, Mekelle University (MU-IRB 2411/2024). Orally informed written consent was obtained from all participants before enrollment. Confidentiality and anonymity were maintained throughout the study.

6.3 Results

Sociodemographic characteristics

Table 11 summarizes the sociodemographic and obstetric characteristics of the participants. The mean maternal age was 24.78 years (SD ± 5.04), with a median of 24.0 years (range: 15–38). More than half of the participants (55.0%) resided in urban areas, while the remainder lived in rural settings. A substantial majority (71.0%) perceived themselves as economically poor, highlighting socioeconomic barriers that may impact maternal nutrition and access to healthcare services.

The occupational profile showed that 74.8% of participants were housewives, reflecting a high level of economic dependence. Educational attainment was relatively low. Only 12.0% had achieved a college diploma or higher. Most participants had completed high school (44.5%) or elementary school (34.3%), and 9.3% reported having no formal education. These educational patterns suggest potential limitations in health literacy, which could influence dietary habits and healthcare-seeking behaviors.

Table 11: Sociodemographic characteristics of first trimester pregnant women in a neural tube defect high-risk area of Tigray, Ethiopia.

Characteristics		Frequency	Percent
Materna age	15-19	57	14.2
	20-24	153	38.3
	25-29	105	26.3
	30-34	63	15.8
	>=35	22	5.5
Marital status	Married	373	93.3
	unmarried	22	5.5
	Divorced	5	1.3
Residence	Rural	180	45.0
	Urban	220	55.0
Educational status	No formal education	37	9.3
	Elementary school	137	34.3
	Highschool	178	44.5
	College diploma and above	48	12.0
Occupation	House wife	299	74.8
	Labor	14	3.5
	Student	22	5.5
	Merchant	48	12.0
	College educated professional	17	4.3
Assumed economic status	Poor	284	71.0
	Medium	105	26.3
	Rich	11	2.8

Maternal age: mean 24.78 ± 5.04 years; median 24.0 years (range 15–38). Standard Deviation (SD): 5.04 years.

Obstetric characteristics

Healthcare engagement was suboptimal. About 51.0% of women reported rare or limited use of healthcare services, and only 28.5% attended regular antenatal check-ups. Similarly, only 28.5% reported receiving family support for healthcare utilization, indicating a lack of strong social support systems, which may adversely affect both maternal and fetal outcomes. In terms of obstetric history, 52.5% of participants were multiparous, while 47.5% were nulliparous. Among those with previous pregnancies, 50.0% had experienced full-term live births, 1.8% had a history of stillbirth, miscarriage, or abortion, and 0.8% had preterm births. Notably, 86.8% of current pregnancies were unplanned, indicating considerable gaps in reproductive health education and access to family planning services (Table 12)

Table 12: Obstetric characteristics of first-trimester pregnant women in a neural tube defect high-risk area of Tigray, Ethiopia.

Characteristics		Frequency	Percent
Health care service utilization	Occasional (Visits as needed)	82	20.5
	Rare (Limited visits)	204	51.0
	Regular (Frequent check-ups)	114	28.5
Family support for health care use	No	286	71.5
	Yes	114	28.5
Parity	Multipara (Has given birth before)	210	52.5
	Nullipara (First-time mother)	190	47.5
Use planned pregnancy	No	347	86.8
	Yes	53	13.3
Pregnancy outcome before the current pregnancy	Preterm live birth	3	.8
	Stillbirth/Miscarriage/Abortion	7	1.8
	No previous pregnancy	190	47.5
	Full-term live birth	200	50.0
Sex or previous pregnancy outcome	unknown	4	1.0
	Male	105	26.3
	Female	101	25.3
	No previous pregnancy	190	47.5

Clinical and nutritional characteristics

At the time of data collection, the majority of participants (60.3%) were between the 9th and 12th weeks of gestation, 31.8% were between the 5th and 8th weeks, and 8.0% were in the first four weeks. This distribution reflects the study's focus on assessing folate levels during the critical period of neural tube formation (Table 13). Nutritional status assessments revealed that over half of the participants (56.0%) had a MUAC below 23 cm, while 44.0% had a MUAC of 23 cm or higher (Table 14).

Table 13: Clinical and nutritional characteristics of first trimester pregnant women in a neural tube defect high-risk area of Tigray, Ethiopia.

Characteristics		Frequency	Percent
Gestational age	1-4 weeks	32	8.0
	5-8 weeks	127	31.8
	9-12 weeks	241	60.3
MUAC	Low MUAC (<23cm)	224	56.0
	Normal MUAC (≥23cm)	176	44.0

MUAC: mean 21.75 ± 1.86 cm; median 22.0 cm (range 17.0–26.0). Standard Deviation (SD): 1.86 cm.

Dietary patterns of the study participants

The analysis of food group consumption among participants reveals significant insights into dietary diversity within the study population. A striking 99.25% of respondents reported consuming grains, white roots and tubers, and plantains, indicating that these staples form the foundation of the typical diet. Pulses—such as beans, peas, and lentils—were consumed by 66.75% of participants, suggesting a moderate inclusion of plant-based protein sources. Consumption of nutrient-dense food groups such as nuts and seeds (26.25%), dark green leafy vegetables (39.25%), and other vitamin A-rich fruits and vegetables (27.00%) was comparatively lower, pointing to potential gaps in micronutrient intake. Alarmingly, the intake of animal-source foods was particularly limited: only 9.75% consumed dairy, 12.50% consumed meat, poultry, or fish, and 12.00% consumed eggs. (Table 14).

Table 14: frequency and percentage of food group among the participant mothers consumed in 24 hours dietary recalls period.

Food Group	Count	% of Respondents
Grains, white roots and tubers, and plantains	397	99.25%
Pulses (beans, peas and lentils)	267	66.75%
Nuts and seeds	105	26.25%
Dairy	39	9.75%
Meat, poultry and fish	50	12.50%
Eggs	48	12.00%
Dark green leafy vegetables	157	39.25%
Other vitamin A-rich fruits and vegetables	108	27.00%
Other vegetables	67	16.75%
Other fruits	67	16.75%

The distribution of Dietary Diversity Scores (DDS), based on the FAO's 10-food group classification, reveals a concerning trend in the dietary patterns of the study population. The majority of participants (84.5%) fell within the low DDS category (consuming fewer than five food groups in the previous 24 hours), indicating a significant lack of dietary variety. Only a small proportion of respondents (3.25%) achieved a medium DDS (consumption of 5–7 food groups), while just 12.25% attained a high DDS (8–10 food groups) (figure 14). These figures reflect a limited inclusion of nutritionally important food groups such as fruits, vegetables, dairy, and animal-source proteins, corroborating the low intake patterns observed in the food group frequency analysis.

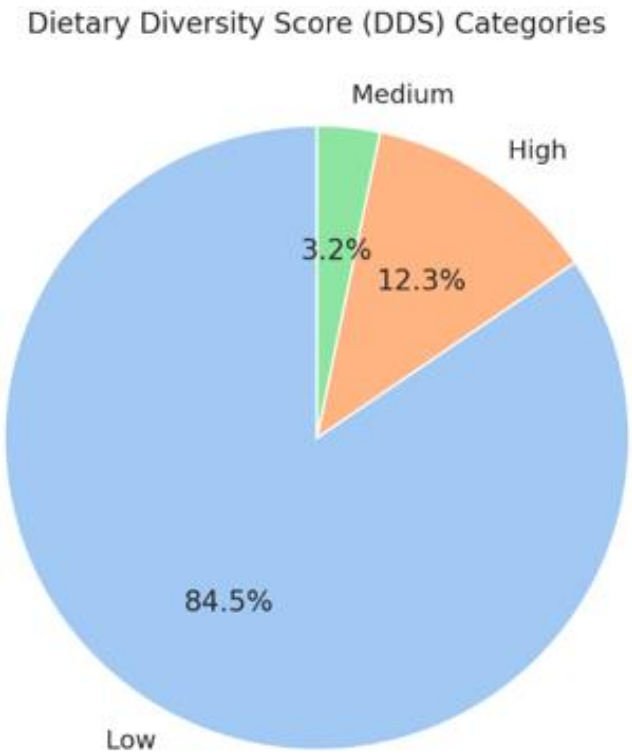

Figure 14: Minimum dietary diversity for women among first trimester pregnant women from Tigray, Ethiopia.

RBC folate status among first-trimester pregnant women

The findings indicate a critically low red blood cell (RBC) folate status among first-trimester pregnant women in the study area. The mean RBC folate concentration was 221.6 ± 68.6 ng/mL, with a median value of 204.9 ng/mL (range: 120.0–439.3 ng/mL). Of the 400 women assessed, 395 (98.75%) had RBC folate levels below the World Health Organization's recommended threshold of 400 ng/mL for the prevention of neural tube defects (NTDs). Only 5 participants (1.25%) had folate levels considered sufficient (Table 15).

Table 15: RBC Folate status of first Trimester pregnant women in a neural tube defect high-risk area of Tigray, Ethiopia (n = 400).

Folate Status	RBC folate level (ng/mL)	Number of women	Percentage (%)
Deficient	< 400	395	98.75
Sufficient	≥ 400	5	1.25

The mean RBC folate concentration was 221.6 ± 68.6 ng/mL, with a median of 204.9 ng/mL (range: 120.0 – 439.3 ng/mL). Standard Deviation (SD): 68.64 ng/mL. Shapiro-Wilk test indicated that RBC folate levels were not normally distributed (W = 0.929, p < 0.0001).

Gaussian distribution of the RBC folate levels

The Gaussian distribution **of** RBC folate concentrations shows a widespread and consistent folate insufficiency in the study population, pointing to systemic nutritional deficits rather than isolated deficiencies. A Shapiro-Wilk test confirmed that RBC folate concentrations were not normally distributed (W = 0.929, p < 0.0001), with a skewed distribution indicating a strong clustering of values in the lower range (Figure 15).

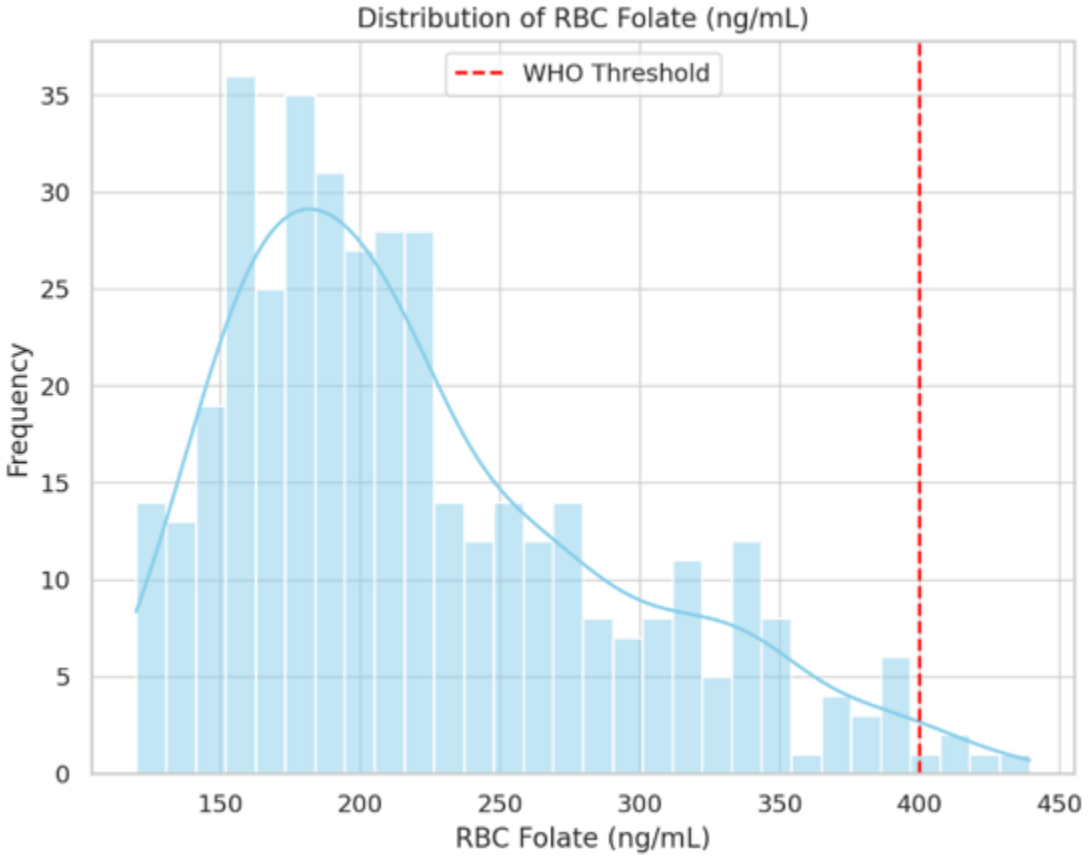

Figure 15: RBC folate distribution among pregnant women in their first trimester, Tigray, Ethiopia.

