Neural Tube Defect: Burden, Determinants, Dietary practice and Effect of Picture Based Nutrition Education toward women's Adherence to Preconception Iron-Folic Acid Supplementation in Eastern Ethiopia

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Acronyms and Abbreviations

ANC	Ante-Natal Care
ASF	Animal Source of Food
BD	Birth Defect
BMI	Body Mass Index
CI	Confidence Interval
CONSORT	Consolidated Standards of Reporting Trials
CSA	Central Statistics Agency
DDS	Dietary Diversity Score
DDU	Dire Dawa University
DHS	Demographics of Health Survey
DNA	Deoxyribonucleic Acid
EDHS	Ethiopia Demographic and Health Survey
EMDHS	Ethiopia Mini Demographic and Health Survey
EPHI	Ethiopia Public Health Institution
EU	European Union
FAS	Folic Acid Supplement
FMoH	Federal Ministry of Health
FVS	Food Variety Score
IFAS	Iron Folic Acid Supplement
IOM	Institute of Medicine

IRB	Institutional Review Board
JU	Jimma University
LB’s	Life Births
MCH	Maternal and Children
MRN	Medical Record Numbers
NICU	Neonatal Intensive Care Unit
NTDs	Neural Tube Defects
OR	Odds Ratio
PACTR	Pan African Clinical Trial Registration
PCA	Principal Component Analysis
PCC	Preconception Care
Ph.D.	Doctor of Philosophy
RCT	Randomized Controlled Trial
SD	Standard Deviation
SE	Standard Error
SPSS	Statistical Packaging for Social Science
UNICEF	United Nations International Children’s Emergency Fund
WHO	World Health Organization

Abstract

Introduction: Globally, neural tube defects (NTDs) are the top five causes of infant mortality, morbidity, and disability. NTDs are invisible to policy-makers yet have a significant emotional, economical psychological, impact on families and society while also contributing to the loss of human potential and associated with substantial mortality, morbidity, disability problems. Due to the sheer number of its population, Ethiopia is one of the four sub-Saharan African countries with the largest number of NTDs cases. However, the burden and determinants of NTDs are not sufficiently studied and the data is largely remains unknown. In addition, there is no data has been found the linkage between maternal dietary practice and developing NTDs. Furthermore, there is a lack of evidence about the practice of preconception folate supplementation to reduce the risk of NTDs among women planning to get pregnant in the existing health care system. Despite the fact that numerous evidence-based studies have been conducted to investigate the effects of preconception folic acid supplementation on reducing the risk of NTDs. However, no or a few studies has been conducted in Ethiopia to examine the effect of preconception picture-based health education on the adherence of iron folic acid supplementation to reduce the risk of NTDs among women planning to become pregnant.

Objectives: To assess the burden, determinants, dietary practice and to examine the effect of pictured based nutrition education knowledge and adherence to preconception iron-folic acid supplementation among women planning to be pregnant in Eastern Ethiopia. This study also aimed to explore the practice of preconception folate supplementation among women planning to get pregnant in the Ethiopian health care system.

Methods: The study is used both quantitative and qualitative components. The quantitative study included retrospective cohort study, matched case control, comparative and parallel randomized controlled trail. A structured checklist questionnaire with open and close-ended questions were used for data collection. Data were collected by interviewing mothers/caretakers and reviewing retrospective medical records. The study subjects were recruited in purposively-selected hospitals from Eastern Ethiopia based on caseloads. Data retrieving form was used to collect the data from neonates and terminated with neural tube defects medical records of the period 2017-2019. Data collectors were trained midwives nurses, health extension workers and other health professionals working at the selected study hospitals. For matched case control and

comparative cross sectional study, cases were ascertained using a prospective study approach, whereas controls were randomly selected from the same selected health facilities. For the case control and comparative cross sectional study, 276 pregnant women (138 cases who delivered or terminated the pregnancy due to NTDs and 138 women who gave birth apparently healthy neonate) were studied. For parallel RCT, 244 women (122 interventional and 122 control groups) who have a plan to pregnancy were included. The incidence (burden) of each case is calculated by dividing the number of cases per year by the total number of births in each hospital. A linear trend of NTDs over time and Extended Mantel-Haenszel chi-square was performed. The dietary practice was assessed using modified food frequency questionnaire (FFQ). The dietary practice of women was determined by using meal frequency, dietary diversity score (DDS), food variety score (FVS), and animal food source (ASF). Statistical procedures, such as frequency, percentage, chi-square, bivariate, and multivariate were used to analyze the data and determine the frequency of NTDs, associated factors and Odds ratio.

The qualitative study was aimed to explore the practice, challenges, and opportunities for preconception folate supplementation to reduce the risk of NTDs. In this study 45 participants included pregnant women who followed ANC, women who had a plan to be pregnant, health care providers who work at family planning service, gynecology ward and experts of maternal and child health care services (MCH). A purposive sampling method was used to select the study participants from health facilities. Content validation of the instruments was done by two experts after which the instruments were pretested and ambiguous questions were removed or revised. An in-depth interview was conducted. Field notes and audio recordings were transcribed verbatim, translated, and analyzed by inductive thematic analysis using Atlas ti.7.1.

Result: For retrospective cohort study a total of 48,567 newborn were participated and 522 newborns with NTDs were identified. The linear trends of NTDs over the three years of 2017-2019 were OR of 1 (base year), 4.3, and 8.3, respectively, [Extended Mantel-Haenszel chi-square for linear trend = *200.53 (P<0.0001)*]. Thus the burden of NTDs showed that a statistically significant increased trend over the three years. For the case control study, Illiterate (AOR=0.34, 95% CI: 0.12-0.92, P=0.034), rural residents (AOR=3.4, 95% CI: 1.18-9.78, P=0.023), having a history of elective termination (AOR=2.95, 95% CI: 1.15-7.55, P=0.023), those who suffered severe anemia in pre or early pregnancy (AOR=3.4, 95% CI: 1.17-9.87, P=0.024), having history

of fever in pre or early pregnancy (AOR=2.75; 95% CI: 1.05-7.15, P=0.038), and mothers who had an exposure to various agrochemicals (AOR=3.39, 95% CI: 1.11-10.3, P=0.032) were significantly associated with occurrence of NTDs. For comparative cross sectional study, the prevalence of good dietary practice in the study population was 29% (95% CI = 23.6%–34.3%). Illiterate women were 84% less likely to adhere to good dietary practice than women with higher education (AOR=0.16, 95% CI, 0.03-0.8), and respondents in the comparison group are more likely to have a good dietary practice than compared to these case groups (AOR=2.1, 95% CI 1.07–4.1). For parallel RCT study, the intervention group (42.6%) had adherence to iron-folic acid supplements compared to the control group (3.3%); this difference was significant ($P<0.0001$). History of NTDs affecting pregnancy, history of spontaneous abortion, and knowledge was independently associated with adherence to iron-folic acid supplement ($P<0.05$). In the qualitative study, there was no provision of preconception folic acid supplementation for those who planned to be pregnant. The challenges for this disruption in the continuum of care include the absence of clear policy, program, and guidelines, lack of knowledge among caregivers and women, unfavorable attitude and belief, the high workload of providers, and poor adherence.

Conclusion: NTDs is a significant public health burden in the study area, with the most common forms being anencephaly and Spinal bifida. Illiterate, rural residents, having a history of elective termination, those who suffered severe anemia in pre or early pregnancy, having history of fever in pre or early pregnancy, and mothers who had an exposure to various agrochemicals were significantly associated with occurrence of NTDs, history of NTDs affecting pregnancy, history of spontaneous abortion, and knowledge was independently associated. The findings suggest that primary preventative strategies should be strengthened by actively promoting preconception care services, such as preconception iron folic acid supplement, as well as food fortification and good dietary practices. This study highlighted the need for policy, program, guidelines, and a structure for preconception care services in the country.

Key words: Neural tube defects, Burden, Determinants, Dietary practice, matched case control, RCT, qualitative study, Ethiopia

Chapter 1: General introduction

1.1 **Overview**

1.1.1 General overview of Neural tube defects

Neural tube forms are a central issue in developmental biology. Vertebrate neurulation is a complex morphogenesis process that requires the coordination of many cellular and molecular events and is regulated by more than 300 genes in mammals (Wilde et al., 2014). Neural tube closure is dependent on the methionine cycle and folate cycle (Leung *et al*., 2017). Folate acts as a cofactor for an enzyme involved in DNA and RNA biosynthesis and is also a supplier of methyl groups to the methylation cycles (Scott et al., 1994).

NTDs arise secondary to abnormal and complicated embryonic development of the spinal cord and brain due to the failure of neurulation or alterations in the morphogenesis or histogenesis of the nervous tissue (Blencowe et al., 2018a, Moore et al., 1982). NTDs can occur in the cranial region (anencephaly, encephalocele), spinal region (spina bifida), or in combination (craniorachisis or complex phenotypes) (Copp and Bernfield, 1994, Wallingford *et al*., 2013). In spinal Bifida, the unborn baby's spine does not close completely to protect the spinal cord (Lindsay et al., 2016). Anencephaly is fatal; many children with anencephaly are stillborn or die shortly after birth because they are often born without part of the skull and brain and die shortly after birth (Hussain et al., 2012, Saheb et al., 2012, Brierley, 2013, Flores et al., 2014). Fifty percent have a life expectancy of between a few minutes and one day, and 25% only live up to 10 days (Jaquier et al., 2006). Encephaloceles are rare congenital disabilities associated with skull defects characterized by a partial lack of bone fusion, leaving a gap through which a portion of the brain sticks out. In some cases, cerebrospinal fluid or brain members may also protrude through this gap. Encephaloceles may be classified as occipital (Manning et al., 2000, Sdano and Pensak, 2005).

NTDs occurs early during pregnancy and happens when the neural tube, which encases the brain and spine, does not close properly between 21 and 28 days following conception; they happen in the first month of pregnancy, often before a woman even do not knows that she is pregnant (Greene and Copp, 2014, Persad et al., 2002, Lazareff, 2010, Butterworth and Bendich, 1996). It is one of the significant causes of infant and child mortality, morbidity, and long-term disability, as well as emotional, psychological, and significant emotional impact on affected families (Greene and Copp, 2014).

1. 2. The burden of NTDs (Epidemiology of NTDs)

NTDs are the leading cause of fetal loss and disabilities in neonates, and it is considered a significant public health problem (Nasri et al., 2014, Bourouba et al., 2018). Morbidity due to NTDs is a crucial consideration since, as the development of public health continues, the prevalence rates among babies born with NTDs are likely to increase. NTDs are relatively uncommon, with a global prevalence among live births. Among the 194 WHO member states, the percent reporting within each region is as follows: African (8/47; 17%), Eastern Mediterranean (12/21; 57%), European (26/53; 49%), Americas (15/35; 43%), South-East Asian (4/11; 36%) and Western Pacific (9/27; 33%) (Zaganjor et al., 2016). In addition, in the US of 1 in 1200 and a worldwide prevalence ranging from 1 in 1,000 (in Europe and the Middle East) to 3-5 in 1,000 (in northern China as of 2014 with folate supplementation campaigns, bringing the prevalence down from 10 per 1,000 for years 2000-2004) (Blencowe et al., 2018a, Salih et al., 2014b, Khoshnood et al., 2015, Liu et al., 2016).

WHO estimates that, every year, globally, congenital anomalies affect an estimated 1 in 33 infants, result in 3.2 million birth defect-related disabilities, and account for an estimated 270,000 newborn deaths. NTDs are among the top five most congenital severe anomalies (Organization, 1987, Blencowe et al., 2018b). Every year worldwide, NTDs develop in about 300,000 pregnancies (Organization, 2013b, Turin, 2010, Mehl and Labrique, 2014, Blencowe et al., 2018b). The incidence of NTDs in developing countries has been up to four-fold higher than in developed ones. Of the 300,000 new NTDs annually occur, 40,000 of these, over 13%, occur in Sub-Saharan Africa alone, though this region has only 13 percent of the global population affected with NTDs (Ren et al., 2006, Cherian et al., 2005). The prevalence of NTDs in African births high prevalence is found in Algeria, Ethiopia, Eritrea, and Nigeria (Oumer *et al*., 2021).

In Kenya a study showed that 6% of deaths in children aged less than three years are due to NTDs, and the prevalence is 20 per 10,000 live births (Githuku et al., 2014). Preliminary studies done in different regions of Ethiopia revealed that the incidence of NTDs is dramatically increasing in recent years. The burden of NTDs is relatively high in eastern Ethiopia (107.5 per 10,000) (Berhane and Belachew, 2022), next to northern Ethiopia as compared with the other regions (131 per 10,000) (Berihu et al., 2018). A low incidence rate is reported in a retrospective study conducted in two teaching hospitals in Addis Ababa, Ethiopia (61/10,000) (Sorri and Kassa, 2015).

1.3. Risk Factors for NTDs

Any woman who could become pregnant is at risk of having a baby with NTDs. It is caused by several etiologic factors. The etiology of NTDs is complex, multifactorial in origin, and poorly understood, and it including environmental (Greene and Copp, 2014, Sun et al., 2011, Nnadi and Singh, 2016), nutritional, lifestyle, and genetic factors or a combination of these components that (Greene and Copp, 2014, Sun et al., 2011, Nnadi and Singh, 2016, Githuku et al., 2014, Greene et al., 2009, Aguiar et al., 2003, Salih, 2014, Gelineau-van Waes et al., 2005, Missmer et al., 2006, Gelineau-van Waes et al., 2009, Turner et al., 2012) contribute to the elevated incidence of NTDs. However, even if these risk factors are identified, different risks may exist even within the same region or country.

1.3.1. Nutritional factors

.An increasing body of evidence suggests that folic acid may help prevent NTDs (Lanphear *et al*., 2005, Coppen and Bolander-Gouaille, 2005, Wald *et al*., 2010),(Wu et al., 2006). The deficiency of folates at the cellular level may be responsible for NTDs due to disturbed bioavailability of folates, and other nutritional factors, such as trace elements, also influence the incidence of NTDs (Copp *et al*., 2013).

Folic acid is necessary for cell division and development because it aids purine, pyrimidine, and nucleoproteins formation as well as methylation processes. Folic acid deficiency hinders DNA synthesis, mainly affecting rapidly dividing cells, such as bone marrow and the gut. Reduced folic acid levels lead to higher homocysteine levels in pregnancy and are also linked to the development of NTDs (Gupta, 2004).

However, several lines of evidence suggest that not only folates but also choline, B_{12}, and methylation metabolisms are involved in NTDs. Decreased vitamin B_{12} and increased total choline or homocysteine in maternal blood have been shown to be associated with increased the risk of NTD. Women with the lowest blood level of B_{12} shortly before and after conception had five times the risk of having a child with an NTDs compared to women with the highest blood level of B_{12} levels (Wang et al., 2012, Molloy et al., 2009, Senousy et al., 2018, Chen et al., 2019, Wahbeh and Manyama, 2021, Fofou-Caillierez et al., 2019). Women who consume little or no meat or animal-based foods are the most likely group of women to have low B_{12} levels, along with women who have intestinal disorders that prevent them from absorbing sufficient amounts of B_{12}.

From large population-based case-control and different experimental studies investigating that methylation of DNA can be influenced by dietary contributions of methyl donors other than folic acid offer additional clues about the complex etiologies of NTDs such as choline, betaine, and methionine, which are highly interrelated in methyl-group metabolism, and an alteration in one affects the others (Shronts, 1997, Genisca et al., 2009). These nutrients, similar to folic acid, are involved in one-carbon (methyl donors) metabolism for methylation of homocysteine to methionine and are utilized for the synthesis of cell membrane phospholipids; choline is a precursor of the neurotransmitter acetylcholine (Zeisel, 2008, Shronts, 1997, Mason, 2003).

Thus, choline deficiency could impact folate and homocysteine metabolism (Zeisel, 2006). Other dietary parameters are also involved in DNA methylation, including vitamin B_6, and serine (Slattery *et al*., 1997). Evidence from both experimental and epidemiological studies shows that inositol supplementation can reduce the risk of NTDs (Levy et al., 2019, Greene et al., 2017, Jin, 2017, Viswanathan et al., 2017, Wilde et al., 2014).

Iron is an essential nutrient in pregnancy, and pregnant women are vulnerable to low iron status. Studies reported that low maternal iron intake (ferritin) (<12 μg/L)during early pregnancy is an independent risk factor for NTDs (Kakebeen and Niswander, 2021, Tsiklauri et al., 2019, Al Zoubi et al., 2019, Nakade et al., 2020, Kenar et al., 2020, Kancherla et al., 2021, Bhadra and Deb, 2020, Felkner et al., 2005, Groenen et al., 2004). There is some evidence that iron may affect other nutrient levels. Studies have indicated a relationship between folate and ferritin in that ferritin can modulate folate availability via cellular one-carbon pathways (Oppenheim et al., 2001a, Suh et al., 2000). Much of the experimental evidence supporting the role of iron in the prevention of NTDs is derived from studies of mice with hypomorphic mutations in the gene encoding the iron transporter ferroportin gene. Adding iron to folic acid for periconceptional use may improve iron status and is likely to prevent NTDs.

Consumption of sprouted potatoes was associated with elevated odds of total NTDs (Ni et al., 2018b, Cuschieri and Calleja-Agius, 2020, van den Brand et al., 2022). Maternal consumption of sprouted potatoes during the periconceptional period may increase the risks of NTDs and OFCs. Potato is commonly consumed worldwide; improper preservation and use should be a matter of concern regarding the potential teratogenicity (Li et al., 2006, Ni et al., 2018a, Liao et al., 2010).

Fumonisin B_1 (FB_1) is a mycotoxin produced by the fungus Fusarium verticillioides, a common contaminant of maize worldwide. An association between ingestion of FB_1 contaminated maize during early pregnancy and increased risk of congenital disabilities, specifically NTDs (Marasas et al., 2008, Eze et al., 2018, Chen et al., 2018, Oliveira and Vasconcelos, 2020, Awuchi et al., 2022, Wu, 2021). Aflatoxin to even low doses may increase vulnerability to congenital disabilities. For example, when diets are deficient in vitamins A, C, or E, or selenium (all of which protect against the toxic effects of aflatoxin), the detoxifying system for aflatoxin may be impaired, increasing the production of epoxides (Galvano *et al*., 2001, Alpsoy and Yalvac, 2011).

1.3.2. Environmental factors

Even though attention has been paid to carbon metabolism, especially the finding that maternal supplementation with folic acid has reduced the risk of NTDs, environmental and genetic factors also influence neural tube closure (Njamnshi et al.) through a direct effect on the biology of embryonic metabolism. Environmental factors influence neural tube closure (Wilde *et al*., 2014) and failure of closure leads to neural tube defects (NTDs), the second-most common congenital disability worldwide.

Children of mothers with insulin-dependent diabetes mellitus and obesity have a risk of NTD. A twofold to threefold (4–9%) increased incidence of NTDs compared with the general population (Mills et al., 1988, Agopian et al., 2013a, Vena et al., 2021, Bourouba et al., 2018, Mowla et al., 2020, Facchinetti et al., 2020). On the other hand, women who had a previous history of abortions are associated with the development of NTDs (Gashaw et al., 2021, Pei et al., 2019, Tesfay et al., 2021, Tadesse et al., 2020, Gedefaw et al., 2018).

The prevention and treatment of NTDs with folic acid anti-folate drugs used during pregnancy had deleterious effects and increased the incidence of NTDs and other congenital defects (Hernández-Díaz et al., 2000). A number of therapeutic drugs have been implicated as teratogens and their adverse effects linked to the timing of use during pregnancy, dose, genetic susceptibility and other factors (McLone, 2003, Tung and Winn, 2011, Yerby, 2003). These drugs include thalidomide (which causes severe limb reduction birth defects and was formerly used to combat morning sickness, but is also used to treat leprosy, macular degeneration in HIV/AIDS and psoriasis); misoprostal (an anti-gastric ulcer medication that is used illegally to induce early abortion, and is linked with several birth defects associated with vascular disruption); anticonvulsant drugs (associated with major malformations, including neural tube defects, microcephaly, intellectual disability, growth restriction, and malformations of the face and fingers); and anticoagulants (linked to nasal hypoplasia, stippling of bones, optic atrophy, microcephaly, growth and intellectual disability, and fetal and neonatal hemorrhage) (Adab *et al*., 2001, Arpino *et al*., 2000, Achon *et al*., 2000, Dos Santos *et al*., 2009, Belcastro and Striano, 2012, Holmes *et al*., 2001, Leamon and Low, 2001, Werler *et al*., 2011). Another risk factors for neural tube defects are maternal exposure to valproic acid taken by mothers who have epilepsy (Agopian et al., 2013a, Tung and Winn, 2011, Yerby, 2003).

Maternal hyperthermia in early pregnancy is an increased risk factor for NTDs (Kerr *et al*., 2017, Pei *et al*., 2015, Suarez *et al*., 2004, Graham Jr and Ferm, 1985, Lundberg *et al*., 2003, Li *et al*., 2007). Other associated risk factors include hyperthermia following episodes of maternal fever or heat exposure (Milunsky et al., 1992, Asamoah et al., 2018, Kerr et al., 2017, Salih et al., 2014a).

Different experimental studies in providing evidence that hyperglycemia lies within the pathogenic pathway of NTDs, and increasing diet quality based on either index is reduced risks of NTDs (Scott et al., 2015, Yazdy et al., 2009, Loeken, 2005, Vena et al., 2021, Bitew et al., 2020, Wahbeh and Manyama, 2021, Rosenbluh and Walfisch, 2021). Significant risks for anencephaly included the area of residence, family history of anencephaly, use of folic acid in pregnancy, maternal age born from women living in urban and in Dega, intake of herbal medicine; drinking alcohol, Tela (Local Area), high BMI index (obesity) (Shabana et al., Tsehay et al., 2019, Ornoy et al., 2015, Taye et al., 2018a, Vena et al., 2021, Bitew et al., 2020, Facchinetti et al., 2020, Corona-Rivera et al., 2021, Takougang, 2018).