Factors associated with RBC folate levels among first trimester pregnant women

In a sample of 400 women, 98.8% (n=395) were classified as folate deficient using the WHO threshold of 400 ng/mL for red blood cell folate. Due to the pronounced class imbalance, a penalized logistic regression with L2 regularization (Ridge) was applied, and model performance was evaluated both in the original sample and after resampling to balance folate status categories.

Adjusting for class imbalance in the dataset using upsampling significantly influenced the estimated associations between key predictors and RBC folate deficiency status. In the balanced model, low DDS**,** medium DDS**,** and urban residence, older age were the strongest risk factors for RBC folate deficiency (Table 16, figure 17). The most notable changes were observed for dietary diversity, mid-upper arm circumference (MUAC), and age group. In the original unbalanced model, low dietary diversity score (DDS) was moderately associated with increased odds of RBC folate deficiency ($\beta = +0.47$), while this relationship became substantially stronger in the balanced model ($\beta = +1.39$). Similarly, individuals with medium DDS also saw an increase in the estimated risk from $\beta = +0.36$ to $\beta = +0.98$, highlighting a graded association between dietary diversity and RBC folate status. These findings suggest that the original model likely underestimated the contribution of poor dietary diversity to folate deficiency due to the overwhelming majority of participants being deficient. MUAC-based nutritional status also demonstrated a more pronounced effect in the balanced model. While being undernourished has shown a negative association in the original model ($\beta = -0.46$), and Stronger negative association after balancing ($\beta = -1.33$). Age-related effects also became more apparent in the resampled model. The older age group, which showed only a weak association with deficiency in the original model ($\beta = +0.07$), exhibited a stronger positive relationship in the balanced model ($\beta = +0.85$), suggesting that age is a relevant risk factor that was previously masked by the skewed distribution of RBC folate status. The middle-aged group's association shifted toward neutrality, moving from a slightly negative coefficient ($\beta = -0.16$) to nearly zero ($\beta = +0.03$), indicating minimal influence on RBC folate status. Overall, these findings underscore the importance of addressing class imbalance when modeling rare outcomes such as RBC folate sufficiency. By balancing the data, key predictors such as DDS, MUAC, and age group showed stronger and more interpretable associations with RBC folate status, offering clearer insights for targeting public health interventions.

Table 16: A penalized logistic regression with L2 regularization (Ridge) showing Factors associated with RBC folate levels among first trimester pregnant women

Feature	**Original** Coefficient	**Balanced** Coefficient	**Change**	**Interpretation**
DDS Category: Low	+0.47	**+1.39**	+0.92	Stronger positive association with deficiency after balancing.
MUAC: Under-nourished	−0.46	**−1.33**	−0.87	Stronger negative association after balancing
Age Group: Older	+0.07	**+0.85**	+0.79	Much stronger association with deficiency among older adults.
DDS Category: Medium	+0.36	**+0.98**	+0.62	Stronger association with deficiency even in medium DDS.
Age Group: Middle	−0.16	**+0.03**	+0.19	Association weakened toward neutrality.
Residence: Urban	+0.53	**+0.55**	+0.02	Minimal change – remains a strong risk factor.

Positive coefficients → associated with higher odds of deficiency**; Negative coefficients** → associated with lower odds of deficiency.

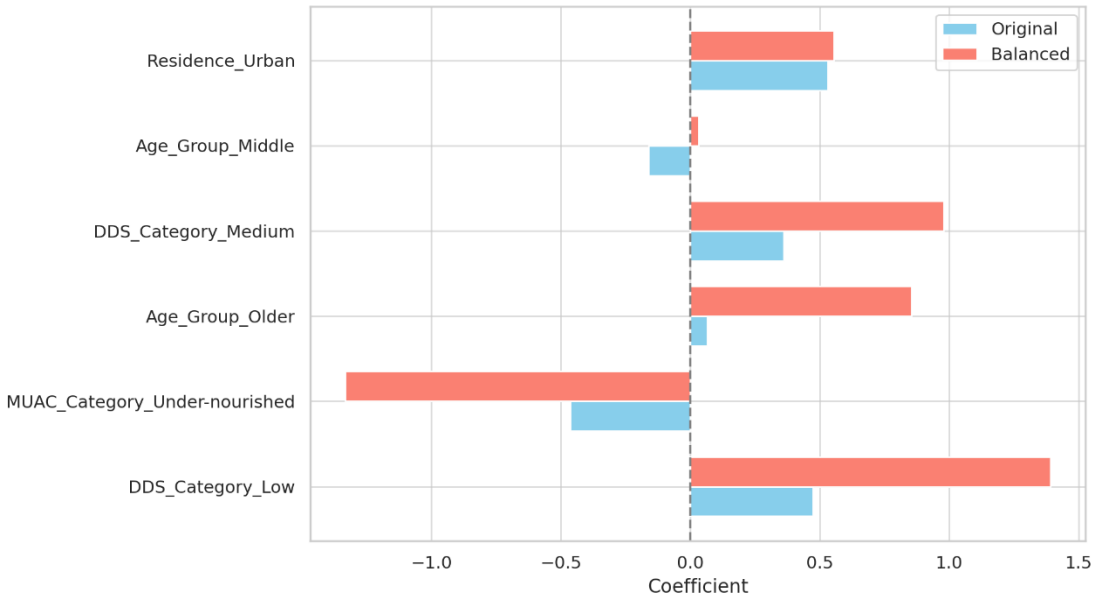

Figure 16: ComparisonPenalized Logistic Regression Coefficients (original versus balanced)

6.4 Discussion

This study reveals an exceptionally high prevalence of red blood cell (RBC) folate deficiency among first-trimester pregnant women in a neural tube defect (NTD) high risk region of northern Ethiopia. Nearly all participants (98.75%) had RBC folate levels below the World Health Organization's recommended threshold of 400 ng/mL for NTD prevention [77]. This widespread deficiency ranks among the highest reported globally and poses serious risks to both maternal and fetal health in the region. The disparity in folate deficiency rates between this study and findings from other regions of Ethiopia is notable. In Addis Ababa, a study using the same biomarker and threshold reported a 27% prevalence of RBC folate deficiency among first-trimester pregnant women [200]. The 2015 national micronutrient survey found that 32% of women of reproductive age had RBC folate levels below the threshold for NTD prevention, with the highest rates reported in northern highland areas, including Tigray [180, 283, 284]. A community-based study in eastern Ethiopia (Haramaya District), using serum folate rather than RBC folate, reported a 49% deficiency rate [181]. These variations may result from differences in biomarkers serum folate reflects short-term intake, while RBC folate indicates long-term status as well as regional disparities in diet, socioeconomic factors, healthcare access, and study design. Urban areas like Addis Ababa likely have better dietary diversity and access to folate-rich foods than rural regions like Tigray and Haramaya, contributing to the differences observed.

Across Africa, the prevalence of folate deficiency among pregnant women varies widely due to differences in assessment methods and cut-off values. For instance, Côte d'Ivoire [285] reported a prevalence of 86.1%, Senegal [148] 54.8%, Benin 18.8% [286], and Nigeria 9% [287]. In Ethiopia, a study conducted among adolescents aged 15–19 found a 31.3% deficiency rate [288], while a national study across nine administrative regions reported 46% among women of reproductive age [289]. In South Africa, the prevalence dropped from 27.6% to 0% following folic acid fortification [290]. In Cameroon, deficiency rates were 17% in women and 8% in children [291]. In Mbeya, Tanzania, 24% of pregnant women had serum folate levels below 3 ng/mL, and a school-based study in Ghana found 53.5% folate insufficiency among adolescent girls [292, 293]. While differences in methodology and population characteristics complicate direct comparisons, the folate deficiency rate observed in Tigray remains exceptionally high by both national and continental standards.

Furthermore, International comparisons highlight the extraordinary severity of folate deficiency in the study area. A global systematic review of folate status among women of reproductive age found that over 20% of women in many low-income countries are affected well above the threshold for public health concern while prevalence in high-income countries typically remains below 5%. Regional studies further illustrate this disparity: in Venezuela, [294] folate deficiency prevalence was reported at 36.3%, and in Turkey, it reached 71.7% among women [295]. These figures underscore the unusually severe nature of the problem in this study setting, underscoring its urgency and the need for targeted intervention.

Our study has shown low DDS, medium DDS, residence, and age was found as the strongest risk factors for RBC folate deficiency among first-trimester pregnant women. Our finding is consistent with findings from Addis Ababa and national studies that link micronutrient adequacy to diets rich in vegetables, legumes, and animal-source foods [180, 200, 283, 284]. The findings carry serious public health implications. The widespread deficiency aligns with persistently high rates of NTDs reported in Tigray, ranging from 130 to 262 per 10,000 births [77, 272], among the highest in sub-Saharan Africa. A critical contributing factor is the lack of pregnancy planning; over 86% of participants reported unplanned pregnancies, reducing the likelihood of periconceptional folic acid supplementation. This is consistent with national trends and highlights the urgent need to integrate micronutrient counselling into family planning and reproductive health services.

Mandatory food fortification presents a promising, evidence-based strategy to address widespread folate deficiency. Global meta-analyses have demonstrated that folic acid fortification of staple grains significantly reduces the incidence of NTDs [296]. Although Ethiopia has drafted fortification regulations, implementation remains pending. Our findings highlight the urgent need to accelerate this policy, particularly in high-risk regions such as Tigray. Meanwhile, promoting the consumption of locally available, folate-rich foods such as dark green leafy vegetables, legumes, and animal-source products is critical. Our finding has shown most respondents (99.25%) consumed staple foods, while intake of nutrient-rich and animal-source foods was low, indicating poor dietary diversity and potential nutrient gaps. The data suggest a heavy reliance on starchy staples and an underrepresentation of several key food groups, particularly those rich in protein and micronutrients. These findings highlight the need for targeted nutrition interventions to promote more balanced diets that include a greater variety of fruits, vegetables, and animal-source

foods to improve overall nutritional status. A tailored food systems approach that improves access to and intake of folate-rich foods is urgently required to complement broader policy interventions.

This study is strengthened by its use of red blood cell (RBC) folate as a biomarker a reliable and stable indicator of long-term folate status and by its relatively large, multisite hospital-based sample. However, several limitations should be considered when interpreting the findings. The facility-based design may limit generalizability to pregnant women who do not access antenatal care. Additionally, the cross-sectional nature of the study prevents causal inference. Inflammatory markers were not measured, although they are not known to significantly influence RBC folate levels.

6.5 Conclusion

The findings of this study reveal a deeply concerning public health crisis unfolding silently among pregnant women in early gestation. Among the 400 participants assessed, an extraordinary 98.75% were found to have red blood cell (RBC) folate concentrations below the World Health Organization's recommended threshold of 400 ng/mL placing nearly the entire cohort at elevated risk for neural tube defects (NTDs) and other folate-related complications. Equally troubling, 85.6% of the women reported low to medium dietary diversity, a factor shown to be strongly and inversely associated with RBC folate levels ($p < 6 \times 10^{-15}$). These results reflect more than just numbers; they point to a profound nutritional vulnerability with potentially life-altering consequences for mothers and their unborn children. The scale and severity of folate deficiency observed in this population may rank among the most extreme ever recorded globally yet it remains largely invisible. This underscores an urgent moral and scientific imperative to strengthen maternal nutrition programs, enhance dietary quality, and implement effective folate supplementation and food fortification strategies. Addressing this hidden emergency is not only a matter of public health policy but a critical step toward safeguarding the well-being of future generations.

6.6 Recommendations

Based on the findings of this study, the following actions are recommended to address the widespread folate deficiency among first-trimester pregnant women in the Tigray region: **Implement Mandatory Food Fortification:** The Ethiopian government should expedite the adoption and enforcement of national legislation mandating folic acid fortification of staple foods

such as wheat and maize flour. This evidence-based, cost-effective intervention has been shown to significantly reduce the incidence of neural tube defects at the population level.

Scale Up Preconception and Antenatal Supplementation: Health authorities should expand access to periconceptional and early antenatal folic acid supplementation, with a particular focus on rural and underserved communities where utilization of health services remains limited. Integrating supplementation into routine maternal and reproductive health services is critical for timely coverage.

Promote Dietary Diversification: Community-based nutrition education programs should emphasize the importance of consuming folate-rich foods such as dark green leafy vegetables, legumes, eggs, and other animal-source foods. Local agricultural and food systems should be strengthened to enhance the availability, accessibility, and affordability of these nutrient-dense foods.

Strengthen Family Planning and Reproductive Health Services: In light of the high prevalence of unplanned pregnancies, it is essential to improve access to family planning services and reproductive health education. Empowering women to plan their pregnancies allows for better nutritional preparation during the critical preconception period.

Provide Targeted Support for Rural Populations: Tailored interventions should address the specific challenges faced by rural women, including limited health literacy, poor access to healthcare, and economic constraints. Strategies such as deploying mobile health units and leveraging community health workers can help bridge service gaps and deliver targeted nutrition support.

Enhance Research and Surveillance: Continued monitoring of folate status among women of reproductive age is vital for tracking progress and guiding interventions. Further research should explore other coexisting micronutrient deficiencies and evaluate the effectiveness of food-based, educational, and policy-level interventions.

Chapter seven: General Discussion

7.1 Overview

This dissertation exposes the alarming scale of neural tube defects (NTDs) in war-torn Tigray, Ethiopiareaching as high as 262.5 per 10,000 births, among the highest ever recorded. Through three interlinked studies, it investigates how conflict-driven folate deficiency, the collapseof maternal health services, food insecurity, and socio-demographic risk factorsparticularly affecting rural, first-time mothers, and less-educated mothers have fueled this crisis. Biomarker analysis show nearly 99% of pregnant women suffer from critically low folate levels. The findings underscore the silent yet devastating toll of “hidden hunger" and the shortcomings of emergency nutrition efforts in addressing essential micronutrient needs. Together, these studies present a compelling, evidence-based call to action-demanding urgent policy reforms to restore maternal health services, strengthen folate interventions, and build long-term nutritional resilience in conflict-affected regions.

7.2 Prevalence and Types of Neural Tube Defects

The prevalence of neural tube defects (NTDs) in war-affected Tigray is estimated at 262.5 per 10,000 birthssurpassing Ethiopia’s pre-war average of 71.48 [8] and far exceeding the global average of 10–20 per 10,000 in countries with established folic acid programs [69].This exceptionally high burden aligns with patterns observed in other conflict zones, such as Fallujah, Iraq [97], and Gaza [98], where the collapse of healthcare and environmental exposure have contributed to surges in birth defects.

Anencephaly was the most frequently observed NTD, followed by spina bifida and encephalocele-consistent with global trends [38]. The high rate of stillbirths, particularly among anencephalic fetuses, underscores both the lethality of the condition and the lack of prenatal diagnosis or early intervention, echoing findings from Sudan and Nigeria [133, 297].Co-occurring anomalies such as hydrocephalus and clubfoot point to potential syndromic or nutritional etiologies, likely linked to folate and B12 deficiencies [298, 299], as well as possible teratogenic exposures from war-related toxins [300].Additionally, the frequent inability todetermine fetal sex-especially in case of macerated stillbirths—highlights both the severity anatomical damage and the absence of basic diagnosticcapabilities. In contrast, high-income countries facilitate early detection and surgical management of NTDs [135]. These findings reveal a convergence of conflict, malnutrition, and

diagnostic inadequacy, reinforcing the urgent need for folic acid fortification, targeted micronutrient interventions, and the restoration of maternal health system in Tigray.

7.3 Risk factors for developing a neural tube defect (NTDs)

Maternal and Socio-Demographic Risk Factors

This study underscores key maternal and socio-demographic factors linked to the high prevalence of neural tube defects (NTDs) in war-affected Tigray, revealing how conflict, inequality, and limited health access intersect to endanger fetal health.

Low maternal education emerged as a major risk factor: women with minimal schooling were up to 20 times more likely to experience NTD-affected pregnancies compared to those with secondary or higher education, largely due to poor awareness of folic acid and limited utilization of antenatal care services [301, 302]. Rural residence further amplified risk as women encountered significant barriers to healthcare, micronutrient access, and diagnostic services-challenges exacerbated s worsened by war-damaged infrastructure [303, 304]. Environmental exposures and traditional practices may also contribute to the elevated risk.

NTDs were most prevalent among women aged 20–29, particularly first-time mothers, suggesting that limited health literacy and lower nutritional preparedness-rather than age-related biologicalvulnerability-may be key drivers [22, 160, 238].

A prior history of miscarriage or stillbirth also correlated with increased NTD risk, indicating possible underlying health or nutritional deficiencies that often go unaddressed in the absence of follow-up care [160].

These findings underscore the urgent investments in maternal education, expanded rural healthcare access, and targeted preconception care in post-conflict settings.

Conflict and Siege as Catalysts for Neural Tube Defect Risk

The armed conflict in Tigray has driven neural tube defect (NTD) rates to crisis levels by dismantling health systems, worsening food insecurity, and exposing women to trauma and environmental hazards. Over 79% of health facilities were destroyed or rendered non-functional [110, 305], cutting off access to essential antenatal care, diagnostics, and folic acid supplementation—interventions that are critical for NTD prevention [77]. Similar health system collapse linked to increased birth defects have been reported in Syria and Yemen [306, 307, 308].

More than half of study participants reported war-related trauma, displacement, or sexual violence [309, 310]. Such trauma can trigger physiological stress responseresponses-particulrly elevated cortisol-that disrupt fetal development and impai neural tube closure [311]. Unintended pregnancies, often resulting from violence, further delay the initiation of antenatal care [312], mirroring trends observed in conflict zones like the DRC and South Sudan [313].

Widespread famine, food blockades, and reliance on unfortified staples foods have led to critical folate deficiencies, akey contributor to NTDs. Many women reported consuming fewer than two meals per day, echoing patterns of food insecurity seen in Somalia and South Sudan [314, 315]. While not directly measured in this study, prolonged exposure to shelling and war debris may pose toxicological risks in Tigray region. Comparable conditions in Fallujah and Gaza have linked elevated levels of heavy metals from munitions to increased rates of birth defects [98, 147, 300].

Overall, our finding highlights thatwar functions as a direct, multifactorial catalyst for NTDs-through systemic healthcare collapse, nutritional deprivation, psychosocialtrauma, and likely environmental toxicant exposure. Tigray now bears one of the highest recorded NTD rates globally, underscoring the urgent need for integrated interventions spanning health, nutrition, mental health, and environmental monitoring.

7.4 Biochemical Findings: Severe RBC Folate Deficiency in Tigray

This study presents critical biochemical evidence linking the exceptionally high prevalence of neural tube defects (NTDs) in Tigray— reaching up to 263 per 10,000 births [8, 9, 237]—to widespread red blood cell (RBC) folate deficiency among pregnant women.

RBC folate, a keybiomarker of long-term folate status, must exceed 400 ng/mL to effectively reduce NTD risk, as established by WHO and global research [77, 117, 317]. Alarmingly, 98.75% of first-trimester women in Tigray had levels below this threshold, placing nearly all pregnancies at elevated risk.

Low dietary diversity, rural residence, and young maternal age emerged as the strongest predictors of deficiency-patterns consistent with national data linking micronutrient adequacy to diets rich in vegetables, legumes, and animal-source foods [171]. The situation is further compounded by the high rate of unplanned pregnancies (over 86%), which limits timely uptake of folic acid supplementation.

In contrast to folate deficiency rates of 27% in Addis Ababa and 49% in Haramaya [197, 316], Tigray stands as a global outlier—its crisis driven by conflict, healthcare system collapse, food insecurity, and blockade-induced nutritional deprivation. Similar patterns have been observed in other conflict-affected settings such as Nigeria [318], Yemen [307, 308], and Sudan [319], reinforcing the devastating impact of war on maternal nutrition and fetal development.

These findings underscore the urgent need for integrated interventions that combine folic acid supplementation, comprehensive nutrition support, and strengthened reproductive health services to prevent further avoidable birth defects in Togray and other conflict-affected regions.

7.5 Implications for Policy and Emergency Response

The findings of this study carry profound implications for health policy and humanitarian response in conflict-affected and fragile regions. In Tigray- where war and blockage have dismantled health systems; the extraordinary burden of neural tube defects (NTDs) should be recognized as a sentinel indicator of systemic collapse. Th is not only a biomedical crisis but a humanitarian failure, demanding urgent policy recalibration and a coordinated, multisectoral response.

Emergency Restoration of Health System: The destruction of antenatal care (ANC) services-particularly the disruption of periconceptional folic acid supplementation-highlights the urgent need to restore core reproductive, maternal, neonatal, and child health (RMNCH) services. Immediate strategies should include deploying mobile health units, implementing task-shifting to community health workers, rapidly rehabilitating health infrastructure to re-establish essential preventive and diagnostic care.

Nutrition-Focused Humanitarian Aid: The near-universal folate deficiency among first-trimester pregnant women calls for integrating micronutrient supplementation (especially folic acid and vitamin B12) into all emergency food aid. Humanitarian agencies must prioritizethe distribution of fortified foods and micronutrient powders in regions facing high rates of congenital anomalies.

Birth Defect Surveillance in crisis Settings: The lack of real-time monitoring for congenital anomalies in Tigray represents a critical gap in global emergency health systems. Anestablishing a birth defect surveillance program as part of post-conflict health recovery is essential to guide evidence-based interventions and track outcomes over time.

Environmental Health and Risk Assessment: Although not directly measured in this study, the potential contribution of environmental teratogens (e.g., heavy metals from munitions) to NTDs require urgent investigation. Environmental health risk assessments and the remediation of contaminated zones must be integral to public health recovery efforts.

Protection of Maternal Health Rights: The study also reinforces the necessity of safeguarding maternal and reproductive health rights during and after conflict. This includes ensuring access to safe pregnancy care, reproductive autonomy, and protection from gender-based violence-rights that are not only medical priorities but also legal and ethical obligations.

In Sum, this study calls for urgent, integrated interventions, including:

- Restoration of reproductive and maternal health services
- Emergency folic acid supplementation
- Implementation of birth defect surveillance systems
- Environmental risk assessments and remediation
- Protection of maternal health and reproductive rights

Together, these findings underscore the devastating intersection of conflict, malnutrition, and preventable birth defects—making a compelling case for immediate public health and humanitarian action.

7.6 Public Health and Scientific Implications

This dissertation contributes important empirical evidence to the global discourse on congenital disorders in humanitarian crises. Its implications extend across maternal health, nutrition, global health security, and developmental biology.

Folate as a Biomarker for Health System Functionality: The pervasive RBC folate deficiency documented here suggest that folate status could serve as a proxy indicator of systemic public health breakdown, particularly in conflict settings. This finding underscores the value of incorporating biochemical surveillance into humanitarian health metrics.

Conflict as a Determinant of Congenital Anomalies: While war is widely recognized as a driver of malnutrition and mortality, this study demonstrates that armed conflict should also be understood

as a direct risk factor for congenital anomalies-mediated through psychosocial, nutrition, and environmental pathways.

Reframing “Hidden Hunger” in Crisis Contexts: The findings call for an expanded definition of “hidden hunger” that includes micronutrient crises with developmental and intergenerational consequences, not just chronic food insecurity. This reframing has implications for the design of nutrition interventions in emergency responses.

Maternal Stress Biology and Fetal Risk: The observed links between trauma exposure and NTD outcomes highlight an urgent research frontier in stress biology-particularly how cortisol, inflammation, and nutrient interarctions contributing to teratogenic risk. Future studies should integrate these variables in both field- based and laboratory investigations.

7.7 Public Health Recommendations and Strategic Priorities

The findings demand urgent, multi-tiered public health action relevant to Tigray and other conflict-affected or nutritionally vulnerable regions:

Emergency Folic Acid Supplementation and Fortification: Rapidly deploy folic acid supplementation for all women of reproductive age, including adolescent. where feasible, implement mass fortification of staple foods such as wheat or maize flour-a proven, sustainable intervention to reduce NTD prevalence (Hernandez-Diaz et al., 2000). These measures should be standard in humanitarian nutrition programs.