Exposures toward organic solvents; agricultural chemicals, including pesticides; water nitrates; heavy metals such as mercury; ionizing radiation; and water disinfection by-products (Yang et al., 2014, Rana et al., 2017, Kalra et al., 2016, Suarez et al., 2012, Blatter et al., 1994, Berihu et al., 2019, Rull et al., 2006b, Finnell et al., 2021) are independent factors for incidence of NTDs. Most food products are produced using these agrochemicals, and people are exposed to food and water consumption.

Studies have shown that families of low socioeconomic status have increased rates of NTDs. (Boulyjenkov, 1998, KIng, 2008, Sikander et al., 2022, Bhandari and Thada, 2021, Wahbeh and

Manyama, 2021, Salazar-Reviakina et al., 2021, Gashaw et al., 2021, Bitew et al., 2020, Singh et al., 2019). This finding has particular significance for middle- and low-income countries.

Sex is a determinant for developing NTDs and is more prevalent in male babies than in females (Liu et al., 2018a, Poletta et al., 2018, Liu et al., 2018b, Berihu et al., 2019). Cousin marriages with 1st degree and maternal relationship with a cousin (BUTT et al., 2013) increased after exposure to folate antagonist medications; and the presence of high anti-bodies titer to folate receptors in women with a previously affected child with NTDs (Vergel *et al*., 1990, Al Rakaf *et al*., 2015). Having a previous pregnancy with a NTDs or having a female relative with a baby with a NTDs increases the risk (Viswanathan et al., 2017, Donini et al., 2013). Racial-ethnic (van der Linden et al., 2006a) were identified as potential sources of variability in the prevalence of NTDs. Maternal diabetes (Kappen et al., 2011), and maternal "flu" in the first trimester (Luteijn et al., 2013, Oster et al., 2011), certain parental occupations (Podgórski et al., 2017, Fathe, 2014), are also risk factors of developing NTDs. Omer *et al*., 2016, observed that the intake of folic acid by the mothers usually starts after conception due to a lack of awareness of its importance (Omer *et al*., 2016).

1.3.3. Genetics factors

Chromosomal anomalies can be associated with NTDs, but represent only 2% to 16% of isolated NTDs (Lynch,_2005). In mice, so far there are more than 300 genes have been linked to NTDs (Wilde *et al*., 2014, Padmanabhan, 2006, Lynch, 2005). Moreover, several epidemiological and animal experimental studies reported that the causes of most NTDs are still unknown (Postoev *et al*., 2015, Oliveira *et al*., 2011). Nutritional factors, together with the environmental factors, might interact with genetic factors during the early development of the embryo, which could lead to the occurrence of the NTDs (Li *et al*., 2013, Khattak *et al*., 2008, Nili and Jahangiri, 2006).

1.4. Prevention of NTDs

1.4.1. Implement Preconception care service

Preconception care service (PCC) represents an opportunity for pregnant women who plan to get pregnant and throughout subsequent pregnancies to receive a broad range of health promotion and prevention services and any required medical treatment (Organization, 2013a, Mason et al., 2014). Optimal healthcare before pregnancy is essential for the mother's well-being and the fetus's development. It is also the earliest connection between maternal and infant health and a window of opportunity to reinforce women's health before during, and after pregnancy through early detection and risk management (Van Der Zee et al., 2011, Johnson et al., 2006). Preconception care offers a variety of service packages in the sense of prevention aimed at mothers and newborns (De Weger et al., 2011, Temel et al., 2015), including micronutrient supplementation, including iron-folic acid supplementation (Organization, 2013a). IFA supplementation before pregnancy can improve birth outcomes and increases the iron and folic acid status in women during pre-pregnancy while addressing the iron deficiency that affects some menstruating women and adolescents.

1.4.2. Provision of preconception folic acid and mandatory food fortification with staple foods.

Folate is an essential water-soluble B-vitamin provided in the diet, particularly from fruits and vegetables. One of the major breakthroughs in NTDs prevention has been the evidence that maternal periconceptional folate supplementation can reduce the risk of NTDs (Safi et al., 2012, Scott et al., 1995, Laurence, 1989, Schorah et al., 1980, Group, 1991, Bhide, 2021, de la Fournière et al., 2020).

The clinical application of folic acid supplementation/intake to prevent NTDs has been well proven for the last 20-25 years (Barua *et al*., 2014). Folic acid, a B vitamin, can reduce the risk of NTDs by 50-70% if taken before conception (Cavalli, 2008, Berry *et al*., 2010, Berry *et al*., 2000), and insufficient folate intake is by far the most significant contributor to NTD occurrence worldwide (McNulty et al., 2019, Czeizel, 2010, Czeizel et al., 2011b, Talaulikar and Arulkumaran, 2011). Also, epidemiologic studies have revealed that folic acid supplements and/or a vitamin-B taken before conception and continued for at least three months during pregnancy reduce the occurrence of NTDs (Nasri et al., 2016, Berry et al., 2010). Folic acid/folate intake can be increased either by consuming a folic acid-containing supplement or by consuming staple foods fortified with folic acid and a diet high in natural food of folate (Githuku et al., 2014, Nasri et al., 2015).

1.5. Why this Ph.D. Dissertation Research Necessitated? What is the Gap it addressed?

Worldwide, NTDs are among the top five most congenital abnormalities (Githuku *et al*., 2014). NTDs have become a global problem of public health significance in the past few decades. Because congenital abnormalities are a major cause of under-5 mortality, adequate surveillance data are needed for prevention and evaluation purposes. However, data on NTDs are limited in lower-income countries and many of which member states (120/194) did not have any data on NTDs (Zaganjor *et al*., 2016, WHO, 2010). Ethiopia is one of the countries where population estimates of the burden of birth defects are not routinely collected. WHO estimated the incidence rate of NTDs in Ethiopia to be 22 per 10,000 births (Botto and Mastroiacovo, 2012).

The burden of NTDs data depends on only a few studies, which were conducted primarily in small areas of the country. As a result, the country's toll of NTDs remains underestimated. Therefore, this study was intended to estimate the trend and burden in the area. In Ethiopia few studies are conducted and tried to identify the determinants risk factors in different area. However, these studies are not well established the risk factors of NTDs in the real contextualizes of the whole country including eastern Ethiopia. There is no study conducted about the determinants for development of NTDs in this area. Therefore, this study was intended to investigate the risk factors for development of NTDs in the study area.

Ensuring proper dietary practice is critical to optimizing the health of mothers and newborns. Nutrition has been shown to influence the risk of neural tube defects (NTDs) due to the role of folic acid and other nutrients. It is intervening before pregnancy has been increasingly emphasized to ensure an adequate nutritional status at conception and early pregnancy. This period is critical because it defines mother-offspring nutrient division and the composition of the placenta (King, 2016). We hypothesized that compromised dietary practice would be associated with an increased risk of having offspring with NTDs. Therefore, this study was intended to determine the prevalence of the dietary practice and investigate the association between dietary practice and the occurrence of NTDs in the study area.

Different programs, strategies, and policies at global, regional, and national levels have been tried in the past to address the burden of congenital abnormalities at significant and NTDs in particular. Preconception folic acid supplementation is crucial because of the implications for preventing NTDs and averting adverse pregnancy outcomes (Thompson et al., 2017, Kumar, 2009, Greenberg et al., 2011). In Ethiopia, different strategies are implemented in the health system, and there has been an improvement in maternal and child health status (FMoH, 2015b, UN, 2015, FMoH, 2015a, Flores *et al*., 2015). However, the problem related to preconception folic acid or iron-folic acid supplements to reduce the risk of NTDs has not been properly addressed in developing countries, including Ethiopia (Njamnshi *et al*., 2008, Sharma *et al*., 2018, Morris *et* *al*., 2018, Gedefaw *et al*., 2018). Therefore, this study was intended to evaluate the practice, challenges, and opportunities for preconception folate supplementation to bridge the gap in the continuum of care to reduce the risk of neural tube defects among women planning to become pregnant in the existing health care system.

Recently, the rates of unintended pregnancy have declined worldwide, with increasing access to and use of contraception (Bearak *et al*., 2018, Sedgh *et al*., 2014, UN, 2020) and the proportion of women who have an unmet need for modern contraception is highest in sub-Saharan Africa (UN, 2020). In Ethiopia, the contraceptive prevalence rate (CPR) among married women aged 15-49 is 41% (ICF, 2019). In Ethiopia, 35% of women who are modern contraceptive users discontinued their method before the removal date (ICF, 2016). Many factors were identified, that desire for pregnancy is the second most common reason for early discontinuation, and it accounts for almost 27.3% (Nega *et al*., 2021, Bekele *et al*., 2015, Burusie, 2015, Belete *et al*., 2018). Pregnant women of any age are at risk for developing NTD-affected fetuses. Nonetheless, it is impossible to predict which women will have an NTDs-affected pregnancy (Bibbins-Domingo et al., 2017). Provision of preconception of iron-folic acid supplements intake is crucial and effective in increasing red blood cell folate concentrations, which plays a vital role in lowering the risk of NTDs and anemia (Stevens et al., 2013, WHO, 2012, WHO, 2015b, Cordero et al., 2015).

Different laboratory-based researches provide evidence that intake of IFAS over at least 12 weeks before conception by women of child-bearing age increased the level of red blood cell folate, potentially lowering the risk of neural tube defect-affected pregnancy (Hao et al., 2008, Bationo et al., 2019, Cordero et al., 2015, Crider et al., 2014, Lamers et al., 2006, Organization, 2015, De Bruyn et al., 2014, Brämswig et al., 2009, Norsworthy et al., 2004, Samson et al., 2020, WHO, 2015b, WHO, 2015a). Also, optimal preconception of IFAS could substantially reduce the risk of iron deficiency anemia and congenital anomalies such as NTDs (Stevens et al., 2013, WHO, 2012). Based on this concrete evidence, we expect women who adhere to IFAS to be more protective against NTDs and another adverse outcome if they become pregnant (Stevens et al., 2013, WHO, 2012).

Therefore, the preconception of iron-folic acid or multivitamin supplementation for planned women is a recent critical breakthrough in the primary prevention of NTDs (Czeizel *et al*., 2011a). Prevention ensures that this multifactorial burden does not have to happen (Postma et al., 2002, Yi et al., 2011).

Ethiopia is one of the countries with the most significant burden of micronutrient deficiencies, and as a result, it has become a significant public health problem (Haidar et al., 2010a, Ababiya and Gabriel, Herrador et al., 2014, Tsegaye Ababiya, 2014). Folic acid coverage among reproductive-age women is low, and 1 in 3 women have a folic acid deficiency (Haidar et al., 2010b) and 84% of women of reproductive age had low red blood cell folate (Haidar et al., 2010b, Bationo et al., 2019, EPHI, 2017, Habte et al., 2015). In Ethiopia, the Provision of IFAS is a primary strategy for pregnant women to prevent maternal death due to anemia and other adverse birth outcomes (FMoH, 2014). However, iron-folic acid awareness and adequate intake remain relatively low in different regions, and different barriers to the program's success were identified (Boti et al., 2018, CSA and ICF, 2017). Poor knowledge about IFAS (Sendeku et al., 2020), inadequate counseling (Gebremichael and Welesamuel, 2020, Galloway et al., 2002), and more proximal barriers such as the absence of partner support (Aguayo et al., 2005, Gebremichael and Welesamuel, 2020, Taye et al., 2015), and forgetting (Gebremichael and Welesamuel, 2020, Kulkarni et al., 2010, Zavaleta et al., 2014), negative perceptions from social factors that influence community and individual preferences (Bali *et al*., 2017), non-provision of education for management of side-effects (Galloway *et al*., 2002) are the primary factors for low adherence to IFAS. Lessons learned from existing health care strategy; alternative approaches are required to initiate preconception of IFAS intake (Kulkarni et al., 2010). Evidence from elsewhere shows that nutrition education intervention is an essential step and tool to improve adherence to micronutrient supplementation and has a positive effect on reducing the risk of NTDs (Taye et al., 2015, Girard and Olude, 2012, Nagata et al., 2012, Martin et al., 2017, WHO, 2016, Cena et al., 2008, Temel et al., 2014, Stephenson et al., 2018, Forrest, 1994). Therefore, nutrition education on preconception iron-folic acid supplements is a very critical intervention to minimize the impacts of NTDs. In addition to the implementation of an effective life change intervention is effective to adhere to iron-folic acid supplements (Stoneham and Edmunds, 2020). In Ethiopia, the health care system has a fertile ground to implement for those women who have a plan to become pregnant. However, there is no responsiveness to promote preconception iron-folic acid supplements and preventive measures to reduce the risk of NTDs and other adverse pregnancy outcomes (Wendimagegn and Bezuidenhout, 2019). Therefore, the fourth reason for this study is to demonstrate the effect of picture-based health education and counseling on knowledge and adherence to preconception of iron-folic acid supplementation among women planning to become pregnant to prevent NTDs and others related adverse maternal outcomes (Figure 1.1).

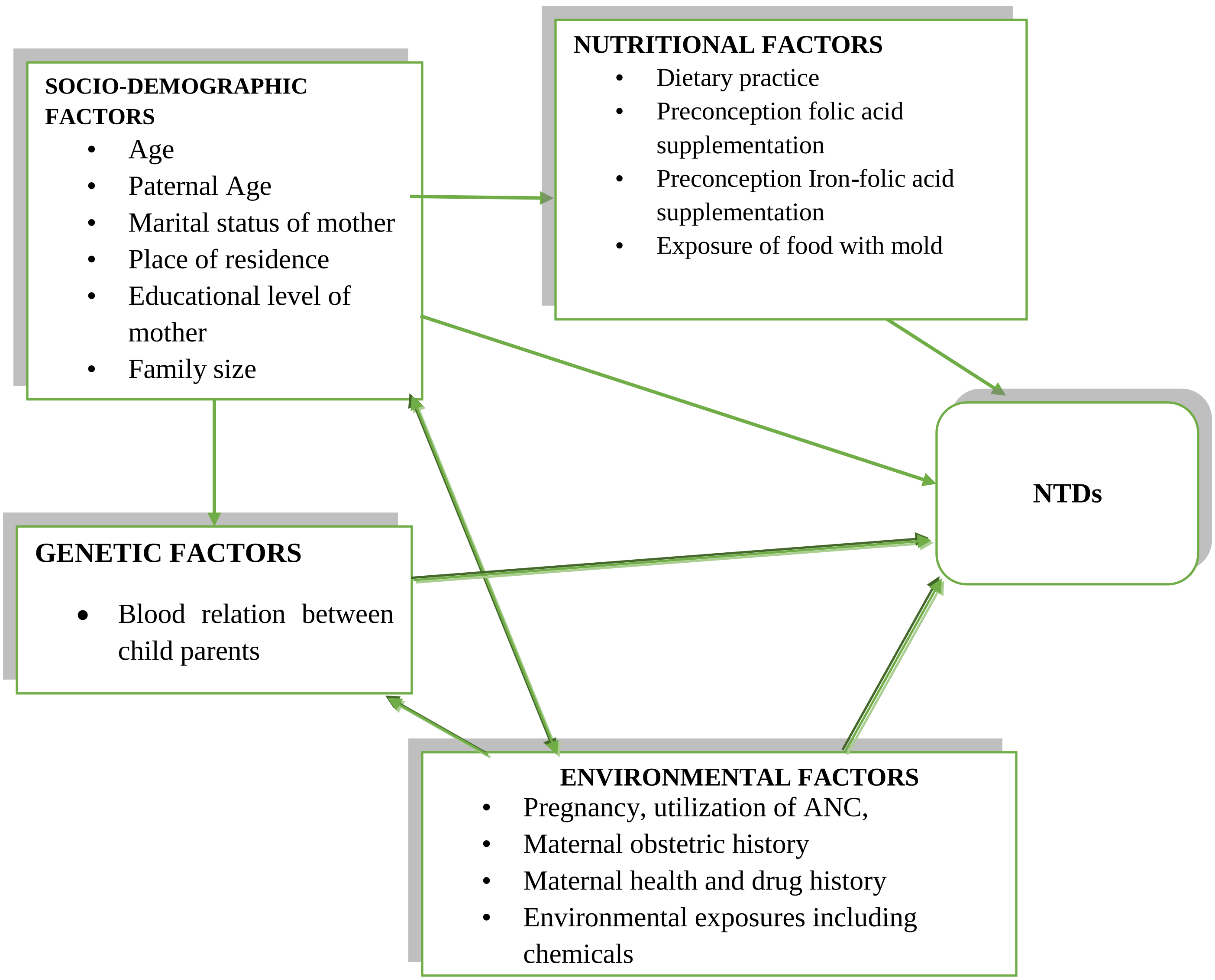

Figure 3: Schematic presentation of conceptual framework of interrelated etiologic factors of NTDs, 2022 (Adapted from different literature review).

1.6. Aims of the study

1.6.1. Overall aims of the study

The overall aim of the study is to obtain an up-to-date picture of the neural tube defect, the dietary practice of women, determinates of NTDs, evaluating the practice, challenges, and opportunities for preconception folate supplementation to bridge the gap in the continuum of care to reduce the risk of neural tube defects among women plan to pregnancy in the Ethiopian health care system, and the examine the effect of a pre-conception picture-based education on knowledge about and adherence to iron-folic acid supplementation among women who had a plan to be pregnant in eastern Ethiopia.

1.6.2. Specific objectives of the study

1. Determine the trend and burden of different type neural tube defects in eastern Ethiopia among neonate with NTDs.

2. Identify the determinants of NTDs among women who gave birth in hospitals in Eastern Ethiopia

3. Identify the prevalence of dietary practice and the association with occurrence of NTDs among case and control groups of women who delivered neonate with NTDs and without NTDs (apparent healthy neonate) counterparts in a setting with high burden of NTDs

4. Evaluate the practice, challenges, and opportunities for preconception folate supplementation to bridge the gap in the continuum of care to reduce the risk of neural tube defects among women plan to pregnancy in the Ethiopian health care system in eastern Ethiopia

5. Determine the effect of picture based health education and counseling targeting on knowledge about and adherence to preconception of Iron-folic acid supplement among women planned to be pregnant in eastern Ethiopia.

1.7. Hypotheses of the study

Research Question

This research focuses on the burden, determinants, and implementation of preconception iron-folic acid. The research questions that this study address is,

1. What is the trend and burden of neural tube defects in eastern Ethiopia?

2. What are the determinants for occurrence of NTDs in eastern Ethiopia?

3. What are challenges and opportunities to practice preconception folic acid supplement in health care system?

Hypothesis for intervention

1. There is a significant difference inn dietary quality among women who delivered newborns with and without NTDs.

2. Preconception picture based health education and counseling for 12 weeks has a positive effect for adherence to iron-folic acid supplement.

1.8. Outline of the Dissertation

Chapter 1: gives the definition, indicators and an overview of NTDs from a global, regional and Ethiopian perspective, **Chapter 2**: outlines the study setting, the methodology and the measurements, **Chapter 3**: presents the trend and the burden of neural tube defects in Eastern Ethiopia in newborns with NTDs, **Chapter 4**: determined the risk factors of NTDs among women who delivered neonate with NTDs and without NTDs, **Chapter 5**: Identified the prevalence of dietary practice and the association with occurrence of NTDs among case and comparison groups of women who delivered neonate with NTDs and without NTDs (apparently healthy neonate) counterpart in a setting with high burden of NTDs, **Chapter 6**: presents the evaluation of the practice, challenges, and opportunities for preconception folate supplementation to bridge the gap in the continuum of care to reduce the risk of neural tube defects among women plan to pregnancy in the Ethiopian health care system,. **Chapter 7**: examined the effect of picture based health education and counseling targeting on knowledge about and adherence to preconception of Iron-folic acid supplement among women planned to be pregnant in eastern Ethiopia using a randomized controlled trial. Finally, **Chapter 8**: integrates the results of the various studies and presents the policy, research, and pragmatic implications of the results for nutrition programming. Schematic representation of the dissertation is shown in Figure 1.2.