Integration of Nutritional and Reproductive Health Services: The restoration of antenatal care services with a holistic approach that combines nutritional supplementation, dietary counseling, and trauma-informed care-particularly for survivors of sexual and gender-based violence. Services should include routine congenital anomaly surveillance using both clinical and biochemical methods to enable early detection and intervention.

Community-Based Health Education: Strengthen culturally tailored health promotion campaigns, led by trained community health workers, to encourage early pregnancy recognition, timely care-seeking, and consumption of folate-rich foods. Campaigns should also address risks linked to substance use and short interpregnancy intervals, engaging local leaders, women’s groups, and faith-based organizations to counter stigma and misinformation.

Ultimately, this study shows how conflict magnifies biomedical risks, dismantles health system, and erodes human dignity-yet also affirms that evidence- based, equity-driven interventions remain possible. It calls for a paradigm shift in mergency health policy that unites biomedical evidence, community resilience and rights-based apparoaches to protect the most vulnerable, especially mothers and children, in times of war.

7.8 Limitations and Future Directions

While this study offers valuable early evidence on the burden, determinants, and biochemical correlates of neural tube defects (NTDs) in war-affected Tigray, several limitations should be acknowledged to contextualize the findings and guide future research.

Limitations

Sample Size and Selection Bias: The study primarily draws from hospital-based deliveries and selected health facilities, potentially, overrepresenting severe cases while undercapturingpregnancies terminated early, home births, or stillbirths in inaccessible rural areas. This limits generalizability and may result in under- or overestimation of true population prevalence.

Cross-Sectional and Observational Design: The cross-sectional, observational nature of the study precludes establishing causality between risk factors and NTD occurrence. Although strong associations emerged—particularly with maternal folate deficiency, low education, and rural residence—the directionality and interdependence of these factors require confirmation through robust longitudinal designs.

Incomplete Phenotypic and Clinical Data: Conflict-related disruptions in documentation and limited resources meant that key clinical information—such as fetal sex, gestational age, precise defect subtype, and associated anomalies—was often missing. This hindered detailed phenotypic classification and limited differentiation between syndromic and isolated NTDs, both of which are important for etiological understanding and program planning.

Potential Underreporting of Maternal Exposures: Sociocultural taboos and the chaotic conditions of displacement may have led to underreporting of sensitive exposures, including substance use,

prior pregnancy complications, and dietary practices. This could introduce misclassification or recall bias.

7.9 Future Research Directions

To build on the foundational insights generated by this study and address critical evidence gaps, we recommend the following avenues for future inquiry:

Genetic and Environmental Etiologies: Future studies should investigate genetic polymorphisms affecting folate metabolism (e.g., MTHFR variants) and assess environmental exposures such as heavy metals (lead, cadmium, uranium) potentially introduced through war-related contamination. Understanding these synergistic pathways could reveal conflict-specific mechanisms of teratogenesis.

Community-Based and Population-Level Surveillance: Expanding surveillance beyond tertiary hospitals to rural health posts, midwife reports, and household surveys is essential to capture unattended deliveries, stillbirths, and early neonatal deaths—events likely underrepresented in clinical records. Such approaches would improve prevalence estimates and shed light on barriers to care among high-risk groups.

Longitudinal follow-up of Affected Infants: Prospective cohort studies are needed to track neurodevelopmental outcomes, access to surgical and rehabilitative services, and quality of life among children with NTDs. These data are critical for designing disability-inclusive health programs, informing social protection policies, and guiding resource allocation during post-conflict recovery.

Evaluation of Nutritional and Policy Interventions: As folate supplementation or food fortification initiatives are implemented, embedded impact evaluations should assess their effectiveness, equity of access, and barriers to uptake. Findings could refine both emergency nutrition strategies and long-term public health policy.

In general, despite methodological constraints, this study lays an essential foundation for understanding congenital anomalies in fragile, under-researched contexts. It underscores the need for a multidisciplinary research agenda spanning genetics, toxicology, public health surveillance, and social science to address the full range of biological, environmental, and systemic contributors to NTDs in conflict-affected populations. Bridging these gaps was not only advance scientific

knowledge but also inform equitable, evidence-based interventions that protect maternal and child health as a fundamental human right.

Chapter eight: Conclusion

This study reveals a disturbing surge in neural tube defects (NTDs) in war-affected Tigray, driven by widespread folate deficiency, severe malnutrition, disrupted antenatal care, and systemic collapse of maternal health services. Nearly all first-trimester women were folate deficient, with key socio-demographic factors such as low education, rural residence, and lack of reproductive autonomy further compounding the risk.

These findings underscorehow conflict exacerbates health crises through the intersection ofnutritional, medical, and social failures. Urgent, multisectoral action is needed: the restoration of maternal health services, implementation of folic acid supplementation and food fortification programs, and targeted support for vulnerable populations-particularly displaced and rural women. Tigray's crisis stands as a stark reminder that in conflict settings, safeguarding maternal health is not only a clinical necessity but a humanitarian imperative.

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Annex-I: Participant information sheet

Project title: Neural Tube Defects in Tigray's Healthcare Facilities Amidst armed Conflict Affected region: An Epidemiological Perspective

Investigators: Birhane Alem Berihu (MSc, Associate professor- PhD student in clinical anatomy and neurobiology): Department of Anatomy, school of medicine, Division of Biomedical sciences, College of Health Sciences, Mekelle University - Mekelle, Ethiopia, Email: birhane.visionary27@gmail.com, Mobile: (251)919447239

Advisor

Main advisor

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Co Advisor

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Professor Tony Magana (MD, neuro surgery): Department of neurosurgery, school of medicine, College of Health Sciences, Mekelle University - Mekelle, Ethiopia, Email: tonymagana1@gmail.com

Introduction

Hello, I am_____, from Mekelle University, College of Health Sciences. I am here today to collect data on “Neural Tube Defects in Tigray's Healthcare Facilities Amidst armed Conflict Affected region: An Epidemiological Perspective. The objective of this study is to determine the prevalence of neural tube defects and hydrocephalous in Tigray regional state, North Ethiopia – retrospective study from October 2020-november 2023, to investigate the neural tube defects and their risk factors among mothers who gave birth in public hospitals prospective study from December 2023- 2024. I kindly request you to take part in this study. Your co-operation and willingness is greatly helpful in identifying information related to neural tube defects and it associated factors. It needs about 30 minutes for the interview. There is no direct benefit and possible risk associated with participating in this study except the time spent for responding to the questionnaire. The information you provide was kept strictly confidential. Your participation is voluntarily and you are not obliged to answer any question that you do not want to answer. If you feel discomfort with the question, it is your right to drop it at any time you want.

Purpose

The overall purpose of this study is investigating the epidemiologic characteristics of neural tube defects in Tigray which help to better understanding of the disease and thus lead to better prevention and treatment strategies. We want to find out the prevalence and factors associated with neural tube defects. With this information we were able to give information to the Government of Tigray and share with the rest of the world to have data on epidemiologic characteristics of neural tube defects in Tigray which helps to better understanding of the disease and thus lead to better prevention and treatment strategies in Tigray, Northern Ethiopia.

Procedure and participation

You receive the Tigrigna version of this information sheet and consent form to read until you completely understand it. If you cannot read, that was not be a problem because we were also provide you oral briefing so that maximum understanding and clarity was created. Then, subjects with interest to participate in our study was asked to sign on the consent form, the investigator was record your personal information. After providing your consent, we were some questions to ask you that we have prepared in advance, and we were ask you to respond what you think about each question.

Confidentiality

We strongly assure you that your name and other identifiers was not be disclosed to anyone outside of the study.

Rights, Risk and Benefits of the Study

Your participation in this study is voluntary and you have the right to refuse to participate or to not answer any questions that you feel uncomfortable. If you change your mind about participating during the course of the study, you have the right to withdraw at any time. By participating in this study and answering our questions you will not receive any direct benefit. However, you will helps to increase understanding about 'neural tube defects and its consequences in Tigray'. The results of this study help to inform the international community and the government of Tigray. We werekeep your information in a safe place, which can only be accessed by the study team. Therefore, I want to assure you that your participation in this study was not involve any risks to you.

Inducement, incentive and compensation

There was not be any monetary payment linked with your participation in this study. The benefit you gain are mentioned in the above under the section ''Benefit''.

Results dissemination

Findings from this study will be disseminated in the form of publication as PhD thesis and conference presentations. Other means of communication methodologies may also be applied when necessary.

Freedom to withdraw

Your participation in this study is completely voluntary. No penalty or loss of benefit is involved if you change your idea that you do not want to participate.

Person to contact

If you have questions regarding this study or would like to be informed of the results after its completion, please feel free to contact the research team leader with the following address: College of Health Sciences, Mekelle University

Team Leader: Birhane Alem Berihu

Address: P.O.Box 1871 Mekelle, Tigray

Cell phone number: 0919447239

II. Informed Consent

I understand that the purpose of the study is to collect information regarding the neural tube defects in the Tigray. I have read the above information, or it has been read to me. I have had the opportunity to ask questions and any questions that I have asked have been answered to my satisfaction. I consent voluntarily to participate in this study and understand that I have the right to withdraw at any time without, in any way, affecting my social life or medical care.

Yes, of course ☐ No ☐

Code of Informant_____Signature _____ Address_____ Date

Name of Witness_____ Signature _____ Address_____ Date

Name of Data Collector_____Signature _____ Address_____ Date

Annex II-Data collection tool

Research Project: Neural Tube Defects in Tigray's Healthcare Facilities Amidst armed Conflict Affected region: An Epidemiological Perspective

Respondent's no **:**_____

zone: _____

wereda:_____

kebele: _____

Date data collection: _____

Name data collatctore): _____

Phone number of data collector

Supervisor name : _____

Phone number of Supervisor

Data collection time: _____

Maternal sociodemographic characteristics

No	Question	Answer	Remark
1	Age in years)	_____	
2	Marital status) **:**	Married Not married Divorced Widowed ____	
3.	Residence	Urban Rural	
	Religion	Orthodox Muslim Catholic Protestant	
4.	Education level	unable to write and read) able to write and read elementary school secondary school college diploma and above	
5.	Occupation	student merchant farmer labor house wife college educated professional	

OBSTETRIC HISTORY

No	Question	Answer	Remark
1	Parity		
2	use planed pregnancies	Yes No	
	ANC	Yes No	
	Number of antenatal care visits	4 visits 1-3 visits 0 visits	
	History of PNC follow up	Yes No	
	Mode of delivery	Vaginal C/S	
	Place of delivery	Home Health facility	
	Gestational age	Term Preterm Post term	
4	the outcome of your latest pregnancy before this)?	Live baby Abortion Still birth Twins/multiple	
	4A. Sex of baby of last pregnancy)	Male Female Ambiguous	
	4B. the birth weight of last pregnancy?		
	4C. If twins/multiple deliveries, what were the sexes of last pregnancy)?		
	4E. What were their birth weights of last pregnancy?		
5.	History of Birth defects?	Yes, No	
6.	ever had child/children with a birth defect)?	Yes, No	
	6A. If yes, please describe the type of birth defects		
7	7A. X-ray /CT scan/ during your recent pregnancy?	Yes No	
	7B. X-ray/ CT scan (how many in your life time)		

Pregnancy intention (only for prospective study)			
S.no	Sections	Response	
1	Pregnancy wanted	A) yes B) No	
2	If no, Reason for unwanted pregnancy	Pregnancy wanted Contraceptive failure Not supported by family Abandoned by partner Extramarital pregnancy Pregnancy from unknown person (rape) Lack of family planning methods No health facility (damaged or looted by invaders) Others	

	7D. If X-ray /CT scan done during pregnancy, when was that ?	1st three months of pregnancy 4th to 6th months of pregnancy 7th to 9th months of pregnancy	

Safe motherhood during genocidal war in Tigray (only for prospective study)		
1	Type of violence event?	Act of war Attack on populations Sexual and Gender-Based violence
2	Exposed to Sexually Transmitted Diseases	A. Yes B. No
2.1	If yes please specify the Sexually Transmitted Diseases	
3	Displaced	A) yes B) No

Health service use and source of food during genocidal war in Tigray (only for prospective study)		
1	Use health services ANC/PNC services	A) yes B) No
1.1	If no reason for not using health service	Not supported by family Lack of family planning methods No health facility (damaged or looted by invaders) Feeling insecure to go health facility by the war

		Lack of interest Others
2	Family food Source	 Cultivation/production Food aid Support from family Purchasing
3	Meal frequency	1 time per day 2 times per day 3 times per day 4 times per day
4	Consumed periconceptional folic acid supplementation	A) yes B) No
5	Consumed fortified	A) yes B) No
6	Consumed multivitamins during first trimester of pregnancy period	A) yes B) No

Recent Pregnancy outcome by type of congenital anomalies

No	Question	Answer	Remark
1	Congenital malformations of the nervous system		
2	Congenital malformations of eye, ear, face and neck		
3	Congenital malformations of the circulatory system		
4	Congenital malformations of the respiratory system		
6	congenital malformations of the digestive system		
7	Congenital malformations of genitourinary organs		
8	Congenital malformations and deformations of the musculoskeletal system		
9	Chromosomal abnormalities, not elsewhere classified.		