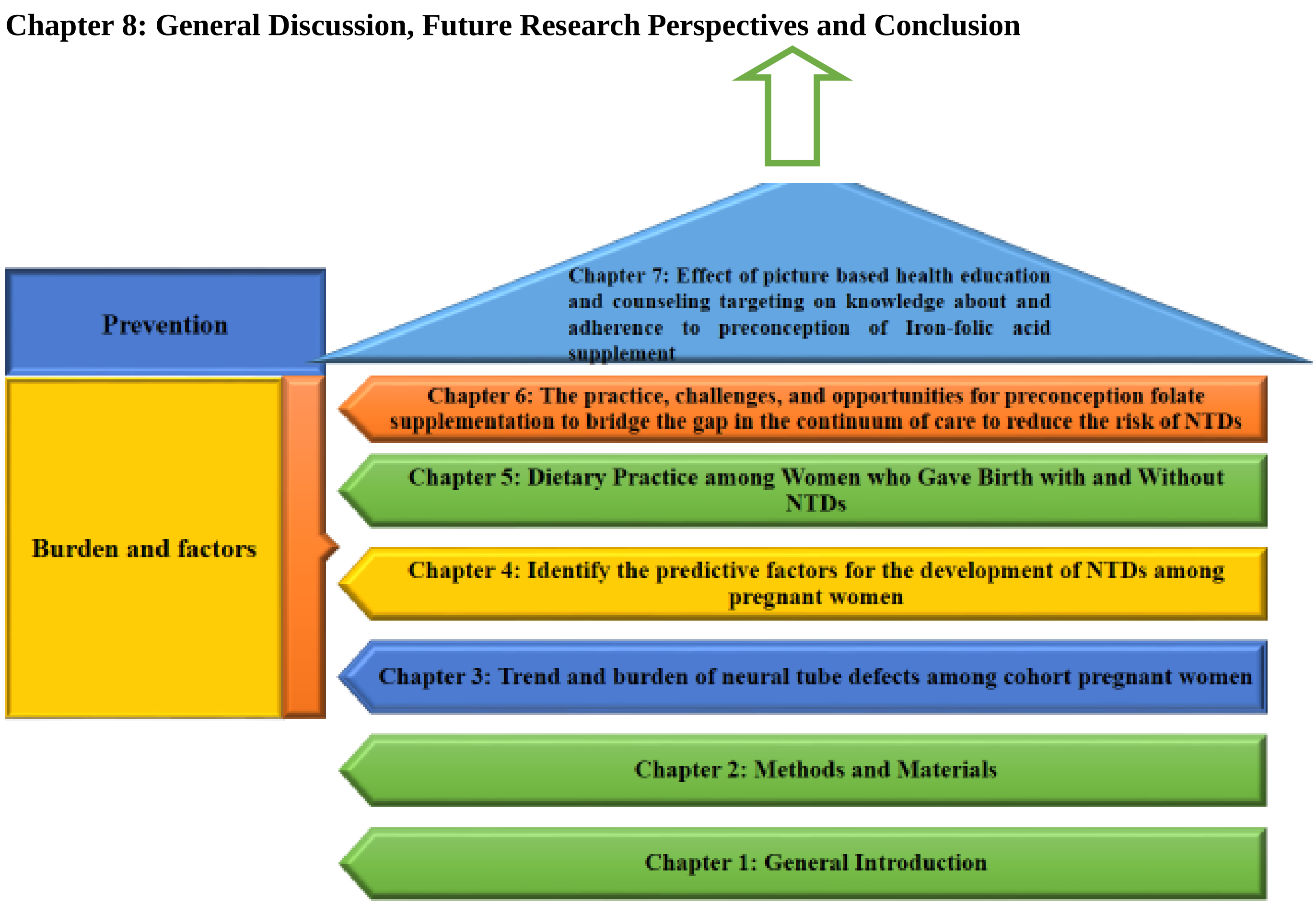

Figure 1.2. Schematic presentation of the dissertation

Chapter 2: Materials and Methods

2. Material and methods

2.1. Study setting

The study was carried out in Dire Dawa City administration, in the regional state of Harari and in the city of Adama in eastern Ethiopia. The Dilchora Referral Hospital is found in Dire Dawa city, 515 kms east of Addis Ababa and cares for about five million people from Dire Dawa and the neighboring Oromia and Somali regions. Hiwot Fana Specialized Teaching Hospital is located in Harar City, 526 kilometers east of Addis Ababa, and provides services to the entire community of Eastern Ethiopia. In addition, the hospitals also serve as teaching centers for students of health and medical sciences. Adama Hospital Medical College serves as a referral center for more than 6 million people from various regions and adjacent areas and regions, including Afar, Amhara and Somali and serve as teaching centers for students of health and medical sciences.

2.2 Data collections tools, measurement and collectors

2.2.1 Quantitative Study

Data was collected through face to face with interview administered pretested structured questionnaire via a mobile phone application with KoBo Tool Collect. A structured, closed and open questionnaire was designed for the quantitative study. It was written in English and translated into the local language, including Amharic, Affan Oromo and Somali language to allow easy and understandable communication with participants. The questionnaire had nine sections including socio-demographic, ANC service, folic acid, IFA utilization, maternal birth history, maternal health and drug history, environmental factors of pregnant mothers and partners, nutritional assessment (using validated qualitative FFQ), knowledge of NTDs, folic acid , IFA and newborn status. For retrospective cohort study, checklist was designed to obtain data that encompasses such as, some demographic, gestational age at the time of birth, use of folic acid and medication during or early pregnancy, hypertension, diabetes and other maternal diseases and time of diagnosis of NTDs. Senior midwife nurses and health extension workers were collected the data.

2.2.1.1 Dietary assessment

A previously validated Food frequency questionnaire (FFQ) was used and the questionnaire included 125 locally available foodstuffs of the most commonly consumed in the community after consultation of key informants. FFQ was pretested and necessary modifications made before actual deployment to generate data.

A Minimum Dietary Diversity for Women (MDD-W) (Custodio *et al*., 2020, FAO, 2016) was used. Food items were grouped into ten food groups based on their nutrients: those include starchy and staple foods and grains, beans and peas, nuts and seeds, dairy, fleshy foods, eggs, vitamin A-rich dark green leafy vegetables, other vitamin A-rich fruits and vegetables, other vegetables, and other fruits (Becquey and Martin-Prevel, 2010, FAO, 2007, Wen *et al*., 2010). Meal frequency, DDS, FVS, ASF score were used to assess the dietary practice. The FVS calculated by counting the frequency of the individual food items consumed by the mothers over the course of a week. The mean FVS of pregnant women was calculated and those of them with FVS greater than the means were labelled as having "high" food variety score whereas those with FVS lower than the means were defined as having "low" FVS. The sum of each food group that the pregnant women consumed over a period of 1 week were calculated to analysis the DDS and converted into tertiles, and the highest tertile used to label "high" dietary diversity score. Consumption of foods from ASF was estimated by counting the frequency of each food from animal sources that women ate over a reference period. ASF score was also converted into terciles and the highest tercile used to label as "high".

Dietary practice categorized as good and poor dietary practice and having good dietary practice when mother had meal frequency > 4, good FVS, high DDS and high ASF score, while poor dietary practice is when women had meal frequency < 4, low FVS, low and medium DDS, and ASF score (Shamim *et al*., 2016, Demilew *et al*., 2020, Belachew *et al*., 2013, FAO, 2016).

2.2.1.2. Anthropometric measurement

Women height, weight, and Middle Upper Arm Circumference (MUAC) were measured after the equipment was calibrated and the data collectors were standardized. In this study, acute malnutrition among pregnant women was defined as MUAC <21 cm (Kahanya, 2016, Thurstans *et al*., 2011).

2.3 Qualitative study

2.3.1 Data collection tool, procedure, quality assurance and collectors

Semi-structured key questions or topics and individual in-depth interviews were used for qualitative data. A description of provision of preconception of folic acid as found in the literature was provided and participants were asked if they knew of any such services. The guides were prepared to cover topics related to a) practice of preconceptional folic acid supplement for reducing the risk of neural tube defects (NTDs) b) challenges for implementation of preconception of folic acid supplement c) opportunities for implementation of preconception folic acid supplement practice.

The sequence of the topics generally moved from the more general to the specific questions. Content validation of the instruments was done by two experts in qualitative research after which the instruments were pretested and ambiguous questions were removed or revised.

A total of five midwives and MCH experts were involved in the in-depth interview and the interviews were conducted in local language depending on the participants' preference by trained data collectors who had prior experience in qualitative data collection. The interview lasted from 45 to 90 minutes. All interviews were recorded and transcribed verbatim.

2.4. Data Quality Control

To ensure data quality, pretest was conducted on different area of the study area which were the same set up of the study area. Data collectors, health educators and supervisors were trained for three days using a training manual included how to enroll women to the study, how to take IFAS and the necessary information included baseline and end line data. In addition health extension workers received training how to provide pictured based nutrition education and counseling, based on the objective of the study, data collection tool and the semantics of each variable on the questionnaire. The data collectors and supervisors were with experienced diploma and degree holder. There was also a demonstration and practical session on interviewing and anthropometric measurements. Standardization exercise was done on anthropometric measurements to reduce inter-observer error. Furthermore, the weight scale indicator was checked against zero reading after weighing every individual. The measurements were also randomly rechecked during data collection. In addition in order to reduce the error during data filled, KoBo Tool Box application was used for data collection. The supervisors and the principal investigator closely monitored the data collection process. Additionally, health educators and counselors also have monthly meetings with supervisors to discuss any problems they are encountering while providing nutrition counseling. Feedback is provided to the counselors in these meetings.

2.5. Data processing and analysis

First, the data were checked for completeness and consistency after exported from KoBo Tool Box to Ms Excel. The data were then exported to the SPSS for Windows version 25 program for analysis purposes. The data were cleaned up by checking outliers and missing values. A descriptive analysis of the background feature was performed. Normality was checked for continuous variables.

2.6. Ethical Approval and consent to participate

Ethical clearance was obtained from Jimma University's Institutional Review Board (IRB) with a reference number of IHR PGY/738/20. Medical directors, administrative offices and deans of studies were briefed on the study objectives through a letter from the Jimma University IRB office to improve collaboration. All data collection procedures were conducted in accordance with relevant guidelines and regulations of Jimma University and the Ethiopian National Research Ethics. For retrospective data, permission to access the data was given by the city administration health bureau and hospital administrations. Written informed consent and verbal ascent was obtained from each selected study participants of mothers and neonate parents or care givers (for those <18 years age and unable to read and write) to confirm their willingness after explaining the purpose of the study and its advantages and risks. The study participants were assured that they were free to withdraw their consent and to discontinue participation in the study without prejudice to it. Data protection and the confidentiality of the data collected were guaranteed throughout the study. Study participants were advised that they have the right to refuse or cancel participation in the study without affecting the service they receive from the respective facilities. This clinical trial was registered prior to data collection for the registration of a pan-African clinical trial with unique identification number of PACTR202104543567379. The study was carried out in accordance with the ethical principles of the Helsinki declaration and the requirement of good clinical practice(Association, 2001).

Chapter 3: Trend and burden of neural tube defects among cohort of pregnant women in Ethiopia: Where are we in the prevention and what is the way forward?

Berhane A, Belachew T. Trend and burden of neural tube defects among cohort of pregnant women in Ethiopia: Where are we in the prevention and what is the way forward?. PloS one. 2022 Feb 18;17(2):e0264005.

Abstract

Introduction: The neural tube defect is one of the five most serious birth defects in the world. In Ethiopia, an accurate estimate of the trend and burden of neural tube defects is still unknown. Not much research has been done on the prevalence and trend of neural tube defects in eastern Ethiopia. As a complement to earlier study efforts, this study is intended to assess the trend and burden of neural tube defects in Eastern Ethiopia and to examine the epidemiological implications of the results.

Methods: A facility-based retrospective cohort study was conducted on cohort pregnant women who gave birth in selected hospitals. Between 2017 and 2019, records of all babies with neural tube defects were obtained. A structured checklist was used to collect the data. The incidence of each case was calculated by dividing the number of cases per year by the total number of live births in each hospital. To determine the linear trend of neural tube defects over time, the linear trend of the Extended Mantel-Haenszel Chi Square was performed. The data were presented with frequencies and percentages. The data were analyzed using SPSS for Windows version 25.

Results: In the three years of the study considered for the analyzes, a total of 48,567 deliveries with 522 women with neural tube defects were recorded, which corresponds to an incidence rate of 107.5 per 10,000 live births in the three years. The linear trends of NTDs over the three year period of 2017-2019 was OR of 1 (base year), 4.3 and 8.3, respectively, [Extended Mantel-Haenszel chi square for linear trend = *200.53 (P<0.0001)*]. Thus, the magnitude of NTDs showed a statistically significant increase trend over the three years. The most common types of neural tube defects found in this area were anencephaly and spina bifida, which accounted for 48.1% and 22.6%, respectively. The distribution of neural tube defects varied between study hospitals, with Adama Medical College Hospital having the highest proportion (46.6%). Over half of the mothers (56.7%) live in cities. Mothers in the age group 25-34 (46.9%) and multigravida mothers had higher proportions (64.4%) of neural tube defects. None of the mothers took folic acid before conception, and only 19% consumed folic acid with iron during pregnancy.

Conclusion and recommendation: The results showed that an increasing trend and exposure to neural tube defects and preconception folic acid supplementation are insignificant in the region. The results suggest that preconception folic acid supplementation should be considered in

conjunction with health services to reduce the risk of neural tube defects in the area. In addition, intensive preventive efforts are required for long-term including, folate intake through dietary diversification and appropriate public health measures. In addition, the data must be properly collected to identify the deaths by NTDs, and the determinants should be investigated through large-scale prospective biomarker studies.

3.1 Background

Neural tube defect (NTD) is one of the five most common and serious birth defects of the brain and spinal cord caused by neural tube failure between 21 and 28 days after conception, usually before a woman realizes she is pregnant. The defect ranges from anencephaly to encephalocoils and spina bifida (Persad et al., 2002, Detrait et al., 2005, Lazareff, 2011, Butterworth and Bendich, 1996, Greene and Copp, 2014). NTDs are one of the main causes of infant and child mortality, morbidity and long-term disability as well as psychological and major emotional effects on affected families (Greene and Copp, 2014). According to the World Health Organization (WHO), approximately 400,000 neural tube defects (NTDs) births occur each year, leading to an estimated 270,000 newborn deaths worldwide (Zaganjor *et al*., 2016) causing more than 10% of newborn deaths. Both developing and developed countries bear the burden of NTDs. In countries where folic acid supplementation is not available, the prevalence is between 0.5 and 2 per 1000 births. Although the burden of NTD varies widely depending on geography and socioeconomic status (Yi *et al*., 2011, Control, 1989), it is the leading cause of neonatal deaths in low and middle-income countries, accounting for 29% of all neonatal deaths (Rudolfson *et al*., 2018).

In Ethiopia, few studies reported that the prevalence increased from year to year, the increase varying geographically. The incidence rate ranged from 61/10,000 in Addis Ababa (Sorri and Kassa, 2015) to 131/10,000 in Tigray (Berihu *et al*., 2018). The total burden of neural tube defects in Ethiopia is unknown and is underestimated due to insufficient and fragmented data. Because NTDs are a leading cause of death in children under the age of five, adequate data is required for informed interventions. There is currently no evidence of the trend and burden NYDs in eastern Ethiopia. This retrospective analysis provides indications of the extent and trend of NTD in the context of the government's intervention efforts since 2005.

3.2 Material and methods

3.2.1 Study setting

The study was carried out in Eastern Ethiopia. Details of the study setting are described in **Chapter 2.**

3.2.2 Study design

A retrospective cohort study was conducted based on a review of the medical records of a cohort of pregnant women delivered at Dil-Chora Referral Hospital, Hiwot Fana Specialized Teaching Hospital, and Adama Medical College Hospital.

3.2.3 Participant selection procedure

The study hospitals were selected purposefully based on referral status and cases load in the eastern part of Ethiopia. From the total delivered babies in the selected hospitals, all recorded babies delivered, treated, and terminated that diagnosed as having NTDs cases were retrieved from medical admission log-book retrospectively from September 1, 2017 to August 30, 2020 were included. Exclusion criteria included absence of client card, unclear recorded or the client card that had incomplete documentation and had more than 50% of the values missing. The detailed methods of define the target participants were as follows (Figure 3.1).

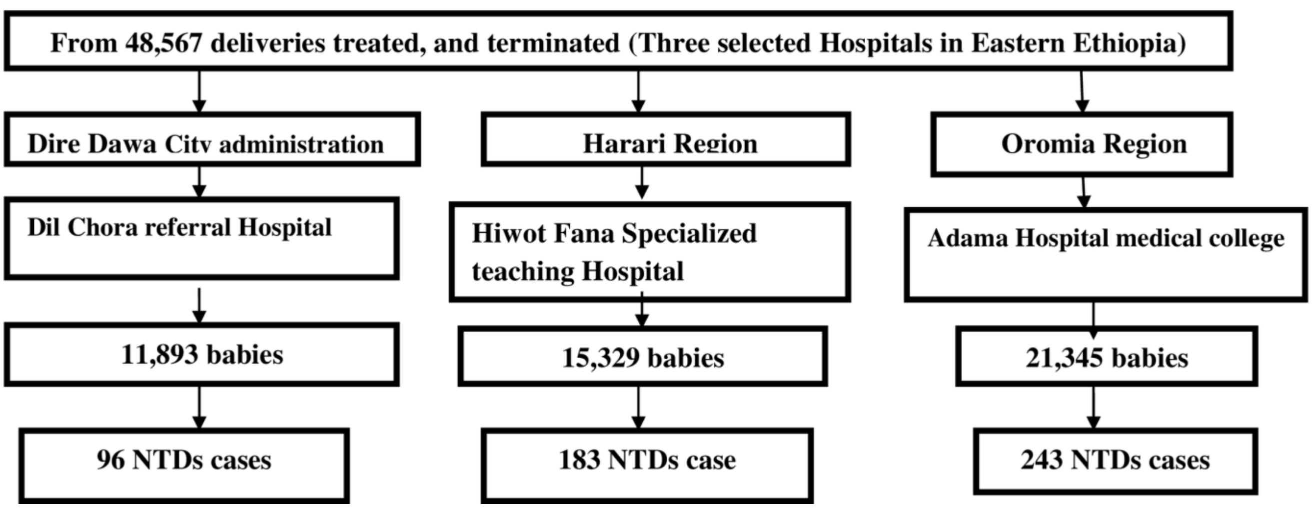

Figure 3.1: Schematic presentation of sampling procedure in Eastern 2017-2019

3.2.4 Data collection tool and method

Details of data collection tool are presented in **Chapter 2.** Data were collected from routine administrative hospital records. All NTDs cases were retrospectively reviewed in a sequential manner. The diagnoses were confirmed by gynecologists, pediatricians, midwives and specialist nurses. Medical Record Numbers (MRN) was used to identify study participants from admission log book.

3.2.5 Variables

Dependent Variable: Trend and burden of NTDs

Independent Variable: Socio-demographic, pregnancy, ANC use, folic acid and IFA, maternal obstetric history, maternal health and drug history,

3.2.6 Operational definitions

NTDs cases: is defined as mothers who gave birth to an alive newborn with any type of NTDs (anencephaly, spina bifida, or encephalocele, or myelomeningocele or meningocele), irrespective of gestational age.

NTDs-affected pregnancy: is defined as one of the following four outcomes: (1) an early fetal loss or miscarriage (defined as a spontaneous pregnancy loss at 20 completed weeks of gestation), (2) fetal death or stillbirth (defined as a spontaneous pregnancy loss at 20 completed weeks of gestation), elective termination of pregnancy for fetal anomaly (eTOPFA), or (4) an affected live birth.

NTDs incidence (burden) was calculated as:

$$= \frac{\text{affected live births + affected still births + eTOPFAs for NTDs}}{\text{Total births}} \times 10{,}000$$

Multiple neural tube defects (MNTDs):-defined by the simultaneous occurrence of more than one NTD in a single case with "normal" neural tissue in between.

3.2.7 Data processing and analysis

Details of the data cleaning and processing are described in **Chapter 2.** Descriptive statistics was employed to summarize socio-demographic characteristics and estimate the incidence of patients with neural tube defects. The trend of NTD was determined for the years between 2017 and 2019. The burden was calculated by dividing the number of NTDs cases identified (numerator) by the total number of births in selected hospitals between 2017 and 2019. Each study site's linear trend was also computed using the corresponding number of live births by year and study site as the denominator. To determine the linear trend of NTDs over time, Extended Mantel-Haenszel chi-square was used.

3.3 Results

Socio demographic characteristics

Between 2017 and 2019, 48,567 pregnant women who delivered in the three selected hospitals, with 522 neonates having one or more types of NTDs. The overall burden of NTDs was 107.5 per 10,000 live births (live birth and stillbirths, foetal deaths). The distribution of NTDs varied between the hospitals studied such that Adama Medical College Hospital accounted for the highest proportion of cases (46.6%). Over half of the mothers (56.7%) lived in urban areas. Nearly one-third (30.5%) of the mothers lived in East Harerghe, and the mean age of the participants was 26.4 (±5.6 SD), with maternal age 25-34 accounting for 46.9% (Table 3.1).

Table 3.1: Background characteristics and proportion of deliveries with NTDs eastern Ethiopia based on hospital data from 2017-2019.

Variables	Frequency	Percent
Study hospitals		
Dil chora referral Hospital	96	18.4
Hiwot fana Specialized teaching Hospital	183	35.1
Adama medical college Hospital	243	46.6
Participant address		
Dire Dawa	86	16.5
Adama	149	28.5
Eastern Harerghe	159	30.5
Hareri	21	4.0
Somali	7	1.3
West Harerghe	5	1
Other (around Adama)	95	18.2
Residence		
Rural	226	43.3
Urban	296	56.7
Mean maternal age (years)	26.4 ± 5.6	
Maternal age		
18-24	205	39.3
25-34	245	46.9
35-45	72	13.8

Reproductive and ANC history

Majority (98.9%) of the mothers gave a single neonate, while 64.4% were multigravida. A little more than half of the mothers (51.1%) had ANC follow-up. All mothers did not receive folic acid supplementation throughout the entire pregnancy. Similarly, 81% of mothers did not receive iron and folic acid supplementation throughout their pregnancy. Whereas, only 5.6% of mothers received folic acid contain multivitamin supplement during their pregnancy (Table 3.2).

Table 3.2: Reproductive and ANC characteristics of pregnant women, Eastern Ethiopia, data from 2017-2019

Variables	Categories	Frequency	Percent
Type of pregnancy	Single	516	98.9
	Twins	6	1.1
Gravidity	Primigravidity	186	35.6
	Multigravidity	336	64.4
History of spontaneous abortion	Not documented	424	81.2
	Yes	98	18.8
Preterm	Not documented	521	97.9
	Yes	1	0.2
Previous history of NTDs	Not documented	511	97.9
	Yes	11	2.1
Sex affected	Male	1	0.2
	Female	3	0.6
	Not documented	7	1.3
Adverse pregnancy	Not documented	518	99.2
	Yes	4	0.8
Type of adverse pregnancy	APH	2	0.4
	Severe preeclampsia	2	0.4
ANC follow	No	255	48.9
	Yes	267	51.1
Place of ANC Visit	Private clinic/hospital	80	15.3
	Governmental health facility	180	34.5
	Non-governmental health facility	3	0.6
	Not documented	4	0.8
Folic acid supplement	Not documented/No	522	100.0
Iron folic acid supplementation	Not documented	423	81.0
	Yes	99	19.0
Multivitamin supplementation	Not documented	464	88.9
	Yes	29	5.6

Illness and drug history

The major illnesses identified in the mothers' morbidity history were spontaneous abortion (18.8%), chronic hypertension (1.1%), diabetic mellitus (1.3%), anemia (2.5%), preeclampsia (2.1%), fever (1.3%), viral infection (1.3%), and parasitic infection (0.8%). Furthermore, 2.1% of mothers had a previous history of NTDs, and 0.6% of mothers were living with HIV/AIDS, only 1.1% used an antiepileptic drug (AED) and 2.1% of mothers used antibiotics (Table 3.3).

Table 3.3. Illness and drug history of pregnant women Eastern Ethiopia data from 2017-2019.

Variables	Categories	Frequency	Percent
History of any infection before/ early during pregnancy	Not documented	515	98.7
	Yes	7	1.3
Type of infection	Hepatitis B	2	0.4
	Respiratory tract	1	0.2
	UTI	2	0.4
	Urinary tract	1	0.2
	Vulvar edema	1	0.2
Chronic hypertension	Not documented	516	98.9
	Yes	6	1.1
Diabetic mellitus	Not documented	515	98.7
	Yes	7	1.3
History of anemia before/ early during pregnancy	Not documented	509	97.5
	Yes	13	2.5
History of preeclampsia	Not documented	511	97.9
	Yes	11	2.2
History of eclampsia	Not documented	519	99.4
	Yes	3	0.6
History of TB	Not documented	521	98.8
	Yes	1	0.2
Living with HIV/AIDS	Not documented	519	99.4
	Yes	3	0.6
History of fever	Not documented	515	98.7
	Yes	7	1.3
History of viral infection	Not documented	515	98.7
	Yes	7	1.3
History of parasite infection	Not documented	518	99.2
	Yes	4	0.8
History of gastric	No documented	518	99.2
	Yes	4	0.8
History of taken Antibiotic	Not documented	511	97.9
	Yes	11	2.1
Utilized Antiepileptic Drugs	Not documented	516	98.9
	Yes	6	1.1

Obstetric History

Extremely preterm (<28 weeks) was the most common gestational age of cases with NTDs. Out of the NTD affected pregnancies, 78.4% were diagnosed by ultrasound before delivery. In terms of mode of delivery, the majority of women had spontaneous vaginal births (87.5%). Nearly equal proportion of males (28%) and females (27.2%) were affected, yielding a sex ratio of 1. Regarding the outcome, 58.2% of NTD-diagnosed pregnancies were terminated medically, while the remaining 27.2 % resulted in stillbirths. Only 1.3% of the total newborns with NTDs were discharged alive with referral based on family consent, while the remaining 98.7% died before referral to NICU, delivery, or medical termination (Table 3.4).

Table 3.4: Obstetric history of pregnant women, Eastern Ethiopia, data from 2017-19.

Variables	Categories	Frequency	Percent
Gestational age	Extremely preterm (< 28 weeks)	254	48.7
	Very preterm (28-31 weeks)	107	20.5
	Moderate preterm (32-36 weeks)	41	7.9
	Extremely term (37-38 weeks)	39	735
	Full term (39-40 weeks)	21	4.0
	Post term (40 weeks)	1	0.2
	Not documented	59	11.3
Mode of NTDs identified	Identified by ultrasound before delivery	409	78.4
	Identified after delivery	58	11.1
	Not documented	55	10.5
Mode of delivery	Spontaneous vaginal	457	87.5
	Cesarean section	49	9.4
	Vacuum	15	2.9
	Forceps	1	0.2
Date of birth	2017	33	6.3
	2018	165	31.6
	2019	324	62.0
Sex of neonate	Male	146	28.0
	Female	142	27.2
	Not documented	234	44.8
Birth or pregnant outcome	Stillbirth	142	27.2
	Alive	62	11.9
	Terminated/elective	304	58.2
	Spontaneous abortion	14	2.7
Status of neonate at discharge	Alive	7	1.3
	Dead	515	98.7

Types of NTDs identified

Anencephaly had the highest proportion (48.1%) of NTDs identified, followed by spinal bifida (22.6 %) and myelomeningocele (10.5%) (Figure: 3.2).

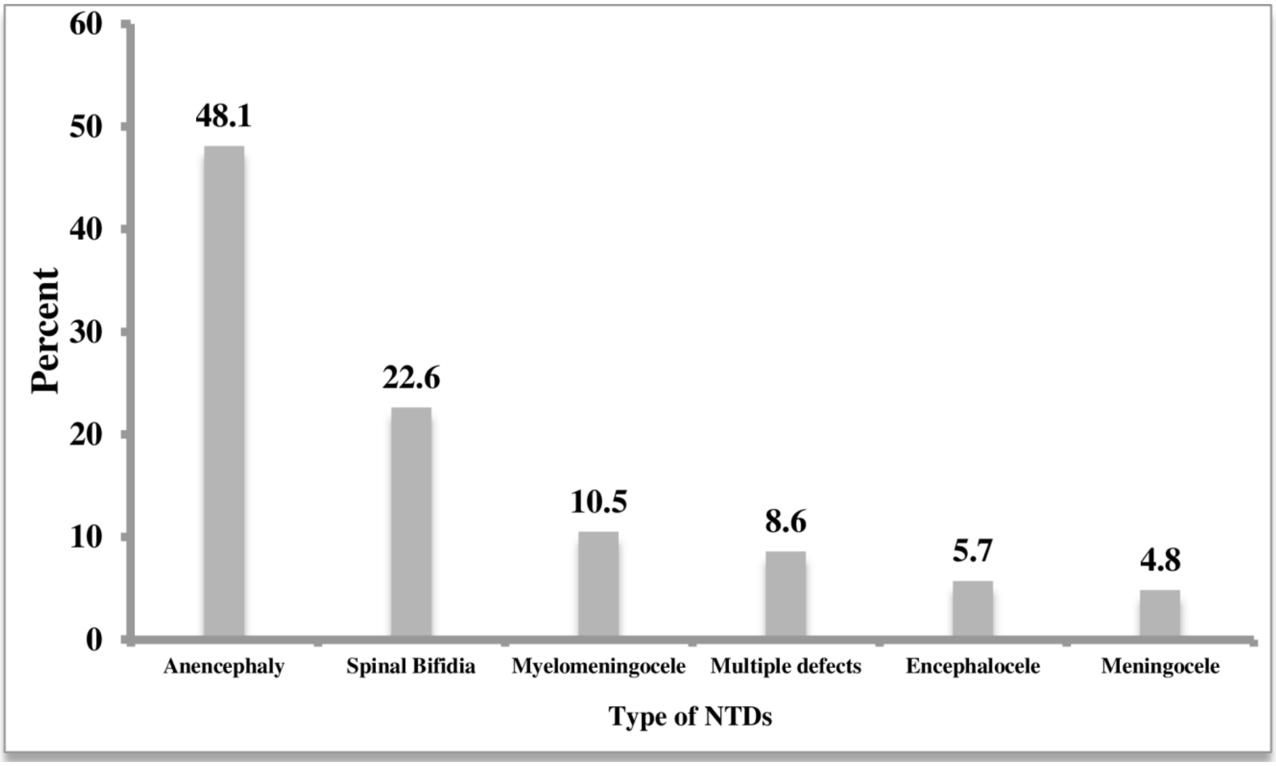

Figure 3.2: Type of identified NTDs in Eastern Ethiopia, Data from September 2017-2019

Nearly a third (27.8%) of the NTD cases were associated with different type of congenital anomalies with most of the congenital anomalies observed in this study being hydrocephalus (79.3%) followed by other type of anomalies (Figure 3.3).

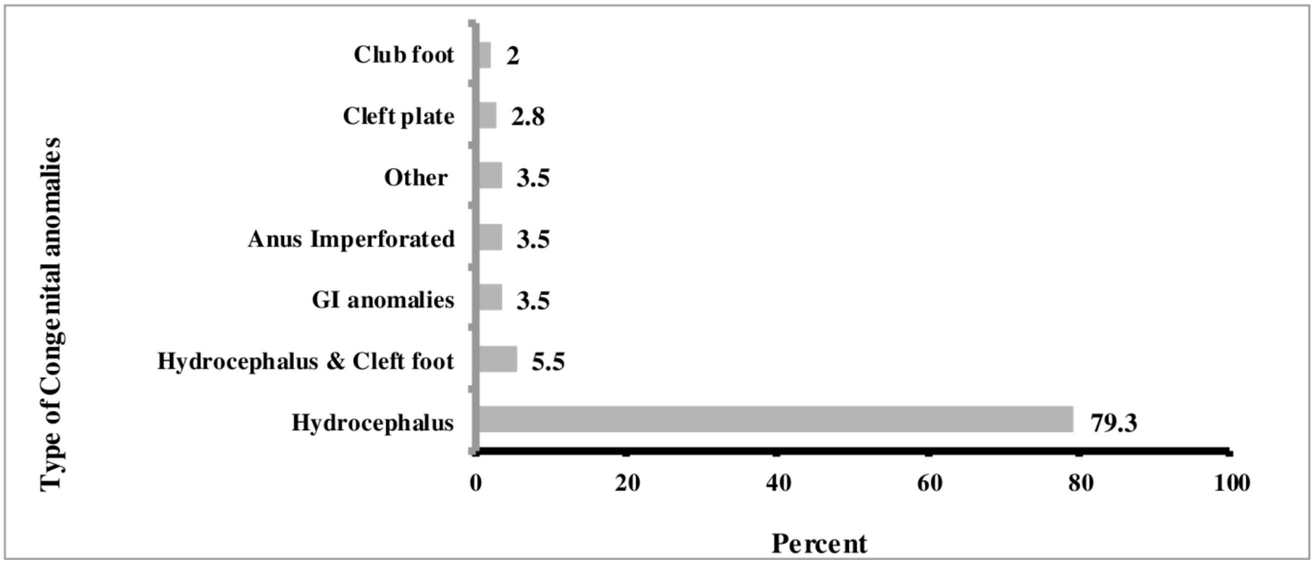

Figure 3.3: Type of congenital anomalies associated with NTDs Eastern Ethiopia, Data from 2017-2019

The overall incidence of NTDs was 107.5 per 10,000 live births with the incidence rate showing an increasing trend over a three-year period. The proportion of NTDs increased linearly over three years, with odd ratios (OR) of 1 (2017) and 4.3, and 8.3 for 2018 and 2019, respectively. Extended Mantel-Haenszel chi-square for linear trend is 200.53 (P<0.0001) (Table 3.5).

Table 3.5: Linear trend of NTDs incidence Eastern Ethiopia, data from September 2017-2019

Year	No. of newborns	No. of newborns with NTDs	Proportion	Incidence per 10,000 births	Mantel-Haenszel Summary Odds Ratio
2017	14479	33	0.22	22.8	1.00
2018	16906	165	0.97	97.6	4.3
2019	17182	324	1.88	188.56	8.3
Total	48567	522	1.07	107.5	

Hiwot Fana Specialized Teaching Hospital had the highest overall incidence of any of the study hospitals (119.4 per 10,000 births). In 2017 and 2018, Dil Chora Hospital had the highest burden of NTDs cases, with an incidence of 51.3 and 115 cases per 10,000 births, respectively .In 2019 the highest burden of NTDs with an incidence of 244 per 10,000 births was found in Hiwot Fana Specialized Teaching Hospital (Table: 3. 6).

Table 3.6: Linear trend of NTD Incidence among study Hospitals eastern Ethiopia, data from September 2017-2019

Year	Study Hospitals								
	Adama medical college Hospital			Hiwot Fana specialization teaching Hospital			Dil Chora Hospital		
	Total delivery	Case	Incidence/10,000	n	Case	Incidence /10,000	N	Case	Incidence/10,000
2017	5455	1	1.8	5124	12	23.4	3900	20	51.3
2018	7584	66	87	5411	54	99.8	3911	45	115
2019	8306	176	211.89	4794	117	244.05	4082	31	75.9
Total	21345	243	113.8	15329	183	119.4	11893	96	80.7

As depicted in table 7, anencephaly had the highest overall incidence, followed by spina bifida and myelomenigocele, with incidences of 51.7 and 24.3/10,000 births, respectively. Encephalocele and meningocele had the lowest incidences, with 6.2 and 5.2/10,000, respectively (Table 3.7).

Table 3.7: Incidence of type of NTDs among study hospitals eastern Ethiopia, data, from 2017-2019

Type of NTDs	Study Hospitals							
	Dil Chora Hospital		Hiwot Fana Hospital		Adama medical college Hospital		Total	
	n	Incidence per 10,000	n	Incidence per 10,000	n	Incidence per 10,000	n	Incidence per 10,000
Myelomeningocele	27	22.7	19	12.4	9	4.2	55	11.3
Anencephaly	36	30.3	109	71.1	106	49.6	251	51.7
Encephalocele	3	2.5	14	9.1	13	6.1	30	6.2
Meningocele	7	5.9	13	8.5	5	2.3	25	5.2
Spinal Bifidia	18	1.5	19	12.4	81	37.9	118	24.3
Multiple defects	5	4.2	9	5.8	29	13.6	43	8.8
Total	96	8.0	183	119.4	243	113.8	522	107.5

Figure 3.4 depicts the linear trend of the different types of NTDs over the study period. The occurrence of anencephaly and spina bifida increased steadily, reaching a peak in 2019 (50.3% and 28.4%, respectively), while the occurrence of multiple defects peaked in 2019 (11.5%) (Figure 3.4).

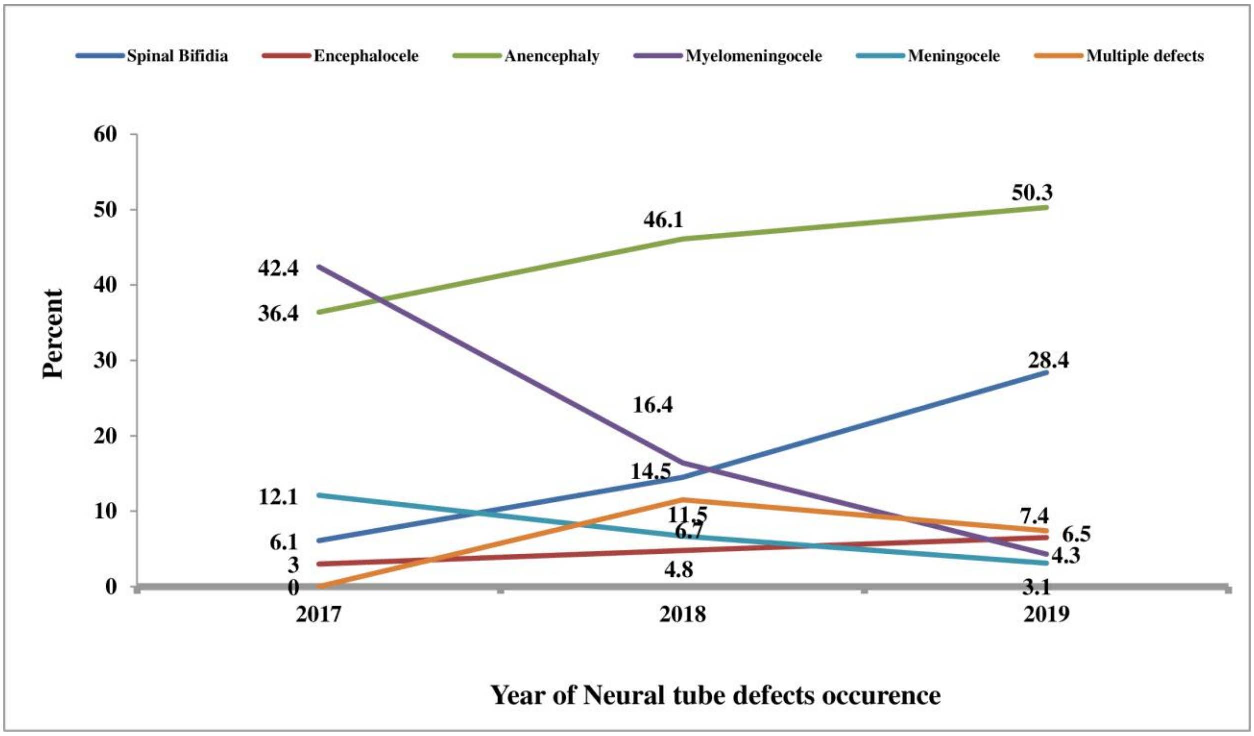

Figure 3.4: Yearly distribution of the occurrence of NTDs in Eastern Ethiopia, data from 2017-2019

Large proportion of anencephaly (43.4%) cases was found at Hiwot Fana Specialized Teaching Hospital, while the majority of spinal bifida (68.6%) were found in Adama Medical College Hospital. Similarly, Dil-Chora Hospital had the highest proportion of myelomenongocele (49.1%). Hiwot Fana specialization teaching Hospital and Adama medical college Hospital each had 46.7 percent and 43.3% of the total cases of enencephale, respectively. Menengocele was found in higher proportions in Hiwot fana (52%) and Dilchora Hospital (28%) hospitals (Figure 3.5).

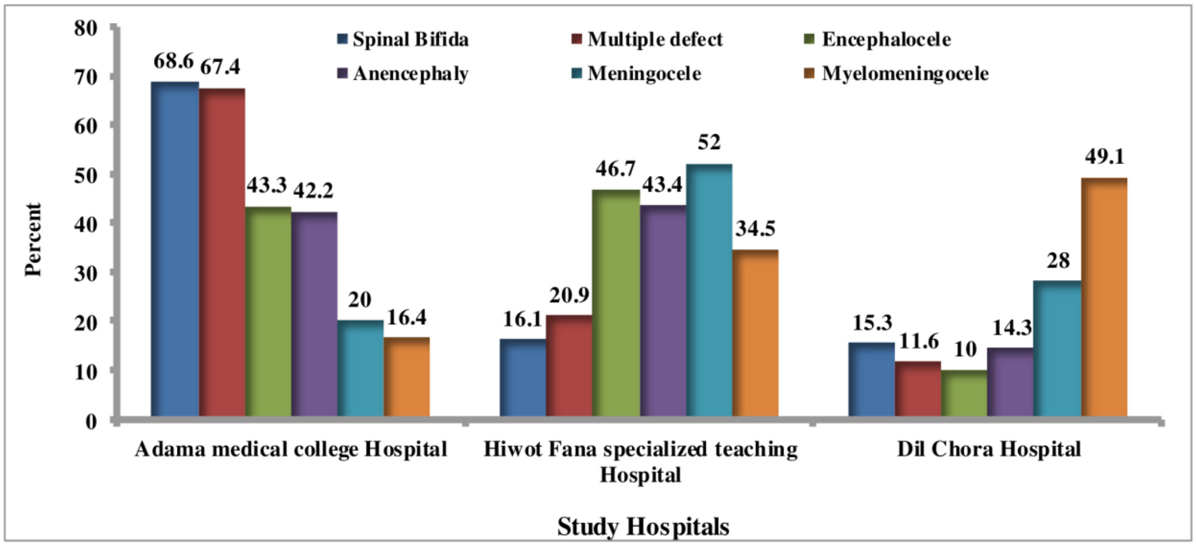

Figure 3.5: Percentage of different type of NTDs among study hospital, Eastern Ethiopia, data from 2017-19

East Harerghe had a higher proportion of pregnancies with anencephaly (18.4%) than Adama (11.4%). Regarding spinal Bifdia, 11.5 percent, 4.2%, and 3.4% of mothers were from Adama, around Adama, and Dire Dawa, respectively. Similarly, the majority of myelomenongocele cases were reported in Dire Dawa and East Harerghe (3.8%), while East Harerghe (46.4%) and Adama (30%) had the highest proportion of enencephale cases, East Harerghe (40%) and Dire Dawa (30 percent) had the highest proportion of menengocele cases (24%) (Figure: 3.6).

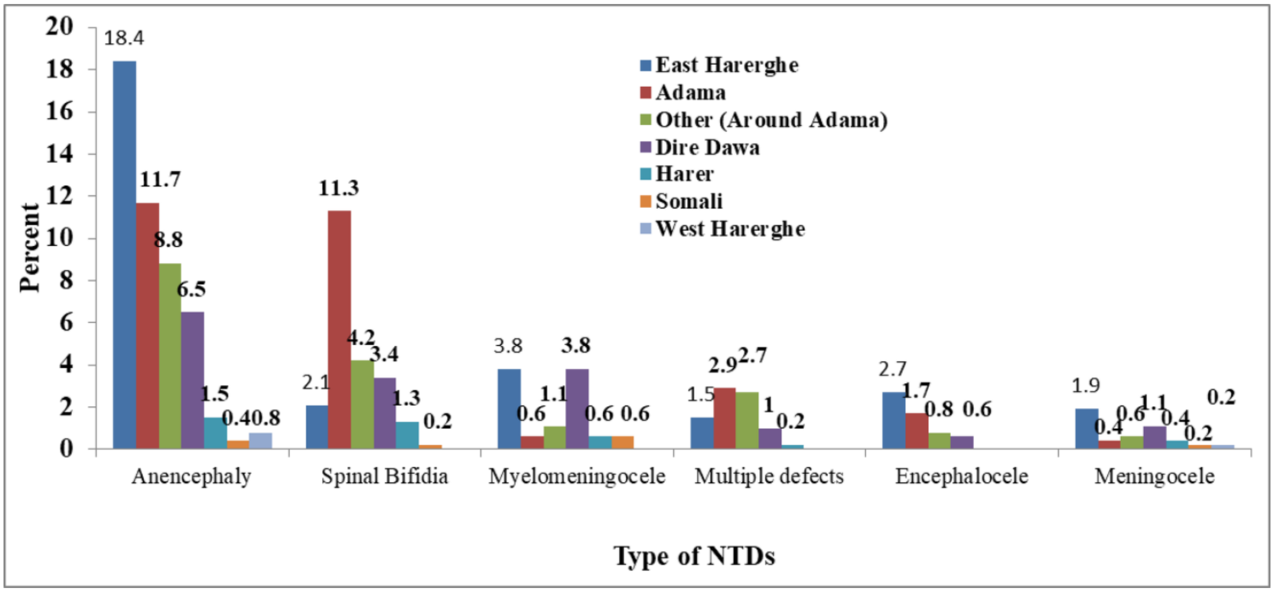

Figure 3.6: Distribution of NTDs among study hospitals Eastern Ethiopia, data from 2017-2019

The proportion of mothers who did not receive iron and folate supplementation and had at least one of the NTDs ranged from 68.0 percent to 88.4%. Similarly, the percentage of mothers with one or more affected NTDs who had a history of spontaneous abortion prior to the current pregnancy ranged from 10% to 25.6% (Table 8).