Annex III- Data collection form for the laboratory investigation

Section	Details
1. Demographic Information	
Patient ID	[Unique Identifier]
Full Name	[First Name, Last Name]
Date of Birth	[YYYY-MM-DD]
Age	[Age in Years]
Gender	Female
2. Sample Information	
Sample Collection Date	[YYYY-MM-DD]
Sample Collection Time	[HH AM/PM]
Type of Sample	[Whole Blood, Plasma, Serum]
Collection Tube Type	[e.g., EDTA Tube, Serum Tube]
Volume Collected	[Volume in mL]
3. **Laboratory Testing Details**	
Method for RBC Folate	[e.g., ECLIA, CLIA]
Calibration Details	[Date and Details of Calibration]
Control Samples	[Details of Control Samples]
4. **Biochemical Analysis Results**	
RBC Folate: Measured Value	[Value in ng/mL]
RBC Folate: Reference Range	[Value in ng/mL]
RBC Folate: Interpretation	[Normal/Low/High]
5. **Quality Control and Assurance**	
QC Data	[Control Results]
Calibration Data	[Details of Calibration Checks]
Notes on Sample Integrity	[Observations on Handling/Storage]
6. **Data Management and Confidentiality**	
Data Entry Person	[Name of Person Entering Data]
Data Review	[Name of Person Reviewing Data]
Confidentiality	[Description of Data Security]

Data collection tool for Sociodemographic and obstetrics characteristics data collection tool for first trimester Pregnant Women

1. Maternal sociodemographic characteristics

No	Question	Answer	Remark
1	Age in years)	_____	
2	Marital status) :	Married Not married Divorced Widowed ____	
3.	Residence	Urban Rural	
	Religion	Orthodox Muslim Catholic Protestant	
4.	Education level	unable to write and read) able to write and read elementary school secondary school college diploma and above	
5.	Occupation	student merchant farmer labor house wife college educated professional	

OBSTETRIC HISTORY

No	Question	Answer	Remark
1	Parity		
2	use planed pregnancies	Yes No	
	ANC	Yes No	
	Number of antenatal care visits	4 visits 1-3 visits 0 visits	

	History of PNC follow up	Yes No	
	Mode of delivery	Vaginal C/S	
	Place of delivery	Home Health facility	
	Gestational age	Term Preterm Post term	
4	the outcome of your latest pregnancy before this)?	Live baby Abortion Still birth Twins/multiple	
	4A. Sex of baby of last pregnancy)	Male Female Ambiguous	
	4B. the birth weight of last pregnancy?		
	4C. If twins/multiple deliveries, what were the sexes of last pregnancy)?		
	4E. What were their birth weights of last pregnancy?		
5.	History of Birth defects?	Yes, No	
6.	ever had child/children with a birth defect)?	Yes, No	
	6A. If yes, please describe the type of birth defects		
7	7A. X-ray /CT scan/ during your recent pregnancy?	Yes No	
	7B. X-ray/ CT scan (how many in your life time)		
	7D. If X-ray /CT scan done during pregnancy, when was that ?	1st three months of pregnancy 4th to 6th months of pregnancy 7th to 9th months of pregnancy	

C: MATERNAL HEALTH AND DRUG USE DURING THE RECENT PREGNANCY

No	Question	Answer	Remark
1.	Did you use any oral herbal medicines during the pregnancy?	Yes No	
	1A. If yes, please state the names of the herbs you used)	_____	
	1B. When did you use the medicines?	1st three months of pregnancy -4th to 6th months of pregnancy 7th to 9th months of pregnancy Throughout the pregnancy period	
	1C. What was the frequency of use of the medicines?	- Daily for a week - Daily for 2-4 weeks - Daily for over a month Other (specify) _____	
2.	2A. - Did you ever smoke during the pregnancy?	Yes No	
	2B. - If yes, when did you do so?	-1st three months of pregnancy - 4th to 6th months of pregnancy - 7th to 9th months of pregnancy - Throughout the pregnancy period	
	2C. What was the frequency of tobacco/cigarette use?	Daily for a week Daily for 2-4 weeks Daily for over a month Other (specify)_____	
	2D. -How many sticks of cigarettes/pipes of tobacco (average) did you consume a day?	_____ _____	
3	3A. - Did you consume any alcoholic drinks during the pregnancy?	Yes No	
	3B. - If yes, when did you consume the drinks?	-1st three months of pregnancy 4th to 6th months of pregnancy 7th to 9th months of pregnancy Throughout the pregnancy period	

	3C. often did you drink the alcoholic beverages?	Daily for a week Daily for 2-4 weeks Daily for over a month Other (specify)	
	3D. How much alcohol (on average) did you consume a day?	_____	
4	4A. What chronic diseases did you suffer during the pregnancy?	_____	
	4B. When were you affected by the diseases?	-1st three months of pregnancy - 4th to 6th months of pregnancy - 7th to 9th months of pregnancy - Throughout the pregnancy period	
	4C. Please name the drugs which were used to manage the diseases in 13 above	_____	
	4D. What were the doses and durations of the drugs listed above?		
	4E. Tick the conditions you had during your latest pregnancy	A painless sore (chancre) in private parts A widespread non itchy body rash Sores in the mouth, nose, throat or private parts	
	4F. Where you treated?	_____	
	4G.When were you treated?	-1st three months of pregnancy 4th to 6th months of pregnancy to 9th months of pregnancy	
5	5A. (Have you ever exposed to pesticide/other chemicals such as fertilizers, paints or others Please describe type the chemical you exposed)		
	5B. (how many times are you exposed to the pesticide/other chemicals)		
	5C. how many times your spouse/ house member been exposed to the pesticide/other chemicals)		

6	What is your source of drinking water? Please circle the most frequent and check if others are used too	City water Bottled water Local river/raw water	
7	7A. Have you suffered by parasitic infection, if yes when and what type of parasitic infection?		
	7B. Did you suffered any of the following symptoms?	Chronic diarrhea- Chronic fever - Chronic weight loss - Chronic abdominal cramps - No -	
	7C. have you ever used khat	Yes No	

Mothers Dietary diversity

	Dietary diversity			
did you eat any of the following during the past 24 hour: (Read each item aloud and record response before proceeding to the next item.)		No	Yes	DK
1	Bread, pasta, rice, noodles, biscuits, cookies or any other food made from oats, maize, barley, wheat, sorghum, millet, or other grain?	0	1	2
2	Any food made from teff, barley, maize, sorghum like injera, kita, or porridge?	0	1	2
3	Any white potatoes or any other foods made from roots?	0	1	2
4	Any pumpkin, carrots or sweet potatoes that are yellow or orange inside?	0	1	2
5	Any dark green, leafy vegetables like kale, spinach or amaranth Leaves	0	1	2
6	Any ripe mangoes, ripe papayas? Ripe Mango	0	1	2
7	Any other fruits or vegetables?	0	1	2
8	Any liver, kidney, heart or other organ meats?	0	1	2
9	Any beef, pork, lamb, goat?	0	1	2

10	Any chicken, or other wild birds?	0	1	2
11	Any eggs?	0	1	2
12	Any fresh or dried fish?-	0	1	2
13	Any foods made from beans, peas, lentils or pulses?	0	1	2
14	Any nuts or seeds such as peanuts, sesame or sunflower seeds?	0	1	2
15	Any cheese, yogurt, milk or other milk products?	0	1	2
16	Any foods made with oil, fat, or butter?	0	1	2
17	Miscellaneous drinks such as cola, coffee, tea, etc	0	1	2
18	Any food fortified with vitamin A	0	1	2
19	Rice -ሩዝ	0	1	2
20	Avocado	0	1	2

Mothers knowledge about Folic acid supplementation and neural tube defects, Services

No	Question	Answer	Remark
1	Access to Access to Healthcare services	Regular (Frequent check-ups) Occasional (Visits as needed) Rare (Limited visits) None (No regular access)	
2	Do you have strong support from family or friends during your pregnancy?	Yes No	
3	Do you know Folic acid	yes B. no	
4	Source of information about folic acid	Healthcare Provider Family/Friends Media Other	If the answer is no please go to next question number

	Do you understand the importance of folic acid during pregnancy?	yes B. no	
5	Do you believe folic acid can help prevent NTDs?	yes B. no	
6	If yes How important do you think folic acid is for preventing birth defects? o	Very Important Somewhat Important Not Important	If the answer is no please go to next question
	What do you know about neural tube defects (NTDs)?	I know a lot I know a little I don't know	
	Do you believe folic acid can help prevent NTDs?	Yes No Not Sure	
7	If yes How important do you think folic acid is for preventing birth defects?	Very Important Somewhat Important Not Important	

Maternal anthropometric and Clinical data

Gestational age	_____ weeks
MUAC	_____ Cm
Height	_____m
Weight	_____kg

ልጋብ 1: ንተሳተፍቲብዛዕባእነካይዶመጽናዕቲሓበሬታወሃቢጽሑፍ

ርእሲ እዚ መጽናዕቲ:- ዝርገሐ ኢተፈጥሮኣዊ ዕቤት ሓንጎልን ሓብለ ሰረሰርን ኣብ ትካላት ክንክን ጥዕና ትግራይ ዝውለዱ ዕሸላት ኣብ ኣብ እዋን ፅንተታዊ ኩናት ትግራይ''

ተመራመሪ(ተምሃራይፒኤችዲ)

ብርሃነ ኣለም በሪሁ (ተመራማሪ ክፍሊ ኣካላት፡መምህርን፣ተሓባባሪ ፕሮፌሰር፣ ተምሃራይ ፒኤችዲ ብክልኒካል ኣናቶሚን፣ኒውሮባዮሎጂን) ፤ email: birhane.alem@mu.edu.et/birhane.visionary27@gmail.com

<u>ዋናኣማኻሪ</u>

ዶክተር ሓየሎም ከበደ (ፒኤችዲ፡ተሓባባሪ ፕሮፌሰር)፡ክፍሊ ኣናቶሚ፡ቤት ትምህርቲ ሕክምና፡ክፍሊ ባዮሜዲካልሳይንስ፡ ኮሌጅሳይንስጥዕና፡ዩኒቨርሲቲ መቐለ - መቐለ፡ኢትዮጵያ

<u>ሓባራዊኣማኻሪ</u>

ፕሮፌሰርኣፈወርቅሙሉጌታ (ፒኤችዲ፡ፕሮፌሰር)፡ክፍሊኣመጋግባ፡ቤትትምህርቲሕብረተሰብጥዕና፡ኮሌጅሳይንስጥዕና፡ዩኒቨርሲቲመቐለ - መቐለ፡ኢትዮጵያ፡ኢመይል፡ afework.mulugeta@gmail.com

ፕሮፌሰርቶኒማጋና (MD, neuro surgery)፡ክፍሊ ኒውሮሰርጀሪ፡ ቤት ትምህርቲ ሕክምና፡ ኮሌጅ ሳይንስ ጥዕና፡ዩኒቨርሲቲ መቐለ - መቐለ፡ኢትዮጵያ፡ኢመይል፡ tonymagana1@gmail.com