Table 3.8: Type of NTDs by FeFol supplementation and history of spontaneous abortion, Eastern Ethiopia data from 2017-19

Type of NTDs	FeFol Supplementation		History of spontaneous abortion		Total
	No	Yes	No	Yes	
	n(%)	n(%)	n(%)	n(%)	
Myelomeningocele	38 (69.1)	17 (30.9)	49 (89.1)	6 (10.9)	55 (10.5)
Anencephaly	203 (80.9)	48 (19.1)	202 (80.5)	49 (19.5)	251 (48.1)
Encephalocele	25 (83.3)	5 (16.7)	27 (90)	3 (10)	30 (5.7)
Meningocele	17 (68)	8 (32)	21 (84)	4 (16)	25 (4.8)
Spinal Bifida	102 (86.4)	16 (13.6)	93 (78.8)	25 (21.2)	118 (22.6)
Multiple NTDs defects	38 (88.4)	5 (11.6)	32 (74.4)	11 (25.6)	43 (8.2)
Total	423 (81)	99 (19)	424 (81.2)	98 (18.8)	522 (100)

Both rural and urban mothers had a high burden of anencephaly, accounting for 61.1% and 38.2%, respectively. Anencephaly was the most frequent NTDS in the age groups of 18-24 and

25-34, accounting for 52.7% and 44.5%, respectively. Multigravida mothers had higher rates of anencephaly (6.7%) and spinal bifida (24.1%) (Figure: 7).

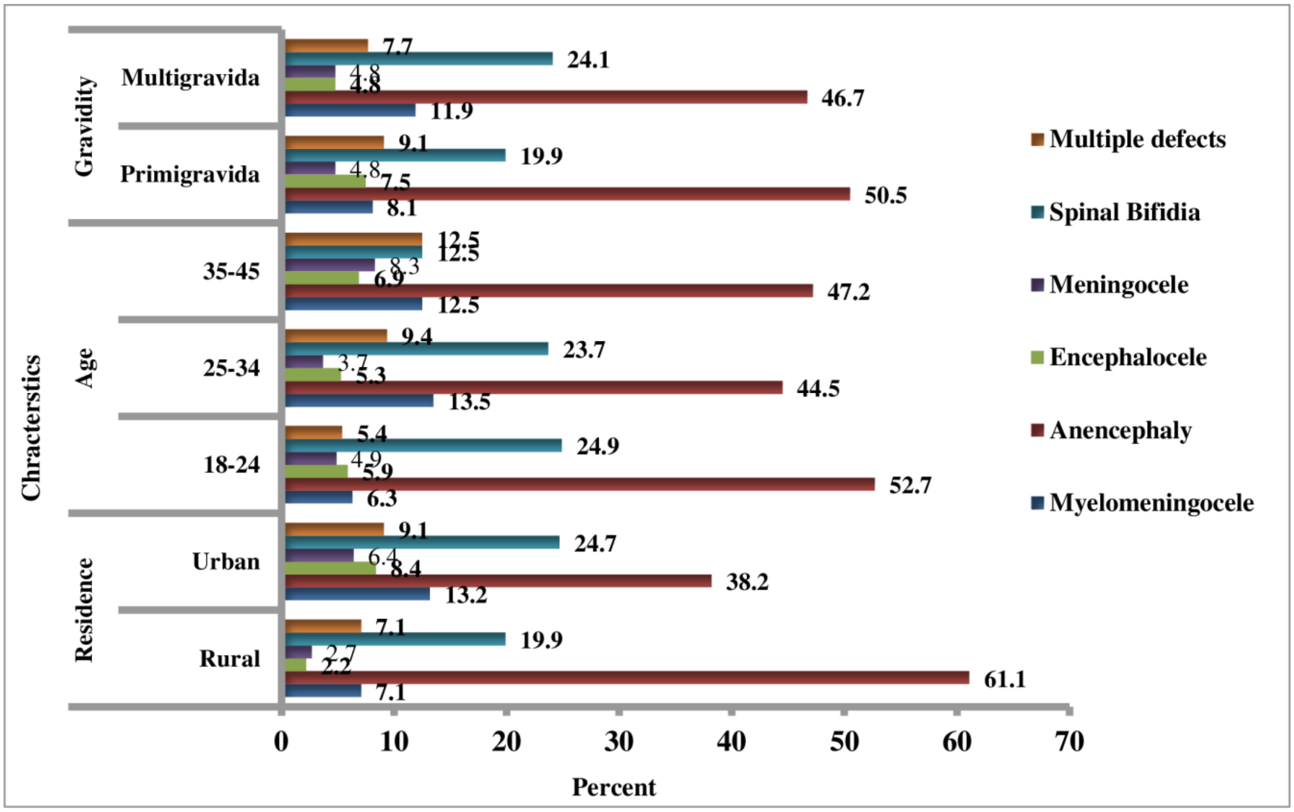

Figure 3.7: Type of NTDs by residence, maternal age and gravidity, Eastern Ethiopia, data from 2017-19

3.4 Discussion

This study recorded a total of 48,567 deliveries from the selected hospitals between 2017 and 2019. Our study found that the overall incidence rate of NTDs was 107.5 per 10,000 live births. The specialized teaching hospital Hiwot Fana had the highest exposure to NTDs (244 per 10,000 deliveries). The incidence of NTDs observed in our study is lower than that reported in prospective studies of births at three teaching hospitals in Addis Ababa (126 per 10,000 births)(Gedefaw *et al*., 2018) and in Tigray Region (131 per 10,000 births (Berihu *et al*., 2018).

The NTD incidence documented in our study is also higher than the report from a systematic review and meta-analysis in Ethiopia (63.3 cases per 10,000 children) (Bitew *et al*., 2020), from a three-year retrospective study at two teaching hospitals in Addis Ababa with an incidence of 61 cases per 10,000 (Sorri and Mesfin, 2015) and according to WHO estimates of 22 per 10,000 births in Ethiopia (Botto and Mastroiacovo, 2012) and eight African countries, which were reported by the WHO with 11.7 per 10,000 births (Zaganjor *et al*., 2016). In Ethiopia, the prevalence of folate deficiency is 46.1%. The prevalence of severe folate deficiency in Dire Dawa and Harer was given as 52.9% and 80.7%, respectively (Haidar et al., 2010b). Thus, the high prevalence of folate deficiency could explain the high exposure to NTDs in Eastern Ethiopia. The low prevalence of NTDs reported in most developed and many developing countries may be due to mandatory folic acid fortification (Arth *et al*., 2016, Williams *et al*., 2015) and increased health-seeking, adequate health and nutrition, planned pregnancies and pre-conception care services. In contrast, the incidence of 107.5 per 10,000 births observed in our study would be a five-fold increase compared to the estimate of the WHO survey in Ethiopia (22 per 10,000) (Botto and Mastroiacovo, 2012). This alerts to the urgent need to implement effective programs to ensure that all women of childbearing age have adequate folic acid, as well as the need to prevent all folic acid preventable NTDs and the urgent need for preconception folic acid supplement services to implement in Eastern Ethiopia.

Anencephaly was the most common form of NTD (48.1%), followed by spina bifida (22.6%) which is consistent with findings from a study conducted at three teaching hospitals in Addis Abeba, Ethiopia (Gedefaw *et al*., 2018) in Tigrai (Berihu *et al*., 2018), Amhara region (Tadesse *et al*., 2020), Bale zone (Atlaw *et al*., 2019), Gujarat hospital, India (26%) (Sharma *et al*., 2018), South west Iran (86.8%) (Ebrahimi *et al*., 2013), Morocco (Forci *et al*., 2021) and in Nigerian (Airede, 1992). These results contradict the results of studies conducted Tikur Anbessa hospital, Ethiopia, Gandhi Memorial, and Ethio-Sewdish hospitals in Addis Ababa reported that the most common NTDs were myelomeningocele and meningocele (Sorri and Mesfin, 2015, Taye and Bedru, 2009). This discrepancy could be attributed to the presence of multifactorial determinants in the different regions and countries in which the studies were conducted. In the retrospective studies of the two teaching hospitals in Addis Ababa, Ethiopia, spina bifida was the most common NTD, followed by anencephaly (Sorri and Mesfin, 2015). The reason anencephaly is more common than in the previous retrospective study in Addis Ababa is that stillbirths were excluded, while our study included stillbirths and accounted for 48.7% of all NTDs.

In the current study, the distribution of NTDs was different between study hospitals, with Adama Medical College Hospital accounting for nearly half (46.6%) of the cases. This discrepancy may be due to the fact that more cases have been referred to this hospital in the Adama area due to the presence of various specialist services such as neurologists and risk factors in the area such as agrochemical exposure. Our study showed that urban mothers were more affected than rural mothers, which made up more than half of all NTDs (56.7%). This inequality could be attributed to greater environmental pressures from risk factors in urban areas compared to rural areas and to differences in lifestyle between the two institutions. This result contradicts the results of a study carried out in the Amhara region by Abay W *et al*. (2020), which found that 59.1% and 36.2% of mothers with NTD pregnancies came from rural and urban areas, respectively (Tadesse *et al*., 2020).

Our study found that the gender distribution of male and female NTD deliveries were 28% and 27.2%, respectively, resulting in a 1: 1 gender ratio. Unidentified gender accounted for 44.8%, which is in line with a study conducted in Thailand in which the gender ratio is 1: 1 (Wasant and Sathienkijkanchai, 2005). This study contradicted the results of previous studies conducted in Addis Ababa and the Amhara region of Ethiopia (Gedefaw *et al*., 2018, Tadesse *et al*., 2020, Sorri and Mesfin, 2015) which described female dominance over males. In contrast, a study conducted by Alem *et al* (2018) that described female dominance over men. In contrast, a study by Alem *et al*. (2018) found male dominance over women in the Tigray region in Ethiopia (Berihu *et al*., 2018). There is no single reason why neural tube defects affect female more than male, or vice versa. Our results also showed that 18.8% of mothers had previously had an abortion. This could be due to trophoblastic cell dormancy from a previous aborted pregnancy. This result is almost identical (17.3%) with the results of Marco *et al*. (2011)(De Marco et al., 2011), and Atilaw *et al*. (2019) in bale zone hospitals in southeast Ethiopia, which accounts for 47.6% (Atlaw *et al*., 2019).

Studies have shown that preconception folic acid supplementation protects against NTDs (Group, 1991, Berry *et al*., 1999, Berry *et al*., 2010). Our research also found that none of the mothers received folic acid supplementation throughout their pregnancy. This finding is in line with studies carried out in Addis Ababa, Ethiopia and Morocco (Gedefaw *et al*., 2018, Forci *et al*., 2021, Sorri and Mesfin, 2015). This could be due to a lack of preconception support in the country as well as a lack of media coverage of the promotion of preconception of folic acid supplementation. This finding has far-reaching practical implications. After 16 years of implementation of the guideline for micronutrient prevention and control in Ethiopia, such a high incidence of NTD above the WHO limit value (6 /10,000 births) (Sorri and Mesfin, 2015) in combination with no supplementation of all cohorts of pregnant women with NTD, even during pregnancy urgent action. Since NTDs occurs on the 28th day of pregnancy, the strengthening of prejudices against folic acid supplementation should be targeted and further researched through different layers.

The following limitations are recognized in this study. Because the study was conducted in only three hospitals, it does not reflect the true prevalence of NTDs in the community. Determinants of NTDs have not been studied or attempted. Since this is a retrospective study, there are significant limitations in the data recorded. In some cases, the required examinations and full medical history were not properly documented. On the other hand, there was a discrepancy between the medical registration number (logbook) and the actual customer card, which led to the fact that the study did not register almost half of the data books, recorded in the study hospitals. As a result, this study did not provide an exact size and number in the study area. Also, as the study focused on the eastern part of the countries, the results may not accurately reflect the national situation and should be interpreted with caution.

3.5 Conclusion

NTDs pose a significant public health burden in the study area, with the most common forms being anencephaly and spinal bifida. The incidence rate is five times higher than WHO estimates for Ethiopia. The results suggest that primary prevention strategies with active promotion of pre-conception care services and the possible implementation of approaches to pre-conception folic acid supplementation and food fortification are needed to reduce exposure to NTDs as a public health emergency in Ethiopia. This will help achieve Sustainable Development Goal 3.2, which states that preventable deaths and disabilities in newborns and children under 5 years of age will end by 2030. It is recommended that the determinants of NTDs in the study area be investigated through large-scale prospective studies with supportive biomarkers.

Chapter 4: Determinants of neural tube defects among women who gave birth in hospitals in Eastern Ethiopia: Evidence from a Matched Case Control Study

(Accepted on Plos One)

Abstract

Background: Neural tube defects (NTDs) are severe birth defects caused by nutritional, genetic or environmental factors. However, determinants for the development of NTDs differ by country, region as well as within the country. The objective of this study was to identify the determinants of NTDs among newborns delivered in three hospitals found in eastern Ethiopia.

Methods: A hospital-based matched case-control study was conducted among 138 cases and 138 control women who delivered a neonate in three teaching hospitals found in Eastern Ethiopia in 2021. Data were gathered using a structured and pre-tested interviewer-administered questionnaire. Cases were mothers who delivered a neonate with any type of NTDs regardless of gestational age or fetal viability, whereas controls were mothers who delivered an apparently healthy newborn. Conditional logistic regression analysis and adjusted odds ratio with its corresponding 95% confidence intervals were used to compare the two groups.

Results: Anencephaly (51.4%) and spinal bifida (34.1%) were the most frequently observed NTDs. None of study participants took preconception folic acid supplementation. Being an illiterate mother (AOR=0.34, 95% CI: 0.12-0.92, P=0.034), rural resident women, (AOR=3.4, 95% CI: 1.18-9.78, P=0.023), mothers with history of spontaneous abortion (AOR=2.95, 95% CI: 1.15-7.55, P=0.023), mothers who suffered with severe anemia (AOR=3.4, 95% CI: 1.17-9.87, P=0.024), mothers with history of fever or cold (AOR=2.75; 95% CI: 1.05-7.15, P=0.038), and mothers who had an exposure to various agro-chemicals (AOR=3.39, 95% CI: 1.11-10.3, P=0.032) were found statistically significantly associated for development of NTDs.

Conclusion and recommendation

In this study, NTDs were associated to a number of determinant factors in the area, including residential area, history of spontaneous abortion, severe anemia, fever/cold, antibiotic use before or during early pregnancy, and exposure to agrochemicals. Furthermore, this study found that among identified determinants, dietary and nutritional related factors played a significant role in the development of NTDs in the study area. Addressing the identified determinants is critical to averting the incidence of NTDs in the study area. Moreover, more research is needed to investigate women's dietary practices as well as the practice of preconception folic acid supplementation for pregnant women in Ethiopia's current health care system.

4.1 Background

Neural tube defects (NTDs) are among the second and most serious congenital anomalies that occur as a result of incomplete closure of the brain or spinal cord between 12 and 28 days of pregnancy. They are associated with a high rate of neonatal death, morbidity, psychological, emotional, economical problem as well as lifelong disability in survivors and their families (Avagliano et al., 2019, ICBDSR, 2014). A recent meta-analysis study estimated that 260,100 NTDs affected birth outcomes worldwide (Blencowe et al., 2018a). Each year, it is estimated that nearly 200,000 neonates are born with NTDs in low- and middle-income countries (LMICs) (Lo et al., 2014). A meta-analysis study published in 2022 found that the pooled birth prevalence of NTDs in eastern Africa was 33 per 10,000 births, with Ethiopia having the highest pooled prevalence (60 per 10,000 births) and Malawi having the lowest (5 per 10,000 births) (Ssentongo et al., 2022). In Ethiopia, the Tigray region had the highest incidence rate of NTDs, accounting for 131 per 10,000 (Berihu et al., 2018) and the eastern part of Ethiopia has the second highest incidence rate (107.5 per 10,000), with the trend increasing alarmingly in recent years (Berhane and Belachew, 2022).

NTDs are caused by complex, multifactorial interactions between several genes, epigenetic, nutritional, and environmental factors (Wilde et al., 2014, Mishra et al., 2020), but mainly dietary factors play a major role. Mishra, P.R. et al., (2020) reported that 70-95% NTDs are linked to genetics and maternal vitamin intake (MVI) (Mishra et al., 2020).

Dietary practice, folate supplementation intake (FSI) with genetics assimilation, hyperglycemia or maternal diabetes, maternal obesity, maternal caffeine use, mutations in foliate-responsive or foliate-dependent pathways, lead in drinking water, in utero arsenic exposure, maternal health conditions, maternal "flu" in the first trimester, geography location, race, parental history, antiepileptic drug use, pesticide exposure, periconceptional second hand smoke exposure (one month prior to conception through the first trimester), indoor air pollution from predominantly biomass heating, hyperthermia during early pregnancy, exposure of x-ray radiation, and history of spontaneous abortion have been reported to contribute to the development of NTDs (Van der Linden et al., 2006b, Tung and Winn, 2011, Demir et al., 2019, Li et al., 2006, Kerr et al., 2017, Rafał Podgórski et al., 2017, Jia et al., 2019, Brender et al., 2010, Milunsky et al., 1992, Wang et al., 2014, Kappen et al., 2011, Asamoah et al., 2018, Luteijn et al., 2014, al, 2017, Oster et al., 2011, Lunau et al., 2015, Berhane et al., 2022, Rull et al., 2006a, Kim et al., 2018, Lassi et al., 2014). However, even if these risk factors are identified, different risks may exist even within the same region or country.

Despite the fact that the burden of NTDs in the eastern part of Ethiopia is the second highest in Ethiopia (107.5 per 10,000), and the trend has increased alarmingly in recent years (Berhane and Belachew, 2022), to the best of literature search and our knowledge, there were no studies conducted on determinants for the development of NTDs in the study area. Thus, the current study aimed to identify the determinants for the development of NTDs among women who delivered neonate in three hospitals that found in eastern Ethiopia.

4.2 Method and Materials

4.2.1 Study design and setting

We used a teaching hospital-based matched case-control design to address the objectives of this study. Cases and controls were enrolled in the obstetrics/gynecology ward and Neonate Infant and Child Unit (NICU). Details of the study setting are described in **Chapter 2.**

4.2.2 Study population and eligibility criteria

In this study, all newborns in the selected hospitals who fulfilled the case and control criteria were included. Newborns whose mothers were very sick, emotionally upset, during data collection, or died after delivery were excluded from this study.

4.2.3 Sample size determination and procedure

The sample size was calculated assuming an equal number of cases and controls (1:1), odds ratio of 3.0, power of 80%, Confidence level of 95% and non-response rate of 5%. The final sample size was 276 (138 cases and 138 controls). Cases of newborns or pregnant women with NTDs were ascertained prospectively until the calculated sample size was reached. Control neonates were randomly selected from the same hospital. Apparently healthy control neonates were matched for a neonate sex, and maternal age.

4.2.4 Data collection procedure and tool

The data from case were collected after the mothers delivered as a neonate or had terminated her pregnancy due to NTDs in the labor, gynecology ward, and NICU. Control mothers were interviewed randomly within 48 hours of birth without discrimination regarding their ethnicity, religion, or marital status. Details of the data collection tools are described in **Chapter 2.**

4.2.5 Operational definition

Neural tube defects (NTDs): is defined as any newborn baby or terminated with anencephaly or spinal bifida, or encephalocele, or meningocele or mylemeningocele.

Cases: Mothers, who gave birth a neonate with any type of NTDs, irrespective of gestational age and fetal survivorship at birth.

Control: Mothers who gave birth a neonate without NTDs that is apparently health newborns.

4.2.6 Data quality assurance, processing and analyses

Details of the data cleaning and processing are described in **Chapter 2.** Descriptive analysis was presented using means, frequencies and percentages. Chi-square was used to assess the significant difference between the two groups. All assumptions were checked. A multivariable conditional logistic regression model was used to identify the independent determinants of NTDs. The measure of association of each variable was determined using a parameter of adjusted odds ratio with 95% confidence intervals. Statistical significance declared at P-value < 0.05. A standard error of > 2.0 was used to test for multicollinearity. Model's fitness was assessed using the Hosmer and Lemeshow tests with P> 0.05 used as fit.

4.3 Results

Of 138 cases, 30(21.7%), 22(15.9%), and 86(62.3%) were from Dil Chora teaching hospital, Hiwot Fana specialization teaching hospital, and Adama medical college hospital, respectively. The overall Mean $\pm$ (S.D.) age of mothers was 26.2 $\pm$ 5.9 years. Rural resident women were found to be more prevalent than urban resident women (53.6% vs. 46.4%) (P =0.023). Besides, maternal age 18-24 years was lower among cases (26.8%) as compared to controls (41.3%) (*P* =0.036). Regarding the family size, the majority of the mothers who were part of households with 1-5 people were higher among cases (79.7%) as compared to controls (64.5%) (P=0.005) **(Table 4.1).**

Table: 4.1. Demographic data of the cases and controls in Eastern Ethiopia

Variables	Categories	Cases		Controls		*P*
		n	%	n	%	
Marital Status	Married	134	97.1	135	97.8	0.605 [a]
	Divorced	3	2.2	3	2.2	
Maternal age	18-24	37	26.8	57	41.3	
	25-34	67	48.6	86	62.3	0.036
	> 34	14	10.1	15	10.9	
Partner age	20-34	110	79.7	111	80.4	0.88 [a]
	> 35	28	20.3	27	19.6	
Partner educational status	Illiterate	9	6.5	8	5.8	
	Formal (1-12)	106	76.8	99	72.3	0.54 [a]
	College and above	23	16.7	30	21.9	
Occupational Status of mother	Governmental	13	9.4	9	6.5	
	Madam	90	65.2	89	64.5	0.695 [a]
	Private	15	10.9	20	14.5	
	Daily worker	20	14.5	20	14.5	
Partner occupational status	Governmental	27	19.6	31	22.5	
	Private	34	24.6	44	31.9	0.259 [a]
	Daily worker	14	10.1	16	11.6	
	Farmer	63	45.7	47	34.1	
Blood relation with your partner	No	133	96.4	133	96.4	1.000 [b]
Family size	1-5	110	79.7	89	64.5	0.005 [a]
	>5	28	20.3	49	35.5	

[a] Pearson chi square; [b] Fisher Exact test. Significant at <0.05

Among cases, 97 (70.3%) of mothers were hade 1-3 years gap between the previous pregnancy. The proportion of primigravida women in the cases was higher than in the control group (18.1% vs 8%) and the difference was statistically significant (p=0.012) **(Table 4.2).**

Table 4.2: Obstetric and health characteristics of case and controls in Eastern Ethiopia

Variables	Categories	Cases		Controls		P
		n	%	n	%	
Gap between the previous pregnancy	Nulligravida	30	21.7	12	8.7	0.011 [a]
	1-3 years	97	70.3	113	81.9	
	4-7 years	11	8	13	9.4	
Gravidity	Primigravida	25	18.1	11	8	0.012 [a]
	Multigravida	113	81.9	127	92	
Family planning	No	87	63	99	71.7	0.123 [a]
	Yes	51	37	39	28.3	
Current Pregnancy	Unplanned	120	87	118	85.5	0.727 [a]
	Planned	18	13	20	14.5	
ANC visit	No	69	50	64	46.4	0.547 [a]
	Yes	69	50	74	53.6	
IFA supplement	No	73	52.9	67	48.6	0.47 [a]
	Yes	65	47.1	71	51.4	
Breast feeding above 2 years	No	128	92.8	130	94.2	0.626 [a]
	Yes	10	7.2	8	5.8	
Male gender predominance	No	126	91.3	122	88.4	0.425 [a]
	Yes	12	8.7	16	11.6	
History of preterm	No	120	87	121	87.7	0.856 [a]
	Yes	18	13	17	12.3	
Chronic hypertension	No	138	100	136	98.6	0.498 [b]
Gastric disease	No	121	87.7	115	83.3	0.305 [a]
	Yes	17	12.3	23	16.7	
Suffered with stress	No	120	87	115	83.3	0.397 [a]
	Yes	18	13	23	16.7	
Suffered with viral infection	No	122	88.4	124	89.9	0.69 [a]
	Yes	16	11.6	14	10.1	
Suffered with malaria	No	132	95.7	136	98.6	0.28 [a]
	Yes	6	4.3	2	1.4	
Suffered from parasitic infection	No	104	75.4	106	76.8	0.77 [a]
	Yes	34	24.6	32	23.2	
Passive cigarette smoker or smoker	No	107	77.5	108	78.3	0.88 [a]
	Yes	31	22.5	30	21.7	
Partner exposure to chemicals	No	127	92	124	89.9	0.52 [a]
	Yes	11	8	14	10.1	
Heating/ cooling fumes in living quarters	No	122	88.4	121	87.7	0.85 [a]
	Yes	16	11.6	17	12.3	
Exposure to radiation	No	125	90.6	124	89.9	0.83 [a]
	Yes	13	9.4	14	10.1	
Inadequate ventilation during heating	No	124	89.9	128	92.8	0.39 [a]
	Yes	14	10.1	10	7.2	

[a] Pearson chi square; [b] Fisher Exact test. Significant at <0.05, IFA=Iron- folic acid, ANC= Antenatal care

Type of NTDs

Out of the total NTDs 51.4% and 34.1% were anencephaly and spinal bifida, respectively (Figure 4.1).

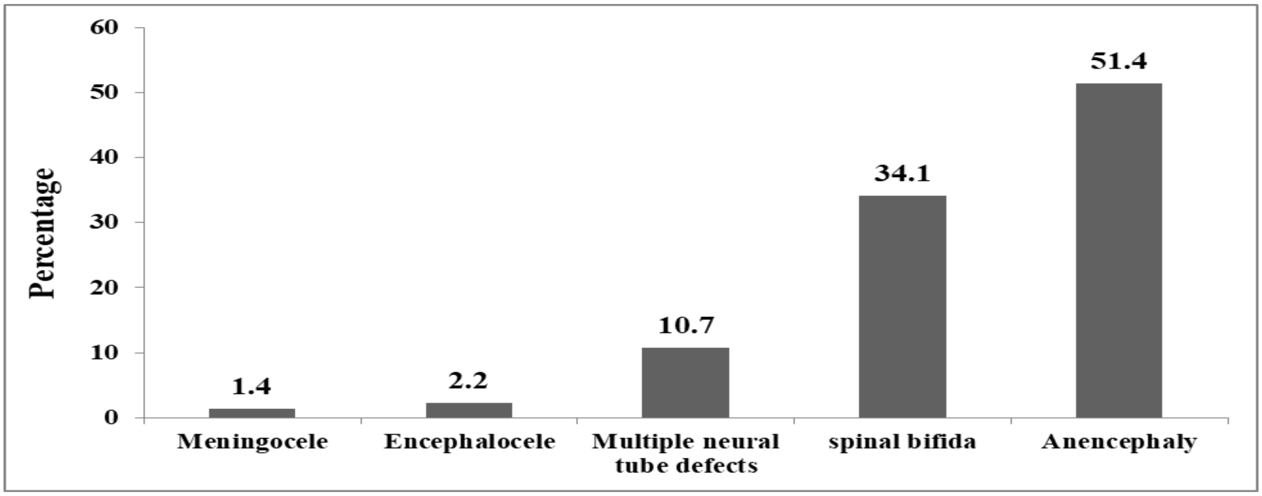

Figure 4.1: Type of NTDs

Some photo from neonate with NTDs taken during data collection

Determinants of Neural Tube Defects

After controlling for confounding variables, multivariate conditional logistic regression analysis identified that mothers who lived in rural areas, had formal education, had a history of elective or terminated abortions, suffered from severe anemia and fever or cold in pre or early pregnancy, and were exposed to various agro-chemicals were statistically significantly associated with the development of NTDs (Table 4.3).

Mothers living in rural areas were 3.4 times at risk for developing NTDs compared to mothers living in urban areas (AOR=3.4, 95% CI: 1.18-9.78, P=0.023). Mothers who had history of elective termination were nearly three times more likely to develop NTDs (AOR=2.95, 95% CI:

1.15-7.55, P=0.023) than those who had no history of elective termination. The risk of having neonates with NTDs was 3.4 fold (AOR=3.4, 95% CI: 1.17-9.87, P=0.024) in mothers who suffered from severe anemia in pre or early pregnancy compared with their counterparts. Mothers who had a history of fever in pre or early pregnancy were 2.75 times more likely to have a neonate with NTDs (AOR=2.75; 95% CI: 1.05-7.15, P=0.038) than mothers who had no history of fever in pre or early pregnancy. The risk of having neonates with NTDs was nearly 3.4-fold (AOR = 3.39, 95% CI: 1.11-10.3, P=0.032) in mothers exposed to various agrochemicals compared with their counterparts. In addition, illiterate mothers had a 66% higher risk of having neonates with NTDs (AOR=0.34, 95 percent CI: 0.12-0.92, P=0.034) than educated mothers (1-12).

Table 4.3: Multivariable analysis for the predictors factors for development of NTDs among women in Eastern Ethiopia,

Variables	Cases	controls	*P*	***AOR(95% CI)***
	n(%)	n(%)		
Residence				
Urban	64(46.4)	86(62.3)		1.00
Rural	74(53.6)	52(37.7)	*0.023*	3.41(1.18-9.78)
Educational status of mothers				
Illiterate	27(19.6)	26(18.8)		1.00
Formal (1-12)	100(72.5)	98(71)	*0.03*	0.34(0.12-0.92)
College & above	11(8)	14(10.1)	*0.272*	0.41(0.08-1.99)
History of elective o spontaneous abortion				
No	111(80.4)	122(88.4)		1.00
Yes	27(19.6)	16(11.6)	*0.023*	2.95(1.15-7.55)
Suffered with Anemia (Before or early pregnancy)				
No	103(74.6)	117(84.8)		1.00
Yes	35(25.4)	21(15.2)	*0.024*	3.4(1.17-9.87)
Fever(hyperthermia)/cold (Before or early pregnancy)				
No	98(71)	107(77.5)		1.00
Yes	40(29)	31(22.5)	*0.038*	2.75(1.05-7.15)
Use any antibiotic (Before or early pregnancy)				
No	112(81.2)	122(88.4)		1.00
Yes	26(18.8)	16(11.6)	*0.003*	6.6(1.89-23.02)
Maternal exposure to agrochemicals				
No	112(81.2)	120(87)		1.00
Yes	26(18.8)	18(13)	*0.032*	3.39(1.11-10.3)

Significant at P <0.05, AOR= Adjusted odd ratio, Max Std.err= 0.785. CI: Confidence interval.

4.4 Discussion

The objective of this case-control study was to identify the determinants of NTDs. In this study, women who resided in rural areas had higher odds of newborns with NTDs compared to their urban counterparts. This finding is supported by a study conducted in Tigray and Amhara Regional State of Ethiopia. This finding is supported by a study conducted in Tigray and Amhara regional state of Ethiopia (Berihu et al., 2019, Tadesse et al., 2020). This disparity in residence area could be attributed to differences in education level, health awareness, workload, and stress, as well as economic and cultural factors.

We also found that illiterate women had greater odds of newborns with NTDs compared to counterparts. This finding is in line with a study conducted in other studies (Li et al., 2006, Tadesse et al., 2020). Lunau *et al*., (2015) also reported that there was a significant relationship between women who had lower education and exposed to higher levels of work stress (Lunau et al., 2015). Experiencing stress before or after early pregnancy could be a predictor for development of NTDs. It was hypothesized that maternal stress could increase the circulating adrenocorticotropin and cortisol levels and affects the fetus's neural development (Gitau et al., 1998). This hypothesis was also supported by a systematic review and meta-analysis conducted by Jia *et al.* (2019) and Suarez and Lucina *et al*. (2003) (Suarez et al., 2003, Jia et al., 2019).

Another potential and possibly underappreciated mechanism associated with the damaging effects of stress may be altered micronutrient concentrations via its influence on nutrient stores in the body (Lopresti, 2020), including folic acid and other nutrients that are responsible for development of NTDs.

Maternal history of elective or termination or spontaneous abortion was significantly associated with having NTDs affected pregnancy. Our study agreed with other studies (Pei et al., 2019, Blanco-Muñoz et al., 2006, Gashaw et al., 2021). The epidemiology basis study showed that a low serum or plasma folate level has been associated with an increased risk of early spontaneous abortion (Nelen et al., 2000, Navarrete-Muñoz et al., 2015, Gaskins et al., 2014), which could be a level of maternal serum folic acid responsible for spontaneous abortions and development of NTDs. Moreover, maternal serum and erythrocyte folate concentration decreases from the twenty weeks of pregnancy onwards and remain low for a long time after delivery (Zheng et al., 2017, Homeister, 1962, Zetterberg, 2004), which could attribute for developing NTDs in the next pregnancy particularly for women with short inter-pregnancy. Hence, periconceptional folic acid supplements can effectively prevent not only the occurrence of NTDs and spontaneous abortion during early pregnancy.

However, Golalipour *et al.* (2014), De marco *et al.* (2014) and Todoroff *et al*., (2000) reported that there was no association between prior spontaneous abortion and development of NTDs (De Marco et al., 2011, Todoroff and Shaw, 2000, Golalipour et al., 2014). This discordant finding between studies could be attributed to the methodological approach used.

Mothers with suffered with chronic anemia was also significantly associated with NTDs in this study. The possible mechanism by which chronic anemia before or during early pregnancy could increase NTDs risk in neonate may be through elevated maternal serum homocysteine and the disturbance of methylation process. Disturbance of maternal fetal serum homocysteine and methylation may be responsible for the development of NTDs in the fetus. Various dietary factors, such as folic acid and vitamin B_{12} influence serum homocysteine levels and play a role in the methylation pathway (Morris et al., 2010, Selhub et al., 2009, Lanska, 2010, Mobasheri et al., 2010, Mahmood, 2014, Smits and Essed, 2001). Ferritin can also modulate folate availability via the cellular one-carbon pathway, implying that low iron status can alter folate utilization even when adequate folate intake and extracellular folate concentrations are present (Oppenheim et al., 2001a, Valberg, 1980). Serum homocysteine levels and methylation seem to be positively correlated with folate deficiency (Felkner et al., 2009). Iron deficiency is the most common cause of anemia (Haider et al., 2013, Iannotti LL, 2005, Pathak et al., 2007), which could possibly play a role in development of NTDs in humans. However, the need for more evidence to substantiate this pathway was suggested (Zeng et al., 2008), and showed no significant difference in maternal ferritin or hemoglobin concentrations between NTD-affected and non-affected pregnancies (Molloy et al., 2014). To know more, further investigation which is the mechanism that connects anemia and NTDs will be needed.

We also observed a significant association between fever/cold before or early pregnancy and developing NTDs, which is consistent with the report of studies conducted in various settings around the world (Shaw et al., 1998, Botto et al., 1999, Dreier et al., 2014). A possible physiological mechanism for the association between fever/hyperthermia and the development of NTDs may be that fever is a marker of another underlying process. Such as specific infections or immune disturbances contribute to increased risk for NTDs. Fever is also associated with increased levels of pro-inflammatory cytokines and other molecules (Romanovsky et al., 2005), that cross the placenta to affect fetal brain development via mechanisms other than hyperthermia. This study also found that the use of antibiotics before or early pregnancy was associated with NTDs. The possible reason is that antibiotic medications may have antifolate effects (de Lourdes Samaniego-Vaesken et al., 2015). Some specific forms of antibiotics such as sulfonamides are risk for fetus developing NTDs (Li et al., 2020, Lassi et al., 2014). However, our findings was not in agreement by a study conducted by Wang M *et al.*, (2014) (Wang et al., 2014). Nevertheless, there is no clear mechanism that antibiotics are risk factors for the development of NTDs.

It was also observed that maternal exposure to agrochemicals before or during the early pregnancy period was associated with increased the odds of NTDs in the offspring, which is supported by other studies (Rappazzo et al., 2016, Rull et al., 2006a, Gashaw et al., 2021). The possible explanation could be that agricultural chemicals are lipophilic and alter cell proliferation and differentiation during neurulation (Slotkin, 2004), averted neurological development and impairment. Agricultural pesticides enter the food chain through animal food sources and crops, including run-off in water bodies (Ben et al., 2019). Agricultural chemicals are used more frequently as part of livelihood activities in the study area, implying the need for more research before dismissing this potentially massive exposure. In contrast, a study conducted from a similar case control study in Tigray, Ethiopia, found no significant association between agro-chemicals and NTDs (Berihu et al., 2019).

Despite all of its significant findings this study has some limitations. The first limitation was the exposure status of study participants was determined, retrospectively, which could have been influenced by recall bias. The second limitation of this study is also that biomarkers and genetic polymorphism were not addressed. Moreover, due to the nature of facility-based research, generalization to the general population is difficult. The third limitation was that there was insufficient evidence to support our claim that fever/cold, anemia, and elective termination were associated with the development of NTDs. Moreover, due to the presence of very small numbers in some categories of predictive variables, the model estimates may be unstable which should be interpreted carefully.

The strength of this investigation was that both cases and controls were drawn from similar settings in a 1:1 ratio to avoid selection bias. Furthermore, a strong case ascertainment was used to identify NTD cases. An effort was also made to investigate several factors that could be potential determinants, which would contribute to a clear understanding of the risk factors for development of NTDs.

4.5 Conclusion

The study found that anencephaly and spinal Bifida are the two most common types of NTDs in the region. The results indicated that the development of NTDs was associated with residential area, history of abortion, history of severe anemia, history of fever, any antibiotics used before or during early pregnancy, and exposure to agrochemicals. Furthermore, among the identified determinant factors, this study revealed that dietary and nutritional related factors play a significant role in the development of NTDs in the study area. As a result, our findings suggest that implementing relevant prevention strategies including a staple food fortification for women of reproductive age, is required to achieve the Sustain Development goal (SDG) related to women and child health in eastern Ethiopia and throughout the country. Moreover, a multi-sectoral effort aimed to reduce the risk of NTDs is needed in the study area.

Future advanced studies including, assessment of biomarkers, dietary practice of women and the practice of preconception folic acid supplementation among women who have planned to be pregnant is needed.

Chapter 5: Dietary Practice among Women who Gave Birth with and Without Neural tube defect: A comparative cross sectional study

Berhane A, Fikadu T, Belachew T. Dietary practice among cohort pregnant women who gave birth to neonates with and without neural tube defect: a comparative cross-sectional study. Journal of Nutritional Science, Cambridge University. 2022 Mar 21;11:e24. doi: 10.1017/jns. 2022.20. PMID: 35399554; PMCID: PMC8943563.

Abstract

The burden of neural tube defects (NTDs) is high in Eastern Ethiopia, but no evidence linking it to maternal dietary practice has been found. It is however unclear if women delivered with NTDs having poor dietary practice than delivered healthy neonate. The objective of this study was to compare dietary practice and women who delivered neonate with NTDs and healthy neonate. In this study we approached comparative cross sectional study design. We recruited two group comprising 138 who delivered neonate with NTDs and 138 comparison group from selected hospitals in Eastern Ethiopia. Dietary practice was assessed using validated food frequency questionnaire (FFQ). The dietary practice of women was determined by using dietary diversity score (DDS), food variety score (FVS) and animal food source (ASF). Chi-square and logistic regression model to determine if there was any significant difference and association between two groups. All tests were two-sided, and p<0.05 was used to declare a statistically significant. The prevalence of good dietary practice in study population was 29% (95% CI = 23.6%–34.3%) and found that significant difference in dietary practice (P=0.034) between case and comparison groups. Significant difference in consumption of beans and peas, flesh food groups, eggs, other vegetables and other fruits between two groups. FVS and DDS were significant difference between two groups. Comparison group had a good dietary practice (AOR=2.1, 95% CI 1.07–4.1). The good dietary practice was more prevalent on rural residents women (AOR= 0.29, 95% CI, 0.1-0.7), and with non-formal education (AOR=0.16, 95% CI, 0.03-0.8). The dietary practice among cohort pregnant women was low in the study area. Nutrition education, folic acid fortification and supplementation are needed for hamper the problem also advanced research is required.

Key words: Dietary practice, pregnant women, developing neural tube defects

5.1 Introduction

Ensuring proper dietary practice is critical to optimizing the health of mothers and new-borns. Poor maternal nutritional status has been linked to a variety of adverse birth outcomes. Nonetheless, the relationship between maternal nutrition and birth outcome is complex and is influenced by a variety of biologic, socioeconomic, and demographic factors that vary greatly across populations (Villar *et al*., 2003). Nutrition has been shown to influence the risk of neural tube defects (NTDs) due to the role of folic acid and other nutrients (Wald *et al*., 1991, Berry *et* *al*., 1999). Folate is an essential nutrient for fetal development because it is a cofactor in many important cellular reactions, including Deoxyribonucleic acid (DNA) and Ribonucleic acid (RNA) synthesis (Czeizel, 2000), other nutrients (Watkins and Carmichael, 2003), poor diet quality(Carmichael *et al*., 2003) low maternal weight gain (Shaw *et al*., 2001), short inter-pregnancy interval (Todoroff and Shaw, 2000) and famine (Wynn and Wynn, 1993, Stein and Susser, 1975). There have been reports of NTDs in the offspring of women who had gastric bypass surgery (Haddow *et al*., 1986), long-term restrictive diets (Sheffer *et al*., 1993), and weight loss due to dieting during early pregnancy (Robert *et al*., 1995). The extent to which these factors explain the population burden of NTDs is unknown due to a lack of knowledge about the underlying mechanisms of action that cause NTDs. Continued research into nutritional exposures related to implicated nutrients will help us understand the nutritional etiologies of NTDs. Understanding the relationship between dietary practice of women is thus critical for preventing negative birth outcomes including NTDs. We hypothesized that compromises dietary practice would be associated with increased risk of having offspring with NTDs. This study thus compared prevalence of dietary practice between groups of women who delivered neonate with NTDs and without NTDs (apparent healthy neonate) counterpart in a setting high burden of NTDs.

5.2 Method and Materials

5.2.1 Study design

This was a comparative cross-sectional study design, which compared the dietary practice between women who delivered neonate with NTDs and their counterparts. The subjects were selected from hospitals which is found in the eastern Ethiopia. Details of the study setting are described in **Chapter 2.**

5.2.2 Study population

The study population consisted of two groups: women who delivered neonate with NTDs and women delivered apparently healthy new-born (without NTDs). NTDs were defined according to the ICD-10 criteria and who has at least with one of the following conditions: anencephaly, spinal bifida, or encephalocele (WHO, 2004). Both groups were recruited from obstetrics/gynaecology department in the same facilities. All women who accepted with written consent included in the study. Women, who were very ill, emotionally upset, mute, or died shortly after delivery, as well cases that were difficult to identify, were excluded. Case group defined as a woman who delivered neonate with NTDs while comparison were randomly selected from the same hospitals of women who delivered neonate apparently health and live born.

5.2.3 Sample size determination

The sample size was calculated assuming an equal number of cases and controls (1:1), odds ratio of 3.0, power of 80% (Taye et al., 2018b), significance level of 5% and non-response rate of 5%. The final sample size was 276 (138 cases and 138 controls).