<u>መእተዊ</u>

እዚ ሓበሬታ ወሃቢ ጽሑፍ ሓፈሻዊ ዕላምኡ ኣብዚ እነካይዶ መጽናዕቲ ተሳተፍቲ ንኽትኮኑ ፍቓድኩም ንምሕታት ኮይኑ ብዛዕባ እዚ እነካይዶ መጽናዕቲ ሙሉእ ሓበሬታ ክዋህበኩም እዩ። ብተወሳኺ እውን ካብ ተሳተፍቲ እንደልዮ ሓገዝ ብዝርዝር ዝተገለጸ ኮይኑ ኣብዚ ከይዲ ድሕንነት፣ ክብርን መሰልን ተሳተፍቲ ብዘረጋገጸ መንገዲ ንኽኸውን እንወስዶም ጥንቃቐታት እውን ኣቐሚጥና ኣለና። ስለዝኾነ ኣብዚ መጽናዕቲ ተሳታፊ ንምኳን ንኽውስኑ/ና እዚ ሓበሬታ ብዕምቐት ምርዳእ ጠቓሚ ይኸውን። ዝኾነ ዓይነት ሕቶ ወይ ከዓ ግልጺ ዘይኮነ ነገር እንተጋጢምዎም/ወን ብዘይዝኾነ ስኽፍታ ክንዲዝደለይዎ/ኦ ግዜ ንኽሓታ/ቱና ይላቦ። ኣብዚ መጽናዕቲ ተሳታፊ ንምኳን እንተወሲኖም/ነን እሞ እዚ ውሳኔኦም/ኣን እቲ መጽናዕቲ ይኹን ንሶም/ንሰን ዝገብሩልና/ራልና ሓገዝን ካልኦት ዝተጠቐሱ ዛዕባታት ሙሉእ ብምሉእ ተረዲእዎም/ወን ብሰናይ ድሌቶም/ተን ፈቂዶም/ደን ምኻኖም/ነን ኣብቲ ዝተዳለወ ናይ ስምምዕ ቕጥዒ ብምፍራም ከረጋግጹልና/ከራጋግፃልና እዮም/እየን። ንዝገብሩልና/ዝገብራልና ምትሕብባርን ሓገዝን ብቕድምያ ምስጋናይ የቕርብ።

<u>ዓላማ ናይዚ መጽናዕትን ናቶም/ናተንተሳትፎን</u>

ሓፈሻዊ ዕላማ እዚ መፅናዕቲ " ጉድለት ኒውራል ቱቦ ኣብ ትካላት ክንክን ጥዕና ትግራይ ኣብ ሞንን ዕጥቃዊ ግጭት ዝተጎድአ ክልል: ኣረኣእያ ለበዳ" ብዝብል ርእሲ ንምፅናዕ እዩ:: ማለት እውን ዝርገሐን ረቐሒታት ሓደጋን ጉድለት ስርዓት መትንን ዕትብቲ ዓንዲሑቐን ኣብ ክልል ትግራይ ሰሜን ኢትዮጵያ፣ ምክልኻል ስጉምትታትን ወሰንቲታቱን ብጥልቀት ንምፅናዕ እዩ:: ካብዚ መፅናዕቲ እዚ ብዝርከብ መረዳእታ ድማ ብቐንዱ ንመንግስቲ ትግራይ ብሓፈሻ ድማ ንማሕበረሰብ ዓለም ብዛዕባ ዘሎ ዝርገሐ ሕማም ጉድለት ስርዓት መትንን ዕትብቲ ዓንዲሑቐን ምክልኻል ስጉምትታትን ወሰንቲታቱን ብዝተፈላለየ መንገዲ ሓበሬታ ብምሃብ ዝክኣል ግንዛበ ንኽፍጠር ክግበር እዩ::

<u>ተሳተፍቲ እዚ መጽናዕቲ ብኸመይ ይሕረዩ?</u>

<u>ዒላማዊ መረፃ ተሳተፍቲ ምስኣካየድና እቲ ሓበሬታ ወሃቢ ወረቐት ክተንብብዎ ክወሃበኩም እዩ። ድሕሪ እዚ ተወሳኺ መብራህርሂ ክዋሃበኩም እዩ። ዝተወሰነ ሕቶታት ብምሕታት ሕድሕድ ነጥቢ ክምዝተረደኣኹም ምስ ኣረጋገጽና ኣብዚ መጽናዕቲ ንምስታፍ ድሌት ዘለዎም ሰብ ሞያ ጥዕና ብድሌቶምን ፈቒዶምን ኣብቲ ናይ ስምምዕ ቕጥዒ ብምፍራም የረጋግፁ። ብድሌትኩም ምስገለፅክሙልና ነዚ መፅናዕቲ እዚ ዝተዳለዉ ሕቶታት ብቐደም ሰዓብ ብምሕታት ዝከኣል መልሲ ክንረክብ ኢና:: ልቢ በሉ:- ንእትህቡና መልስታት ብናይ ኣብ ዝተዳለወ ቅጥዒ መሕተት ወረቀት ንቐድሑ ምኳና ኣቐዲምና ክነፍልጠኩም ንደሊ::</u>

ኣብዚ መጽናዕቲ ብምስታፍካ/ክን እንታይ ጥቕሚ ይረኽባ/በሉ?

ምስ እዚ መጽናዕቲ ዝተተሓሓዘ ንተሳተፍቲ ዝኽፈል ቀጥታ ክፍሊት ከምዘይህሉ ክንገልጽ ንፈቱ። ኮይኑ ግና ካብዚ መፅናዕቲ እዚ ብዝርከብ ሓበሬታ መሰረት ኣብ ምምሕያሽን ምግታእን ጉድለት ስርዓት መትንን ዕትብቲ ዓንዲሑቕን ኣብ ስርዓተ ክንክን ጥዕና ትግራይ፣ ምኪልኻል ስጉምትታትን ወሰንቲታቱን ብዝግበር ናይ ግንዛበ መፍጠሪ ከይድታት፣ ክትትላትን መስተኻኽሊ ስጉምትታትን ከም ውልቀ ሰብ ኮነ ከም ዓዲ ተረባሒ እዮም/እየን ኢልና ንኣምን።

ኣብዚ መጽናዕቲ ብምስታፈይ እንታይ ጉድኣት ክበጽሐኒ ይኽእል?

እዚ መጽናዕቲ ናይ ተሳተፍቲ ድሕንነት፣ ክብርን መሰልን ብዝለዓለ ደረጃ ብዘረጋገፀ ከይዲ ንኽፍፀም ዓብዪ ጥንቃቐ ኽንገብር ኢና። ብስሩ እውን ግን ኣብዚ መጽናዕቲ ንተሳተፍቲ ጉድኣት ዘብጽሕ ወይ ከዓ ናብ ሓደጋ ዘጋልጽ ተግባር የለን ነዚ ቃለ መሕትት 30 ደቂቃ ግዜኡም/ኣን ክህቡ/ባና እዮም።

እቲ መጽናዕቲ ክቋርፆ ይኽእል ‘ዶ?

ቅድም ኢሉ ከምዝተገለፀ ኣብዚ መፅናዕቲ ምስታፍ ሙሉእ ንሙሉእ ኣብ ሰናይ ድሌት ተሳተፍቲ ዝተመርኮሰ እዩ። ኣብ ከይዲ ካብቲ መጽናዕቲ ምቁራጽ ይከኣል እዩ። ስለዘቋረፀ/ፃ ዝበጽሖም/ሐን ዝኾነ ዓይነት ክፍሊት ይኹን ቅጽዓት ኣይህሉን።

ካብ ተሳተፍቲ እንወስዶ ሓበሬታ ምስጢራውነት ብዝምልከት

ናይዚ መጽናዕቲ ውፅኢት ዓላማ ንምስኻፅ ንዝምከቶ ኣካል ኮነ ብሕትመት መልክዕ ክዳሎ ንኽእል ኢና። እዙይ ግን እቲ ሓበረታ ናይመን ምኻኑ ብዘየፍልጥ መንገዲ እዩ ክፍፀም። ብኻሊእ ኣበሃህላ ካብቲ ዋና ተመራማሪይ ወጻኢ ዝካየድ መፅናዕታዊ ስራሕቲ ኩሉ መለለዩ ተሳተፍቲ ዝኾኑ ሓበሬታታት ብምጥፋእ እዩ ክትግበር። እዙይ እቲ ውጽኢት መጽናዕቲ እንትሕተም እውን ክትግበር እዩ። ካብዚ መጽናዕቲ ዝእከብ ሓበሬታ ይኹን ዝርከብ ውጽኢት ከይጠፍእ ኣብ ኮምፑተርን ሰርቨርን ክቕመጥ ይኽእል እዩ። ስልጣን ዋና ተመራማሪይ እቲ ሓበሬታ ይኹን ውጽኢት ናይዚ መጽናዕቲ ንምጥቃም ግዜ ገደብ የብሉን።

ምዝርጋሕ ውፅኢት ናይዚ መፅናዕቲ ዝምልከት

ውፅኢት ናይዚ መፅናዕቲ ብመልክዕ ሕታም ወይ ከዓ ኣብ ኮንፈረንስታት ብምቕራብ ክሰራጭ/ክዝርጋሕ እዩ። ከከም ኣድላይነቱ ካልኦት ሜላታት እናተጠቀምካ እውን እቲ ውፅኢት ናይ ምዝርጋሕ ስራሕቲ ክስራሕ ይኽእል እዩ።

ንመን ክረኽቡ/ባ ይደልዩ/ያ?

ተወሳኺ ሓበሬታ እንተደልዮም/የን ካብ ዋና መተሓባበሪ ናይዚ መፅናዕቲ በቲ ዝተጠቀሰ ኣድራሻ ኣቢልኩም/ለን ክትረክቡ ከም ትኽእሉ/ላ ኢኹም/እኽን።

ናይዚ መፅናዕቲ ዋና መተሓባበሪ ሽምን ኣድራሻን

ሽም፡- ብርሃነ ኣለም በሪሁ

ቤት ትምህርቲ፡- ኣናቶሚ

ኮሌጅ፡- ጥዕና ሳይንስ

ዩኒቨርስቲ፡- መቐለ ዩኒቨርስቲ

ኢ-መይል፡- birhane.alem@mu.edu.et ወይ birhane.vionary27@gmail.com

ሞባይል፡- +251919447239

ኣብ ኣፍልጦ ዝተመስረተ ናይ ተሳታፋይነት ስምምዕነት

ዓላማ ናይዚ መፅናዕቲ ብዛዕባ ጉድለት ኒውራል ቱቦ ኣብ ትካላት ክንክን ጥዕና ትግራይ ኣብ ሞንነ ዕጥቃዊ ግጭት ዝተንድአ ክልል: ኣረኣእያ ለበዳ ንምእካብ ምኽኑ ብዝግባእ ተረዲአ ኣለኹ። ዝርዝር ብዛዕባ ንተሳተፍቲ ዝዋሃብ ሓበሬታ ወሃቢ ጽሑፍ እውን ኣንበበን ተነቢቡለይን ኣሎ። ዘይበረሀለይ ሕቶ ናይምሕታት እኹል ዕድል ዝረኸብኩ እንትኸውን ዝግባእ እኹል መልሲ እውን ተዋሂቡኒ እዩ።

ብተወሳኺ ብሰናይ ድለየተይ ኣብዚ መፅናዕቲ እዚ እናተሳተፍኩ እንትኾን ኣብ ዝደለኽዎ ግዜ ብዘይዝኾነ ምቅዋስ/ሃሰያ ማሕበራዊ ሂወት ወይካዓ ጥዕናዊ ኣገልግሎት ካብዚ መፅናዕቲ ምስታፍ ዓርሰይ ከማልል ዝኽእል ምኻነይ ተረዲአ ኣለኹ።።

እወ፣ ብዝግባእ

ኣይፋል

ምሽጥራዊ መፍለይ

ተሓታቲ/ት_____ፈርማ_____ኣድራሻ_____ዕለት_____

ሽም ምስክር_____ፈርማ_____ኣድራሻ_____ዕለት_____

ሽም ኣካቢ መረዳእታ_____ፈርማ_____ኣድራሻ_____ዕለት_____

ልጋብ 2:ቃለመሕተት- ትግርኛቅጥዒ

ርእሲ መፅናዕቲ: ዝርገሐ ኢተፈጥሮኣዊ ዕቤት ሓንሳልን ሓበለ ሰረሰርን ኣብ ትካላት ክንክን ጥዕና ትግራይ ዝውለዱ ዕሸላት ኣብ ኣብ እዋን ፅንተታዊ ኩናት ትግራይ''