5.2.4 Data collection tool and procedure

Minimum Dietary Diversity for Women (MDD-W) and anthropometric measurements were taken from participants. Details of the data collection tool and procedures are presented in **Chapter 2.**

5.2.5 Data quality assurance, processing and analysis

Details of the data quality assurance, cleaning and processing are described in **Chapter 2.** The chi-square and the independent t-test test were used to observe for any statistical significant differences in prevalence and mean intensities between the two groups. Logistic regression model was fitted to compare the prevalence of dietary practice between the two groups. The best fit model was determined using Akaike's Information Criteria (AIC). A lower AIC suggested a better fitting model. An odds ratio at 95% confidence intervals was used for measuring the association. All assumptions were checked and standard error of >2.0 were used to test for multicolliniarity. All tests were two-sided, and statistical significance was determined using p values of 0.05.

5.3 Results

Figure 5.1 depicts the contribution of each food group to the development of FVS, DDS, and ASFS. All subjects in both groups consumed more starch and staples food group. Consumption of beans, peas, dairy, flesh, vitamin A rich DGLV, other vegetables and fruits were higher in the counterpart group than case group, while nuts and seeds, eggs were comparatively higher in the case group (**Fig 5.1).**

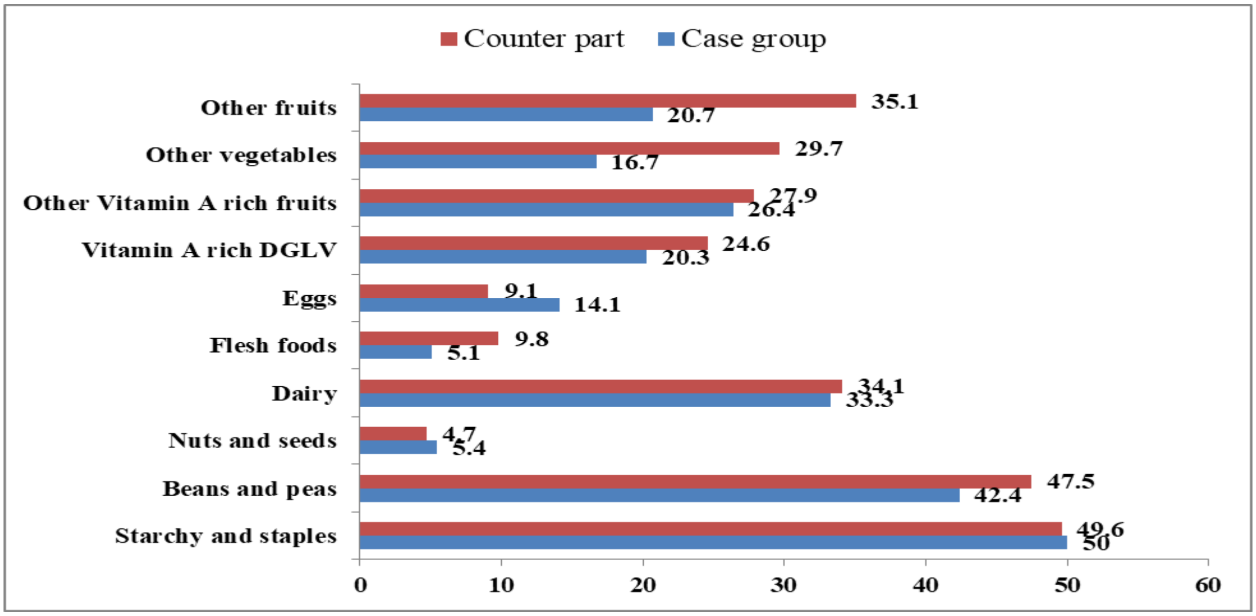

Fig 5.1: Percentage of foods groups consumed

There was significant difference in consumption of beans and peas (p=0.005), flesh food groups (p=0.028), eggs (p=0.046), other vegetables and other fruits (p<0.001) between case and comparison group. Women who delivered a neonate without NTDs were consumed more flesh and fruits than comparison group (Table 5.2).

Table 5.2: Food groups consumed in three months dietary recalls, among of participant women's of cases and comparison group.

Characteristics	**Case group (n=138)**	**Comparison(n =138)**	**Mean difference**	***P***
	Mean + Std	**Mean + Std**		
Starchy and staple foods	5.5+2.13	5.4+ 2.11	0.00725	*0.31*
Beans and peas	5.5+3.75	5.7+3.5	0.10145	*0.005**
Nuts and seeds	0.1 + 0.3	0.1+0.3	0.01449	*0.42*
Dairy	1.01+ 1.0	1.16+0.89	-0.01449	*0.6*
Flesh foods	0.18+0.38	0.13+0.4	-0.0942	*0.028**
Eggs	0.25+0.43	0.19+0.39	0.10145	*0.046**
Vitamin A rich DGLV	0.64+0.49	0.83+0.5	-0.08696	*0.14*
Other vitamin A rich fruits & vegetables	0.73+0.9	0.76+0.89	0.26087	*0.36*
Other vegetables	0.54+0.51	0.55+0.6	0.26087	*<0.0001***
Other fruits	0.57+0.49	0.41+0.49	0.28986	*<0.0001***

*Independent Student t test and *Chi square test, DGLV=Dark Green Leaf and Vegetables*

The prevalence of good dietary practice in study population was 29% (95% CI = 23.6%–34.3%) (Fig: 5.2).

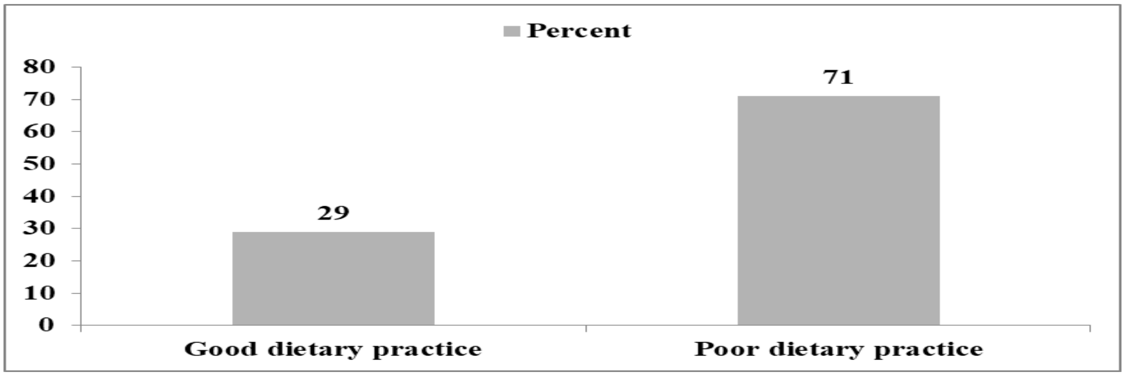

Fig 5.2: Prevalence of dietary practice study participants in eastern Ethiopia

Dietary practice (P=0.034) showed significant difference in the two groups. In addition FVS (P=0.01) and DDS (P=0.045) were significant difference between case group and comparison group (Table 5.3).

Table 5.3: Dietary practice between case group and counterpart

Characteristics	Case group (n=138)	Comparison group (n=138)	*P*
	n (%)	n (%)	
Dietary practice			
Poor	106(76.8)	90(65.2)	*0.034**
Good	32(23.2)	48(34.8)	
Food variety Score			
Low	70(50.7)	49(35.5)	*0.01**
High	68(49.3)	89(64.5)	
Dietary Diversity Score			
Low	39(14.1)	25(9.1)	
Medium	64(23.2)	68(24.6)	*0.045**
High	35(12.7)	45(16.3)	
Animal Source Food			
Low	27(9.8)	34(12.3)	
Medium	80(29	69(25)	*0.79*
High	31(11.2)	35(12.7)	
Body Mass Index			
Under weight	8(2.9)	4(1.4)	
Normal weight	126(45.7)	131(47.5)	*0.49*
Over weight	4(1.4)	3(1.1)	
MUAC			
Under-nourished	75(27.2)	82(29.7)	*0.39*
Well nourished	63(22.8)	56(20.3)	

** Chi square test: Significant at 0.05*

Women who lived in rural were 71% less having a good dietary practice than those compared to women who resident in urban (AOR= 0.29, 95% CI, 0.1-0.7). Study participants with non-formal education were 84% times less having a good dietary practice than compared to those women with tertiary education (AOR=0.16, 95% CI, 0.03-0.8), while respondents with comparison group were 2.1 times more odds of having a good dietary practice than compared to those case groups (AOR=2.1, 95% CI 1.07–4.1) (Table 5.4).

Table 5.4: Parameter estimates from multivariate regression predicting dietary practice among participant women, Eastern Ethiopia

Parameters	Dietary practice status		β	SE	AOR(95% CI)	*P*
	Poor n(%)	Good n(%)				
Participants group						
Case group	106(54.1)	32(40)			1.00	
Comparison group	90(45.9)	48 (60)	0.75	0.34	2.1(1.07-4.1)	0.029
Residence						
Urban	96(49)	63(78.8)			1.00	
Rural	100(51)	17(21.2)	-1.2	0.49	0.29(0.1-0.7)	0.013
Educational status of women						
No formal education	18(9.2)	23(28.7)	-1.7	0.8	0.16(0.03-0.8)	0.02
Primary education (1-8)	102(52)	22(27.5)	0.8	0.7	2.2(0.5-8.9)	0.25
Secondary education (9-12)	67(34.2)	19(23.8)	1.07	0.6	2.9(0.8-10.4)	0.09
Tertiary education	9(4.6)	16(20)			1.00	

AOR= Adjusted odds ratio, Maximum Standard error (SE)= 0.8., Significant at P<0.05

5.4 Discussion

The present study has compared the dietary practice of women who delivered a neonate with NTDs and apparently delivered healthy comparison. The dietary practice was assessed in terms of meal frequency, food variety score, dietary diversity score and animal source food score. In the current study, the overall proportion of good dietary practice was low (29%). The finding of this study was higher than that of a study done in Haramaya district, eastern part of Ethiopia (15.18%) (Fite *et al*., 2022), and West Gojjam Zone, Ethiopia (19.9%) (Demilew *et al*., 2020).

The present study showed that case groups had poor dietary practice (40%) than the comparison group and there is a significant difference in the dietary practice between two groups (P=0.034). Poor dietary practice of pregnant mother significantly affects fetus health and may result poor birth outcomes (Kramer, 1987, Gogoi and Prusty, 2013, Evans *et al*., 2018). Also, it contributes to micronutrient deficiency (Lee *et al*., 2013, Torheim *et al*., 2003) and may be associated with folate deficiency (Gibson, 2005) and could be leads to the occurrence of NTDs.

In this study, nearly half of the case group (50%) and 49.6% comparison group women was consumed starchy and staple food groups, while flesh and nuts and seeds foodstuffs were the lowest food groups consumed in the two groups (5.1% of cases and 5.4% of comparison groups). This finding implies that foodstuffs that consisting of folate are rarely consumed among the study participants. There was a significant difference in the consumption of beans and peas (P=0.005), flesh food groups (P=0.028), other vegetables and other fruits (P<0.001) between case group and comparison group. This study is in line with other study conducted in Ethiopia (Aliwo *et al*., 2019, Demilew *et al*., 2020). The fact that flesh food, nuts and seeds are good source of folate (Paik, 2008), it could explain the difference in the likelihood of NTDs risk between the two groups. It has been demonstrated that folate has health benefits and plays an important role in one-carbon metabolism, and that its deficiency is primarily linked to defects in the development of the foetal neural system, leading to NTDs and megaloblastic anemia (Smithells and Seller, Mulinare *et al*., 1988, Bower and Stanley, 1989). The fact that none of the pregnant women in this study took folic acid before and during pregnancy is an issue that requires immediate policy attention.

There were statistically significant difference in DDS (P=0.01) and FVS (P=0.045) between case and comparison group. The possibility of this finding might be dietary diversity is correlated with the nutrient adequacy, increased nutrient intake, and better nutritional status of pregnant women (Tsegaye *et al*., 2020). In our study women who lived in urban area is significant association with dietary practice. This might be due to the fact that urban dwellers is exposed to dietary information and will more enforced to practice adequate diet. In addition, this study found that women's dietary practice significantly associated with women educational status. This finding is supported by different studies (Shamim *et al*., 2016, Kiboi *et al*., 2017, Kever *et al*., 2015, Alemayehu and Tesema, 2015, Shehab, 2012). This could be attributed to the role of education in informing people about the importance of eating a diverse diet. On the other hand, educated women can have better employment and income opportunities (Revenga and Shetty, 2012), which could be improve their household food security and consumption of a diverse diet.

In general, the findings have far-reaching practical implications. Folate can be getting through dietary sources because folate is found in many foods sources, and the findings revealed that women with poor dietary practice imply that the intake of folate is low among study participants. This necessitates efforts to galvanize public health measures aimed at increasing consumption of folate-rich foods in particular and improving good dietary practice in general through a food-based approach. This is clearly stated in Ethiopia's newly developed food and nutrition policy and strategy (FMoH, 2019, Duflo, 2012).

There is no or rare comparative studies conducted in Ethiopia, also in Eastern Ethiopia, regarding to dietary practice between women who delivered neonate with NTDs and comparison groups which makes this study is unique. We acknowledge the study's limitations, including the inability to assess nutrient content of foods as a component of dietary practice. Nonetheless, it has been demonstrated that these qualitative measures, such as DDS, ASF, and FVS, are highly correlated with nutrient levels and can be used as a surrogate marker of dietary practice.

5.5 Conclusion

This study highlighted that large proportion of the study participants had poor dietary practice with regards of folate-source foodstuffs groups. Dietary practice was significantly associated with occurrence of NTDs. This finding implies the need for considering food fortification with basic food, pre-conception folic acid supplementation, and nutrition education intervention. Further research is required to find out the gene-nutrient and gene environment interactions, as well as particular causative factors associated with NTDs in Ethiopia.

Chapter 6: The practice, Challenges, And Opportunities For Preconception Folate Supplementation To Bridge The Gap In The Continuum Of Care To Reduce The Risk Of Neural Tube Defects among women plan to pregnant in the Ethiopian Health care system

(Submitted)

Abstract

Background: Provision of preconception folate supplementation is a promising continuum of services to prevent the neural tube defects and to improve the health of future children. A weekly or daily intake of iron folic acid prior to pregnancy is effective in increasing folate levels in red blood cells and reducing the risk of neural tube defects. This study aimed to evaluating the practice, challenge and opportunities of preconception folate supplementation to reduce the risk of neural tube defects in health care facilities among women plan to pregnancy in Ethiopia.

Method: A qualitative study was conducted and pregnant women who followed ANC, women plan to be pregnant, health care providers and experts of maternal and child health care were participated in the study. A purposive sampling method was used to select the study participants from health facilities. A total of 45 in-depth interviews were conducted. Filed notes and audio-recordings were transcribed verbatim, translated and analyzed by inductive thematic analysis using Atlas ti.7.1.

Results: It was observed that there was no provision of folic acid supplementation to prevent neural tube defects for those who had planned to be pregnant in Ethiopia. The challenges for this disruption include: absence of clear policy, guideline, negligence from providers side, lack of knowledge among care givers and women, unfavorable attitude and belief, payment of user fee by pregnant women, high work load of providers, unplanned pregnancy and poor adherence.

Conclusion: There is no provision of folic acid supplementation before pregnancy for those women having a plan to pregnancy. The findings imply the importance of developing clear, program guideline capitalizing on the existing opportunities to prevent the incidence of neural tube defects and reduce the neonatal morbidity and mortality that to achieve the sustainable development goal. Moreover, advanced experimental study design will be needed to start the preconception iron-folic acid supplement with other care service for women who planned to pregnant.

6.1 Introduction

The Ethiopian constitution, health policy and different health strategies give a strong emphasis to prevention and early intervention as an approach (Asresu et al., 2019, Goshu et al., 2018, Demisse et al., 2019), through the health extension programs (Gavin et al., 2014).

However, there is lack of evidence about preconception folate supplementation to prevent neural tube defects among women who plan to be pregnant in the study area. Therefore, this qualitative study evaluating the practice, challenges and opportunities of provision of preconception folate supplementation to prevent neural tube defects in health care facilities among women who plan to pregnancy.

6.2 Methods and Materials

6.2.1 Study design

A qualitative study design with in depth interview and description of factors associated with service utilization among health care providers and women's in eastern Ethiopia. This study followed the standard protocol for qualitative studies, COREQ (consolidated criteria for reporting qualitative research) for reporting qualitative findings.

6.2.2 Participants, Recruitment

In this study women who planned to be pregnant and pregnant women who visited antenatal care (ANC) services were a study participants and recruited from health facilities. In order to assess the challenge and opportunities of preconception folic acid to study participants, key informants were recruited from health facilities and health bureau. The key informants were health care professionals (midwives, senior nurses) who were working in Obstetrics and Gynaecology (Ob/Gyn), department of family planning and experts of Maternal and Child Health care service (MCH) from Regional Health Bureau. Purposively a total of 45 study participants including key informant interviews (KII) were selected.

6.2.3 Data Collection and measurement

To fully understand the utilization behavior of women's before preconception to reducing the risk of NTDs, three different semi-structured questionnaire (for women, health care provider and health experts) with open-ended questions were developed after reviewing different literature. Details are presented in **Chapter 2.**

6.2.4 Data Analyses

Transcripts were analyzed using thematic framework Atlasti version 7.0.71 software. Transcripts were firstly read thoroughly to have sense of the whole content of discussions and become familiar with the data. For each transcript, significant statements were identified and highlighted and were then extracted for each interview and meanings formulated. These formulated meanings were then further clustered into theme and emergent sub-themes, under which the study findings were described.

To maintain the trustworthiness of the data, different techniques were used. At the outset, the interviewer guide was carefully tested in order to ensure that it worked as intended. To ensure the credibility of the peer debriefing process, it was done by members of the group. A summary of the key points and some confusing ideas were presented to check the interpretations, critiques, and confirmation. All participants were made aware of the final results of the study in order to ensure accuracy of their ideas. The transferability was verified by informing the entire research process, different perspectives of the participants, experience, methodology and interpretation of the results. To ensure the reliability and coherence of the data, participants and the findings were checked and verified. To maintain confidentiality, all records were kept anonymous marked with codes and made available for cross-checking. The validity, steadfastness, adaptability, and conformability of the investigation were safeguarded by various strategies. Specialists were engaged in information assortment and examination and the authors made summarization at the end of each interview. Detailed approaches and search processes were certainly recorded and used to help translate the results. The data were deciphered, analyzed and checked against sound tapped information and field notes prior to delivering the last report. Partners were asked to share their impressions of the information that had been analyzed and deciphered. The findings of this study were considered and checked by experienced people in the subjective study. Finally, the results were presented in narratives using well said verbatims as illustrations.

6.3 Results

A total of 45 participants participated with an overall maternal age ranging from 18 to 40 years and mean (±SD) age was 26.2(±5.9) years. As indicated in Table 1, 97.3% of participants were females and 84.4% were living in urban areas. Larger proportion (42.2%) of the study participants were in tertiary educational level, 48.9% were housewives and had between 1-3 family members (Table 6.1).

Table: 6.1. Demographic data of study participants, Eastern Ethiopia

Variables		Frequency	Percentage
	Female	43	97.3
Living area	Urban	38	84.4
	Rural	7	5.6
Age	18-24	5	11.1
	25-34	35	77.8
	> 34	5	11.1
Mean age		**26.2(±5.9)**	
Educational status	No formal education	7	15.6
	Primary (1-8)	8	17.8
	Secondary (9-12)	11	24.4
	Tertiary	19	42.2
Occupational status	Governmental	8	17.8
	Housewife	25	55.5
	Private	12	26.7
Family size	1-3	22	48.9
	4-6	18	40
	>7	5	11.1

All groups of study participants are described in three sections, different themes and subthemes. Thorough reading of the verbatims and transcriptions generated themes and sub themes are summarized in Table 6.2. The first section included issues such as: preconception care practices on reducing the risk of neural tube defects. The second theme is challenges for preconception care practice on reducing the risk of neural tube defects and the third is the opportunities of implemented of preconception care practice on reducing the risk of neural tube defects prevent by uptake of iron folic acid or folic acid supplement.

Table 6.2: Themes and subthemes identified for preconceptional care for reducing the risk of NTDs

Theme	Sub-theme
Practice of preconception of FAS	**Provision of FAS**
Challenges for implementation	Absence of clear policy, program and guideline
	Lack of Knowledge among health care givers and women
	Program and Adherence
	Unplanned pregnancy
	Work load
Opportunities for implementation of preconception care for reducing the risk of NTDs	Established health extension program
	Family planning program

Practice of preconception of FAS

Provision of FAS

Almost all health care providers and experts stated that there is no provision of preconception of FAS to prevent the risk of NTDs for women who removed a modern contraception and those aborted spontaneously and refused to take contraceptive for intention to pregnancy at family planning service and gynecology ward. They explained that the majority of women turned to health facilities only after confirmed pregnancy and when there is a problem with the fetus. Public health expert stated that,

"….Generally speaking we didn't provide a preconception FAS to prevent NTDs. We only provide IFAS at ANC" (**MPH, public health expert from health bureau).**

Similarly, senior midwifery added that,

"..In Ethiopia, preconception care service is not part of maternal service in health facilities so, there is no provision of FAS for woman who having a plan to pregnant either women who removed modern contraception or spontaneous aborted and refuse to take modern contraception" (**Midwifery from family planning service).**

All of the women participants described that they didn't take a preconception of FAS to prevent NTDs affected pregnancy and added that, they didn't visit health care facility before conception, "...the reason is due to the unavailability of the services in health facilities. For example, women should go to the health facilities for ANC because this service is available at health centers" **(Women who visit ANC).**

Challenges for implementation

Absence of clear policy, program and guideline

All public health experts explained that in our country, there is no clear guideline and program on preconception care service with the provision of IFAS or FAS for prevention of NTDs affected pregnancy. They pointed out that delivery of any program is based on a guideline. In Ethiopia, health policies clearly stated about maternal and child health services focus on the use of focused antenatal care (FANC) and skilled delivery services. They stated that since they don't have a clear guideline, they can't do anything. So, they always practice what we are accountable for. Every health care provider automatically performs what he/she is expected to do. Otherwise; nobody will go beyond that. Public health expert added the following to corroborate this idea.