ናይ ምላሽወሃቢ/ት መለለይ ቁጽሪ፡_____

ዞባ፡_____

ወረዳ፡_____

ጣብያ/ቁሸት፡ _____

መረዳእታ እተኣከበሉ ዕለት፡_____

ናይ መረዳእታ መለዩ ኮድ፡_____

ናይ መረዳእታ ኣካቢ ፌርማ: _____

ናይ ተቐፃሪ መለዩኮድ: _____

ናይ ተቐፃሪ ፌርማ::_____

ዕለት: _____

ናይ ኣዶታት ድሕረ ባይታ መሕተት

ተ.ቑ	ሕቶ	መልሲ	መብርሂ
1	ዕድመ	_____	
2	ኩነታት ሓዳር	ሓዳር ዘለዋ ሓዳር ዘይብላ ዝተፋትሐት ሰብኣያ ዝሞታ ካልእ እንተልዩ ይግለፅ	
3.	ዝነብሩሉ ቦታ	ከተማ ገጠር	
4.	ትምህርቲ ደርጃ	ምንባብን ምፅሓፍን ዘይኽእል/ዘይትኽእል ምንባብን ምፅሓፍን ዝኽእል/ትኽእል 1-4 ክፍሊዝወደኣ/ት	

		5-8 ክፍሊዝወደአ/ት 9-12 ክፍሊዝወደአ/ት ዲፕሎማ ልዕሊኡን	
5.	ኩነታትስራሕ	ተምሃሪ/ት ነጋዳይ ሓረስታይ/ ገባር ሞያተኛ ዘይሞያተኛ በዓልቲእንዳ ኮሌጅ ሙህር በዓልሞያ ስራሕ ዘይብሉ/ላ	
7.	7A. ዓሌታዊ ውልደት ኢተፈጥሮ ኣልዎንዶ	እወ ኣይፋል	
	7B. እንድሕር ኣለዎን ኣብራህርሃልና		

ስነተ ዋልዶ ድሕረ ባይታ መሕተት

ተ.ቑ	ሕቶ	መልሲ	መብርሂ
1	በዝሒዝተወልዱ ህጻውነቲ		
2	ናይ ጥንሲ ፕላን ዶትክተላ	እወ ኣይፋል	
4	ቅድሚ ሕጂ ዝነበረክን ጥንሲ ውጺኢቱ እንታይ ይመስል	ወሪዱ ብሂወት ተወሊዱ ምውት ኣብ ውሽጢ ማህጸን ሞይቱ ማንታ	
	4B. ቅድሚ ሕጂ ዝተወልደ ህጻን ጾታ እንታይኔሩ	ወዲ ኣን	

		ኢተፈጥሮኣዊ	
	4C. እቲ ዝተወለደ ህጻን ክብደቱ ክንደይ ነይሩ		
	4D. እንድሕር ማናቱን/ካብኡ ንላዕሊ ኮይኖም ዖተኣም እንታይ ኔሩ		
	4E. እንትወለዱ ክብደቶም ክንደይ ዝኣክል ኔሩ		
5.	ውልደተ ኢተፈጥሮኣዊ ኣእምሮ ዝባሃል ሰሚዕክን ዶትፈልጣ	እወ ኣይፋል	
6.	ኢተፈጥሮኣዊ ኣእምሮ ዘለዎ ህጻን ወሊደንዶ ይፈልጣ?	እወ ኣይፋል	
	6A. እንድሕር እወ ኢለን እንታይ ዓይነት ሽግርኔሩ		
7	7A. ጨረር(X-ray /CT scan)ምርመራ ጌረንዶ ይፈልጣ(	እወ ኣይፋል	
	7B. ንክንደይ ግዜ ከጨረር (X-ray/ CT scan) ምርመራ ጌርክን		
	7C. ንክንደይግዜክ ጨረር X-ray / CT scan ምርመራ ጌርክን (how many CT scan in your life time)		
	7D. እንድሕር እወ ኢለን መዓዝ ኔሩ?	መጀመርያ 3 ናይ ጥንሲ ወርሒ 4ይ - 6ይ ናይ ጥንሲ ወርሒ - 7ይ - 9ይ ናይ ጥንሲ ወርሒ	

ናይኣዶታትፎሊክኣሲድክኒን/ ዝተመጣጠነምግቢምምጋብመሕትተ)

ተ.ቑ	ሕቶ	መልሲ	መብርሂ
1	ፎሊክ ኣሲድ ክበሃል ዶ ሰሚዕክን ትፈልጣ?	እወ ኣይፋልጥን	
2	ብፎሊክ ኣሲድ ዝተመጣጠነ ምግቢዶ ይወስዳ ኔረን?	እወ B. ኣይፋል	
	2A. እንድሕር ብፎሊክ ኣሲድ ዝተመጣጠነ ምግቢ ይወስዳ ኔረን መዓዝ ጀሚረን?	ቅድሚ ናይ ጥንሲ እዋን ኣብ መጀመርያ 3 ናይ ጥንሲ ወርሒ 4ይ - 6ይ ናይ ጥንሲ ወርሒ	

		7ይ - 9ይ ናይ ጥንሲ ወርሒ	
3.	3A. -ፎሊክኣሲድከኒንዶይወስዳኔረን?	እወ B. (ኣይፋል)	
	3B. እንድህርፎሊክኣሲድይወስዳኔረንመዓዝጀሚረን?	ቅድሚ ናይ ጥንሲ እዋን ኣብ መጀመርያ 3 ናይ ጥንሲ ወርሒ 4ይ - 6ይ ናይ ጥንሲ ወርሒ 7ይ - 9ይ ናይ ጥንሲ ወርሒ	
	3C. ዓቀን ፎሊክ ኣሲድ ክኒን ዶክንደይ ኔሩ?	4ሚግ B. 0.4ሚግ	

ዕላማ ጥንሲ ኣብ እዋን ህልቂት ኩናት ኣብ ትግራይ

1. ውጥን ጥንሲ - ሀ) እወለ) ኣይፋልን።

2. ጥንሲ ዘይተሓሰበ - ሀ) እወለ) ኣይፋልን።

2.1 እወ እንተኾይኑ ምኽንያት ዘይተሓሰበ ጥንሲ

ሀ. ጥንሲ ዝድለ

ለ/ ውድቀት መከላኸሊጥንሲ

ሐ. ብስድራ ቤት ዘይድገፍ

መ. ብመሻርኽቲ ዝተደርበየ

ሠ ካብሓዳር ወጻኢዝግበር ጥንሲ

ረ/ ካብ ዘይተፈልጠ ሰብ ዝመጽ እጥንሲ (ዓመጽ)

ሰ. ኣገባብ ውጥን ስድራቤት ዘይምህላው

H. ትካልጥዕና የለን (ብወረርቲ ዝተበላሸወ ወይ ዝተዘመተ)

I. ካልኦት

ኣብ ትግራይ ኣብ እዋን ህልቂት ኩናት ውሑስ ኣደነት

1. ዓይነት ናይ ግርጭት ፍጻመ?

ሀ/ ግብረ ሽበራዊ መጥቃዕቲ

ለ/ ተግባርኩናት

ሐ. መጥቃዕቲ ኣብ ልዕሊ ህዝቢ

መ. ምጭዋይ

ሠ ጭፍጨፋ

ረ ብግዴታ ምጥፋእ

ሰ/ ኣብ ንብረት ዝበፅሕ ጉድኣት

H. ጾታ ውንጽታውን ዓመጽ

2. ንጽታዊ ርክብ ዝመሓላለፉ ሕማማት ዝተቓልዑ - ሀ) እወለ) ኣይፋልን።

2.1.1. እወ እንተኾይኑ በጃኹም ብጽታዊ ርክብ ዝመሓላለፉ ሕማማት ግለጹ

3. ዝተመዛበሉ- ሀ) እወ ለ) ኣይፋልን።

ናይቀረባእዋንውጽኢትጥንሲብዓይነትናይተፈጥሮኣዋምዝንባዕ

ተቁ	ሕቶ	መልሲ	መብርሂ
1	ብተፈጥሮ ዝመጽእ ምጉዳል ቅርጺ ስርዓተ ነርቭ		
2	ብተፈጥሮ ዝመጽእ ምጉዳል ቅርጺ ዓይኒ፡ እዝኒ፡ ገጽን ክሳድን		
3	ብተፈጥሮ ዝመጽእ ምጉዳል ቅርጺ ስርዓተ ዑደት ደም		
4	ብተፈጥሮ ዝመጽእ ምጉዳል ቅርጺ ስርዓተ ምስትንፋስ		
6	ብተፈጥሮ ዝመጽእ ምጉዳል ቅርጺ ስርዓተ ምሕቃቕ መግቢ		
7	ብተፈጥሮ ዝመጽእ ምጉዳል ቅርጺ ኣካላት ብልዕቲ ሽንቲ		
8	ብተፈጥሮ ዝመጽእ ምጉዳል ቅርጺን ምቕያር ቅርጺ ጭዋዳታትን ኣዕጽምትን		
9	ኣብ ካልእ ቦታታት ዘይተመደበ ዘይንቡርነት ክሮሞዞም።		

ልጋብ 3: ናይ ላቦራቶሪ ምርመራ ዝግበረለን ጥኡሳት ኣዶታት ማሕበረ ኢኮኖሚያዊ ኩነታት ዝምልከት ቃለመሕተት-ትግርኛ ቅጥዒ

1. ውልቃዊ ሓበሬታ

ተሓካሚ መለለዩ ቁፅሪ.፡ _____

ዕድመ፡ _____

ዕለት ምርግጋጽ ጥንሲ (ዝተከሰተሉ)፡

A: ናይ ኣዶታት ድሕረ ባይታ መሕተት (maternal background characteristics)

ተ.ቑ	ሕቶ	መልሲ	መብርሂ
1	ዕድመ	_____	
2	ኩነታትሓዳር	ዘለዋ ዘይብላ ዝተፋትሐት ሰብኣያዝሞታ ካልእእንተልዩ	
3.	ዝኑበሩሉ ቦታ	ከተማ ገጸር	
4.	ትምህርቲ ደርጃ	ምንባብን ምፅሓፍን ዘይኽእል/ዘይትኽእል- unable to write and read) ምንባብን ምፅሓፍን ዝኽእል/ትኽእል (able to write and read) 1-4 ክፍሊዝወደአ/ት 5-8 ክፍሊዝወደአ/ት 9-12 ክፍሊዝወደአ/ት ዲፕሎማ ዝወደአ/ት ዲግሪዝወደአ/ትወይምካብኡንላዕሊ	
5.	ኩነታት ስራሕ(Occupation)	ተምሃሪ/ት	

		ነጋዳይ ሓረስታይ/ ገባር ሞያተኛ ዘይሞያተኛ በዓልቲእንዳ ኮሌጅ ሙህር በዓል ሞያ ስራሕ ዘይብሉ/ላ	
7.	7A. ዓሌታዊ ውልደት ኢተፈጥሮ ኣልዎንዶ	እወ ኣይፋል	ኣይፋል እንድሕርኮይኑ መልሲ ናብ ናይ ስነ ተዋልዶ ድሕረ ባይታ መሕተት ይስገራ/ሩ
	7B. እንድሕር ኣለዎን ኣብራህርሃልና		

ስነተዋልዶድሕረባይታመሕተት

ተ.ቑ	ሕቶ	መልሲ	መብርሂ
1	በዝሒ ዝተወልዱ ህጻውነቲ	2 B. 3 C. 4 D. 5 E. 6 f. >7	
2	ናይ ጥንሲ ፕላን ዶ ትክተላ?	እወ ኣይፋል	
3	3A. For how many years did you have breast feeding for each child you have?ንሕድሕድ ቆልዑ ክሳብ ክንደይ ዓመት የጡባ ኔረን?	ትሕቲ ሓደ ዓመት ሓደ ዓመት ክልተ ዓመት ልዕሊ ክልተ ዓመት	
	3.B. ኣ ብ እዋን ጥንስክን ንክንደይ ዝኣክሉ ህፃውንቲ ኣጥቢብክን	A. 0 B.1 C. 2 D.>2	

4	4A. ቅድሚ ሕጂ ዝነበረክን ጥንሲ ውጺኢቱ እንታይይ መስል	ወሪዱ ብሂወት ተወሊዱ ምውት ኣብ ውሽጢ ማህጸን ሞይቱ ማንታ	
	4B. ቅድሚ ሕጂ ዝተወልደ ህጻን ጾታ እንታይ ኔሩ (Sex of baby of last pregnancy)	ወዲ ኣን ኢተፈጥሮኣዊ	
	4C. እቲ ዝተወለደ ህጻን ክብደቱ ክንደይ ነይሩ?		
	4D. እንድሕር ማናቱን/ካብኡ ንላዕሊ ኮይኖም ዖተኣም እንታይኔሩ		
	4E. እንትወለዱ ክብደቶም ክንደይ ዝኣክል ኔሩ?		
5.	ውልደተ ኢተፈጥሮኣዊ ኣእምሮ ዝባሃል ሰሚዕክን ዶ ትፈልጣ	እወ ኣይፋል	
6.	ኢተፈጥሮኣዊ ኣእምሮ ዘለዎ ህጻን ወሊደን ዶ ይፈልጣ	እወ ኣይፋል	እንድሕር ኣይፋል ኮይኑ መልሲ ናብ ቁፅሪ 7 መሕተት ይስገራ/ሩ
	6A. እንድሕር እወ ኢለን እንታይ ዓይነት ሽግር ኔሩ	_____ _____ንኣብነት ክፍሊ ሓንጎል ምንኣስ/ዘይምህላው፣ ንኣብነት ክፍሊ ሓንጎል ካብ ዓቕን ንላዕሊ ምዕባይ፣ ክፍሊ ሓንጎል ብኣንዲሑቀ ምውፃእ፣ ክፍሊ ሓንጎል ምውፃእ፣ ካሊእ	

7	7A. ጨረር(X-ray /CT scan)ምርመራጌረን ዶ ይፈልጣ?	እወ ኣይፋል	እንድሕር ኣይፋል ኮይኑ መልሲ ናብ C መሕተት ይስገራ/ሩ
	7B. ንክንደይ ግዜ ከ ጨረር ምርመራ ጌርክን		
	7C. ንክንደይ ግዜ ከ ጨረር X-ray / CT scan ምርመራ ጌርክን		

C ናይኣዶኩነታትጥዕናንኩነታትባህላዊኮነዘመናዊመደሃኒትምጥቃም

ተ.ቑ	ሕቶ	መልሲ	መብርሂ
1.	ናይ ባህሊ መድሃኒት ተጠቂመን ዶ ይፈልጣ	እወ ኣይፋል	2- እንድሕር ኣይፋል ኮይኑ መልሲ ናብ ቁፅሪ 2 መሕተት ይስገራ/ሩ
	1A. እንድሕር ተጠቂመን ሽም እቲ ባህሊ መድሃኒት እንታይ ይበሃል		
	1B. መዓዝ ከ ተጠቂመናሉ		
	1C. ንክንደይ ዝኣክል ተጠቂመናሉ? - What was the frequency of use of the medicines?	በቢመዓልቱንሓደሰሙን በቢመዓልቱ 2-4 ሰሙን በቢመዓልቱንሓደወርሒ ካሊእኣብርህርህኡ Other (specify) _____	
2.	2A. ሲጋራ ኣትኪከን ዶ ይፈልጣ? -	እወ ኣይፋል	3-እንድሕር ኣይፋል ኮይኑ መልሲ ናብ ቁፅሪ 3 መሕተት ይስገራ/ሩ
	2B. እንድሕር ሲጋራ ኣትኪከን መዓዝኔሩ?		

	2C. ንክንደይ ዝኣክል ብተደጋጋሚ ሲጋራ ኣትኪከን? - What was the frequency of tobacco/cigarette use?	በቢመዓልቱ ንሓደሰሙን በቢመዓልቱንሓደሰሙን በቢመዓልቱ 2-4 ሰሙን በቢመዓልቱንሓደወርሒ ካሊእኣብርህርህኦ) _____	
	2D. በቢመዓልቱ ክንደይ ዝኣክል ሲጋራ የትክካ ኔረን?	_____	
3	3A. ኣብ ጥንሲ እዋን ኣልኮል ዶ ሰትየን ይፈልጣ?	እወ ኣይፋል	4 -እንድሕር ኣይፋል ኮይኑ መልሲ ናብ ቁፅሪ 4 መሕተት ይስገራ/ሩ
	3B. እንድሕር ዝሰትያ መዓዝሰት የን?		
	3C. ንክንደይ ዝኣክል ብተደጋጋ ሚሰትየን?	በቢመዓልቱንሓደሰሙን በቢመዓልቱ 2-4 ሰሙን በቢመዓልቱንሓደወርሒ ካሊእ ኣብርህርህኦ _____	
	3D. በቢመዓልቱ ክንደይ ዝኣክል ይሰትያኔረን?	_____	
4	4A. ኣብ እዋን ጥንሲ እንታይ ሕዱር ሕማም ኣጥቂዕወን ይፈልጥ?		እንድሕር ኣይፋል ኮይኑ መልሲ ናብ ቁፅሪ 5 መሕተት ይስገራ/ሩ
	4B. መዓዝ እዋን በቲ ሕማም ተጠቂዐን ኔረን?		

	4C. ካቲ ኣብ ቁፅሪ 13 ዝጠቀስክነኣ ሕማም ንምቁጽጻር ዝተጠቀማሉ መድሃኒት እንታይ ይበሃል?	_____	
	4D. እቲ ዝጠቀስክነኣ መድሃኒት መጠኑን በዝሒ ዝተጠቀምካናሉ መዓልቲ ከ ክንደይኣዩ?		
	4E. ድሕሪ መድሃኒት ምጥቃመን ኣብ እዋን ጥንሲ ዝተሰመዐን ሕማም እንታይ ኔሩ	ኣብ ሓደ ሓደ ቦታታት ቃንዛ ዘይብሉ ናይ ሕበጥ ምልክት ኣብ መላእ ሰውነት ሕከክ ኣብ ከንፈርን ጎሮሮን ከባቢ ምቁሳል	
	4F. ኣበይ ተሓኪመን/ዲሐኒን? - Where you treated?	_____	
	4G. መዓዝን ተሓኪመን/ዲሐኒን? When were you treated?		
5	5A. ንዝኮነ ዓይነት ፀረ ፃህያይ/ ካሊእ ኬሚካል ተቃሊዕክን ዶ ትፈልጣ		ኣይፋል ኮይኑ መልሲ ናብ ቁፅሪ 6 መሕተት ይስገራ/ሩ
	5B. ንክንደይዝኣክል ግዜ ከ ተቃሊዕክን		
	5C. ንክንደይዝኣክል ግዜ ከ በዓል በቤትክን ወይ ደቅክን ተቃሊያም		
6	ፍልፍል መስተ ማይኩም እንታይ እዩ	ናይ ከተማ ቡንባ ማይ) ዝተዓሸገ ማይ) ዋሓዚ ሩባ ማይ)	
7	7A. ንዝኮነ ዓይነት ታህዋስያ ተቃሊዕክን ዶ ትፈልጣ፣ እንድህር ተቃሊዕክን መዓዝ ኔሩ ፣እንታይ ከ ይበሃል እቲ ታህዋስያን		እንድሕር ኣይፋል ኮይኑ መልሲ ናብ መግቢ መሕተት ይስገራ/ሩ
	7B. - በዚ ታህዋስያን ነዞም ዝስዕቡ ምልክታት ሕማም ከ ተጋሊፅክን ዶ ትፈልጣ	ተቅማጥ ሙቀት ሰውነት ክብደት ምቅናስ	

		ከብዲ ቁርፀት ኣይፋል	
	7C. ጫትዶ ይጥቀማእየን	Yes - እወ b. No - ኣይፋል	

ናይስነመዓዘመሕተት)

	ዓይነታት መግቢ			
ካብዞም ዝስዕቡ ዓይነታት መግቢ ዝተመገብክምዎም ምረፁ		ኣይፋል	እወ	ኣይፈልጦን
1	ዳቦ, ፓስታ, ሩዝ, ፓስታ, ብስኩት, ኩኪዎች እና ዳቦን, በቆሎ, ገብስ, ስርናይ, መሸላ, ዳጉሳ, ወይም ሌላ ከብ ጥራጥረ ዝተሰርሑ ዓይነት ምግቢታት?	0	1	2
2	ናይ ጣፍ, ስገም, ዒልቦ, መሸላ ቅጫ, ወይም ገዓት	0	1	2
3	ናይ ድንች ዓይነታት መግቢ	0	1	2
4	ማንኛውም ዱባ, ካሮት ወይም ሽኮር ድንች?	0	1	2
5	ማንኛውም ደማቅ ሓምለዋይ ቅጠላማ አትክልቲ	0	1	2
6	ዝበሰለ ማንጎ/ፓፓያ	0	1	2
7	ዝኮኑ ዓይነት ፍረምረን ኣሕምልቲን	0	1	2
8	ዝኮኑ ዓይነት ፀላም ከብዲ፣ኩሊት፣/ልቢ-ስጋ	0	1	2
9	ዝኮኑ ዓይነት ስጋ ናይ ከፍቲ፣ጠየል/ሓሰማ	0	1	2
10	ናይ ደርሆ ዓይነት ስጋ	0	1	2
11	እንቁላሊሕ	0	1	2
12	ዓሳ	0	1	2
13	ባሎንጋ, አተር, ምስር ወይም ጥራጥረ ኮይኖም ዝተሰርሑ ማንኛቸውንም ምግቢታት?	0	1	2
14	ናይ ኦቾሎኒ, ሰሊጥ ወይም ሱፍ ዘርኢታት?	0	1	2
15	ማንኛውም ኣፅቦ፣, ርሃኢ, ፃባ ምህርትታት?	0	1	2
16	ካብ ዘጥቲ/ጠስሚ ዝተሰርሑ መግቢታት	0	1	2

17	ኮካኮላ, ቡና, ሻይ, መስተታት ወዘተ	0	1	2
18	ብቪታሚን A ዝማዕበለ መግቢ	0	1	2
19	ሩዝ	0	1	2
20	ኣቮካዶ	0	1	2

ናይኣፍልጦኣዶታትፎሊክኣሲድክኒን/ ዝተመጣጠነምግቢምምጋብከምኡውንምስንጣቅዕትብቲዓንዲሑቑንክፍሊሓንልንመሕትተ

ተቁ	ሕቶ	መልሲ	መብራህርሂ
1	ተበፃሕነት ክንክን ጥዕና- ኣገልግሎት ክንክን ጥዕና ምርካብ፤ •	ስሩዕ (ተደጋጋሚ መርመራ) . • ሓሓሊፉ (ከም ኣድላይነቱ ምብጻሕ) • ሳሕቲ (ውሱን ምብጻሕ) • ዝኾነ የለን (ስሩዕ ምብጻሕ የለን)	
2	ኣብ እዋን ጥንስኺ ካብ ቤተሰብ ወይ ፈተውቲ ሓያል ደገፍ ኣለኪ ድዩ?	o እወ o ኣይፋልን። o ብመጠኑ	
3	ኣብ እዋን ጥንሲ ኣገዳስነት ፎሊክ ኣሲድ ይርድኣካ ድዩ?	o እወ o ኣይፋልን	
4	ምንጪ ሓበሬታ ብዛዕባ መመላእታ ፎሊክ ኣሲድ፤	o ወሃቢ ክንክን ጥዕና o ስድራቤት/ፈተውቲ o ሚድያ o ካልእ	
5	ብዛዕባ ጉድለት ሻምብቆ ኒውራል (NTDs) እንታይ ትፈልጡ?	o ብዙሕ እፈልጥ እየ o ቁሩብ እፈልጥ እየ	

		o ብዙሕ ኣይፈልጥን’የ	
6	ፎሊክ ኣሲድ ንNTDs ንምክልኻል ክሕግዝ ይኽእል እዩ ኢልካ ትኣምን ዲኻ?	o እወ o ኣይፋልን። o ርግጸኛ ኣይኮንኩን	
	ፎሊክ ኣሲድ ንመከላኸሊ ስንክልና ውልደት ክሳብ ክንደይ ኣገዳሲ ይመስለኩም?	o ኣዝዩ ኣገዳሲ እዩ o ብመጠኑ ኣገዳሲ እዩ o ኣገዳሲ ኣይኮነን	

ናይ ኣዶ ሕክምናዊን ኣመጋግባ ኩነታት

ግታዊዕድ መጥንሲ	_____ ብሰሙን
ዓቕን ላዕለዋይ መሓው ርድ	_____ ብሴሜ
ቁመት	_____ብሜ
ክብደት	_____ብኪግ
ዝተገበጠ መጠን ሰብነት	_____ኪግ/ሜ^2