"…Health care providers, as part of their clinical practice, may give preconception care service for the prevention of NTDs to their clients informally and inconsistently. For example, a gynecologist may consider FAS for three months as a treatment to mothers whose pregnancy is affected by NTDs. But, the other health care providers do not give this service a preconception care service at family planning clinic and gynecology ward."(**MPH, public health expert health bureau maternal and children department)**

Almost all participants' health care providers described that as we know in Ethiopia there is no guideline for preconception care service with iron folic acid supplement for reducing the risk of NTDs. They deliver FANC and skilled delivery services, in a routine program. For prevention of NTDs affected pregnancy, they are not obliged to provide or prescribe IFAS or FAS before pregnancy unless supported by guideline and established program in health facilities. So, they stated that they can't do anything.

A degree midwifery nurse from family planning service stated about why she did not deliver a preconception care service for prevention of NTDs to those who had a plan to be pregnant as follows.

"…For example, one day a university instructor woman asked me to provide her folic acid supplementation. I don't have any guideline to provide IFAS or FAS to non-pregnant women whether who have a plan to be pregnant or not" **(Degree midwife nurse from family planning clinic).**

Absence of program in health care system

Most participants of health care providers explained that the preconception folate supplementation is a neglected program in health care facilities in the area also in Ethiopia. The mothers are not assessed whether they are in good condition to be a pregnant or not like the level of hemoglobin (anemia), nutritional status or other diseases or consultation on their modifiable lifestyle before conception. They provide only a preconception care consultation if a mother asks for any form of advice or treatment for any form of ailment. However, the consultation provided is based on what they know and not supported by standard guideline.

Similarly, one health care provider from Gynecology Department ward added,

"…We focus only on contraceptive for women who aborted spontaneously. If the mothers refuse to use contraceptive we do not go further that the mothers have get preconception care service with the provision of iron folic acid or folic acid supplement to prevent NTDs affected pregnancy" **(Degree midwifery nurses, from Hospital Gynecology ward).**

This was also substantiated by the following assertion stated by a diploma nurse.

"…For mothers who want to remove the insertion of contraceptive, I take much time to know why the woman wanted to remove. Because officials are always concerned and raise questions on why the number of mothers getting long acting contraceptive removed increased. (**Diploma nurse from Family planning clinic/section)**

A midwife nurse workings in the Gynecology ward stated the following in trying to explain her experience related to preconception care service.

"…I only give counseling to women to take prescribed folic acid tablets properly for three months for women who delivered a baby with NTDs and any type of congenital anomaly, but not linked with health extension **(Degree midwife nurses from Hospital Gynecology ward)**.

Almost of all health care providers who participated in the study explained that there is negligence on preconception care service with IFAS or FAS for prevention of NTDs and not part of education during their degree or diploma courses. She also added that there is a few or no focus area about preconception of iron folic acid or folic acid supplement for prevention of the occurrence of NTDs affected pregnancy. They didn't have a theoretical training as well the setup to practice PCC for prevention of NTDs. After graduation, the facilities where we are currently working also do not have the setup for PCC provision to prevent NTDs and other negative birth outcome. Thus, there is a missed the opportunity on those having a plan to be pregnant.

Nevertheless, very few practitioners responded that

"…They knew little about preconception care with uptake of IFAS or FAS for the prevention of NTDs only but on other service of preconception care service is neglected and lacks of focus on the area."

Lack of knowledge among care givers and women

In all group of the societies including health care providers, there is a big knowledge gap about preconception of IFAS or FAS for reducing the occurrence of NTDs. They don't have any knowledge about what exactly preconception care is all about and lack of detailed information about this issue.

Senior midwifery told that,

"…If you ask any health care providers, hope you do not get answer on what preconception care mean for prevention of developing NTDs. We have not heard about PCC service for prevention of NTDs and other negative outcomes from any media." **(Midwifery nurses from family planning service)**

Similarly, one diploma midwife nurse from Gynecology ward added,

"...This is the first time I heard about the preconception of iron folic acid or folic acid to prevent NTDs affected pregnancy and other negative birth outcomes for those who aborted spontaneously and refused to take contraceptive" **(Diploma midwifery nurse from Gynecology ward).**

The majority of participant mothers who visited ANC clinic stated the following to show why they didn't use the preconception care service for prevention of NTDs affected pregnancy before they are being pregnant, that they don't have any knowledge or information about preconception care service with IFAS or FAS for prevention of NTDs affected pregnancy. Due to this; we haven't done anything before pregnancy even if our pregnancy is planned. Similarly, another woman agreed on majority's idea and added her opinion as follows,

"…the other reason is due to health care providers don't have any knowledge or information about preconception care service with the provision of IFAS or FAS for prevention of NTDs affected pregnancy that is why they didn't deliver the service to us **(Women who follow ANC).**

Women who removed contraceptive for intention to pregnancy described that,

"…We usually relay on health care providers. We assume that all important service is delivered to us" **(Women who have a plan to pregnant).**

Similarly one mother added,

"…why the health care providers didn't provide IFAS or FAS before pregnancy for prevention of NTD affected pregnancy and I didn't hear about it in any media, but she [the nurse] knows why I removed the contraceptive. So, she has to be given preconception care service including IFAS or FAS **(Women who removed long acting contraceptive).**

Program and adherence

Almost all health care providers pointed out that in health facility, IFAS were given only to pregnant women, because of there is ANC program but not preconception care program. On the other hand midwife nurses from Gynecology ward and family planning clinic suggested that even if the service is provided free, women may not use it properly. One public health expert added,

"…You can see the pregnant women attending ANC program were not taking it properly and adhere to IFAS. There is low adherence even among health care providers unless another strategy is employed". **(MPH public health expert)**

Unplanned pregnancy

The majority of women and health care providers described that unplanned pregnancy is one of the factors that hinder women from preparing for pregnancy. They repeatedly mentioned that unplanned pregnancy was a common problem in the community. The participants explained that some women do not know they have conceived and become aware when they visit health facility for other services.

"...It is due to an unplanned pregnancy. For some women, this may be due to menstrual disturbance. For example, women who know about preparation for pregnancy but her menstrual period is disturbed may conceive without getting this care (MPH public health expert and senior midwifery).

Another woman explained that,

"…The majority of women do not do anything before they conceive. Some of them even do not know that they are pregnant. They aware of it after the fetal movement started. Some of them hear that they get pregnant when they attend health facility for some illnesses. They also give birth without knowing their gestational age" (Women who visit ANC).

High staff work load

Almost all of health care providers from family planning clinic explained that work load is one of the factors that hinder proper delivery of PCC service to prevent of recurrence of NTDs or reduce the risk of NTDs.

"… Even if you add another activity, there is high work load in this section i.e, only one health care provider gives a consultation about the choice of contraceptive and then insertion. Frankly speaking, our activities are boring and so difficult." **(Diploma nurse from Gynecology ward and family planning section after briefing what PCC mean)**

Opportunities of preconception care on prevention of NTDs

Health extension program

Almost all public health experts and health care providers agreed that in Ethiopia one of the most important opportunities to prevent NTDs affected pregnancy and other related negative outcomes are the presence of health extension program and delivery service door to door via trained health extension workers. Preconception care service with IFAS or FAS starts at the health facilities for those having a plan being pregnant and aborted spontaneously and refuse to take contraceptive and linked with the established of health extension program that delivery service door to door via trained health extension workers. One public health expert added that:

"…Preconception care service with IFAS or FAS is a crucial time for achieving a sustainable development goal" (Public health expert).

Family planning service

Almost all public health experts and health care providers stated that, family planning clinic gives a golden opportunity to reduce the occurrence of NTDs and other related adverse pregnancy outcome. Yes, women have to be assessed for their current status like nutritional status, lifestyle, diagnosed with diabetes mellitus, asthma or hypertension or women that are on anticonvulsants or others issue. They believe that mothers should get a preconception care service with IFAS or FAS at least for three months before pregnancy occurs**.**

Similarly, another midwife nurse and public health expert from Gynecology ward added,

"…Surprisingly, we miss a great opportunity for the mothers who refused to use contraceptive after spontaneous abortion [indirectly planned to be pregnancy, although this is a good strategy for improving maternal and child health including prevention of development of NTDs pregnancy outcome **(Public health expert and Diploma midwifery nurse from Gynecology ward).**

In summary, the absence of preconception IFAS or FAS care service for prevention of NTDs was mentioned by all participants. Absence of clear guideline, negligence, knowledge, belief and attitude, economic and program and adherence were cited by most participants while the presence of health extension program and family planning service were indicated as an opportunity to be used as a platform to deliver preconception care service (Table 6.3).

Table 6.3: Summary of challenges and opportunities of delivery and uptake of preconception care for women stated by the key informants

Challenges	**Number of Key informants who reported**
Absence of clear program and guideline for preconception care service	All
Lack of knowledge among care givers and women	Most
Program and adherence	Most
Unplanned pregnancy	Most
Work load	Most
Opportunity to be used as a platform for delivery and uptake of preconception care Service for women uttered by health care workers	
Presence of the health extension program	All
Presence of family planning service	All

6.4 Discussion

This qualitative research sought to evaluate where we are in the prevention of NTDs in Ethiopia health care system. To our knowledge, this is the first qualitative study to address the preconception practice mainly provision of folic acid to reduce the risk of NTDs affected pregnancy for women who have plans to pregnant found in Ethiopia. The practices highlighted include services provided within the health care system that aim to improve maternal health for positive pregnancy outcomes. It is therefore understandable that the practices highlighted in this study appear to primarily focus on the woman.

Preconception health care is crucial because of the implications for preventing neural tube defects and averting adverse outcome pregnancy which is related to folic acid supplementation (Thompson et al., 2017, Kumar, 2009, Greenberg et al., 2011). Our finding indicated that there is no preconception folate supplementation practices for women who planned to pregnancy to reduce the risk of neural tube defects in the study area. This is due to the absence of policy, structure or guidelines for uptake preconception care service indicated as predominant challenges in health system. Biratu (2017) reported from a rigorous systematic literature review that there is no any nationally published preconception guideline to guide preconception care service in Ethiopia. But, Ethiopian health policy may potentially give fertile ground and to accommodate the integration of preconception care service. In addition, the mission of the health sector in Ethiopia is stated totally in agreement with the goal of preconception care (Biratu, 2017). The importance of preconception care service been reported by Feuren *et al.,* (2004) (Fleuren et al., 2004). The need for availing preconception care service guideline for health care providers is highly recommended to prevent development of NTDs (Johnson et al., 2006). Consequently, in the absence of preconception care service policy and guideline, there is lack of clarity on the responsibility of providing preconception folate supplementation as part of routine service in health facilities.

This study showed lack of preconception care knowledge to be a challenge to the provision and preconception folate supplementation for prevention of NTDs. This issue has also been discussed in different studies (Sijpkens et al., 2016, M'hamdi et al., 2017, Mazza and Chapman, 2010, Teshome et al., 2020a, Teshome et al., 2020b, Bekele et al., 2020). Lack or low level of knowledge in this study might be due to relatively low health education activities conducted to raise the awareness of women and of the community on the importance of preconception folate supplementation and care service in general during the preconceptionl period. In addition, although Ethiopia's family planning guideline is expected indicate the need for the integration of preconception care service into daily activity of practitioners, there is not such guidance as it stands currently. This gap might have happened as a result of non-familiarity of preconception care service in family planning service in Ethiopia. A systematic review by Poels *et al.*, (2016) reported that, women are unfamiliar with the concept and use of preconception care service (Poels et al., 2016). This was due to the fact that preconception care is a new program in many countries and the existence of knowledge gap among health care providers. Lack of knowledge can deprive health-seeking behavior and service utilization. This suggests the need for developing strong communication for increasing awareness of the community. Different advertising campaigns, such as social media, TV and local media should be emphasized.

In the current study the need to pay user fee was cited as a challenge to uptake of preconception care service. According to WHO, preconception care service largely focuses on the primary prevention through health education and counseling (Organization, 2013a) suggesting the need to making the service free to improve its effectiveness (Zhou et al., 2016). On the other hand, provision of iron folic supplementation for having planned pregnancy was not considered. Conversely, EDHS 2016 reported that the coverage of iron folic supplementation during pregnancy still remains at substandard level as only 5% of pregnant women took iron and folic acid tablets for 90 days (Bhutta et al., 2013, CSA and ICF, 2017). This might be due to the fact that many women are still unaware of how much micronutrients particularly iron and folic acid have an impact on their pregnancy outcomes and future generation heralding the need for improving their nutritional behaviors before conception.

In our study, work load of health care providers was reported as one of the challenges for the provision of preconception care service to women who have a plan to be pregnant directly or indirectly. Luquis and Paz, (2015) also identified lack of time and heavy workload as one of the most important factors that prevented health care providers from engaging in health promotion and prevention (Luquis and Paz, 2015). A study in six European countries also found that mean consultation length in general practices was 10.7 minutes (Deveugele et al., 2002). This might be related to the fact that only one health care provider gives consultation about the choice of type of long acting family planning and then performs the procedure based on what the mothers choose making the consultation time to be long.

The current study showed that unplanned pregnancy is one of the challenges as an important deterrent to the uptake of preconception care which was also reported by other studies (Nwolise et al., 2020, Rahman et al., 2017, Ojukwu et al., 2016). These findings implied that unplanned pregnancy to be one of the factors affecting maternal and child health. There is a need for strengthening awareness creation on the use of PCC through health extension workers during home to home visits, conferences and outreach activities.

The study explored the challenges and opportunities for implementing PCC service, which is believed to give an original clue for revamping the service. However, as study was carried out only in Dire Dawa, eastern Ethiopia, the practices here may not be representative of other areas of the country, but the fact that the health policy which established in the country is implemented in the same fashion throughout the country.

This is the first in-depth qualitative study that explored the practice, challenges and the opportunities of delivering preconception care in Dire Dawa City Administration. The fact that the findings were not triangulated with quantitative study and that it is conducted only at facility level and only conducted in one regional government are limitations of this study authors acknowledge.

6.5 Conclusions

This study highlighted the need for policy, program, guidelines and a structure for preconception care services in the country. Such guidelines can incorporate the existing forms of care that are not within the health system such as preconception counseling. It also requires action including, better communication and link to health extension workers to improve preconception care services. In addition, preconception care service can be used as an opportunity to achieve the sustainable development goal on reduction of neonate mortality and disability. Moreover, other advanced experimental study design such as randomized clinical trial will be needed to start the preconception iron-folic acid supplement with other care service for women who planned to pregnant.

Chapter 7: Effect of Picture-based health education and counselling on knowledge and adherence to preconception Iron-folic acid supplementation among women planning to be pregnant in Eastern Ethiopia: a randomized controlled trial

Berhane, A., & Belachew, T. (2022). Effect of Picture-based health education and counselling on knowledge and adherence to preconception Iron-folic acid supplementation among women planning to be pregnant in Eastern Ethiopia: A randomized controlled trial. Journal of Nutritional Science Cambridge University, 11, E58. doi:10.1017/jns.2022.51

Abstract

This study examined the effect of picture-based nutrition education on knowledge and adherence of iron folic acid supplement intake in Ethiopia, a country where high there is burden of neural tube defects and anemia. Parallel randomized controlled trial design was employed among women planned to pregnant in eastern Ethiopia. The interventional arm (n=122) received a preconception picture-based nutrition education and counseling with iron-folic acid supplement, and the control arm (n=122) received only preconception iron-folic acid supplement. The effect of the intervention between-group differences were assessed using a chi-square an independent t-test. Bivarite and multivariable linear regression analyses modelling was performed to detect the most independent variables affecting the outcome. Outcome measures were knowledge and adherence to iron folic acid supplement intake during three months intervention period. After intervention, in women's who plan to be pregnant most women in the intervention group (42.6%) had adherence with iron-folic acid supplements compared to the control group (3.3%); this difference was significant ($P<0.0001$). In Bivariate and multivariate linear regression analyses history of NTDs affecting pregnancy, history of spontaneous abortion and knowledge were independently associated with adherence to iron-folic acid supplement ($P<0.05$). Pre-conception nutrition education with regular follow-ups could effective on knowledge and adherence to iron folic acid supplementation. This intervention is very short, simple, and cost effective and has potential for adaptation, feasible development to large-scale implementation in the existing health care system among women who plan to be pregnant to prevent NTDs and adverse birth outcomes in Ethiopia.

This clinical trial was registered on 06/04/2021 under the ClinicalTrials.gov with identifier number PACTR202104543567379.

7.1 Introduction

Globally the utilization of modern contraception is significantly increase in sub-Saharan Africa (Bearak et al., 2018, Sedgh et al., 2014, UN, 2020). In Ethiopia, the contraceptive prevalence rate (CPR) among currently married women is 41% (ICF, 2019), however, 35% of modern contraception (medical product including sub-dermal implants, or IUDs or loop which is designed to prevent pregnancy) users were discontinued before the removal date (ICF, 2016). The desire for pregnancy is the second most common reason for early discontinuation and it accounts almost 27.3% (Nega et al., 2021, Bekele et al., 2015, Burusie, 2015, Belete et al., 2018). Any woman who could become pregnant is having a risk of a baby with neural tube defects (NTDs) (Bibbins-Domingo et al., 2017). The etiology of NTDs is complex, multifactorial in origin, but mainly dietary factors play a major role (Berhane et al., 2022, Gupta and Hanson, 2022, Crivellenti et al., 2018, Biagi et al., 2019, Carmichael et al., 2010, Groenen et al., 2004, Safi et al., 2012, Scott et al., 1995, Laurence, 1989, Schorah et al., 1980, Group, 1991, Bhide, 2021, de la Fournière et al., 2020). Optimal intake of IFAS is very crucial for increasing folate concentrations in red blood cells and could significantly reduce the risk of iron deficiency anemia and congenital anomalies such as NTDs (Stevens et al., 2013, WHO, 2012, WHO, 2015b, Cordero et al., 2015). Iron can modulate folate availability via a cellular one-carbon pathway, and low iron status could alter folate utilization despite an adequate folate intake and extracellular folate concentrations (Oppenheim et al., 2001b, Valberg, 1980). Sufficient iron plays an important role in the development of the neural tube and to successful neural tube closure and that deficits in iron might cause some cases of NTDs (Mao et al., 2010, Zohn et al., 2007a, Zohn et al., 2007b).

Ethiopia has one of the highest rates of micronutrient deficiencies, including iron deficiency, making it a significant public health concern (Haidar et al., 2010a, Ababiya and Gabriel, Herrador et al., 2014, Tsegaye Ababiya, 2014). In Ethiopia 84% of women of reproductive age (WRA) had low levels of red blood cell folate (Haidar et al., 2010b, Bationo et al., 2019, EPHI, 2017, Habte et al., 2015, Haider et al.). Because of WRA with low serum folate levels, the incidence of NTDs in Ethiopia has increased alarmingly, recorded the highest rate, and emerged as a public health concern (Berihu et al., 2018, Berhane and Belachew, 2022), so there is an urgent needed to develop effective intervention to address this problem. Despite the fact that the

Ethiopian health care system is fertile ground for the provision of IFAS to reduce the risk of NTDs, there is no room for preconception care services to women who discontinue modern contraception for planned pregnancy, and the golden opportunity for instituting key NTD preventative interventions is lost.

In Ethiopia, providing IFAS to pregnant women is a primary strategy for preventing maternal and newborn morbidity and mortality due to anemia (FMoH, 2014), However, knowledge and adherence to iron folic acid remain relatively low, and various barriers to the program's success have been identified (Boti et al., 2018, CSA and ICF, 2017, Sendeku et al., 2020, Gebremichael and Welesamuel, 2020, Galloway et al., 2002, Aguayo et al., 2005, Taye et al., 2015, Kulkarni et al., 2010, Zavaleta et al., 2014, Bali et al., 2017). Lessons learned from the current health-care strategy for IFAS provision, alternative approaches are required to improve the adherence to preconception of IFAS intake. Evidence from other studies indicates that health education intervention is an important tool to improve knowledge and adherence to micronutrient supplementation (Taye et al., 2015, Girard and Olude, 2012, Nagata et al., 2012, Martin et al., 2017, WHO, 2016, Cena et al., 2008, Temel et al., 2014, Stephenson et al., 2018, Forrest, 1994). For effective intervention, it needs to be locally appropriate, simple practically implemented through the existing health care system. We assumed that preconception of picture based health education intervention package for women plan to pregnancy is the best approach to reduce the risk of NTDs and other adverse outcomes. Therefore, the aim of this study was to evaluate the effect of picture based health education and counselling intervention to increase the knowledge and adherence to IFAS intake among women have a plan to pregnancy.

7.2 Methods and materials

7.2.1 Study area and period

The study was conducted in Eastern Ethiopia. Details of study setting are presented in **Chapter 2**. The study was conducted between May 2021 and October 2021.

7.2.2 Trial design: A double-blind parallel randomized controlled trial was conducted at gynecology and family planning clinic at a healthcare facilities to determine the effect of pictured based nutrition education and counseling on the importance of adherence to IFAS during pregnancy.

7.2.3 Participants: women in the age group of 18-45 years who requested to interrupt contraception due to intention to be pregnant and women with spontaneous abortion who refused to take a contraceptive that had an intention to be pregnant. The inclusion criteria included: currently living in the study area (women who are usual residents and live or intend to live in the area for 6 or more months) and giving consent to participate in the study. Women who took IFAS or any other micro supplements in the past two months and participants who are mentally/physically challenged to provide consent were excluded from the study.

7.2.4 Intervention and its procedure

The details of the study protocol was published elsewhere (Berhane A, 2022). In accordance with the protocol, the midwifery nurses who worked in the gynecology ward and family planning clinics would invite all eligible women to participate in the study and collected the base line data. In addition health extension workers were provided home to home pictured based nutrition education and counseling for women who assigned to interventional arm.

Individual picture based nutrition education and counseling including picture and text messages was delivered to the intervention group using conventional inter-personal communication intervention for 10-20 minutes by trained health extension workers in monthly basis. Education includes warnings about lifestyle, dietary habits, risk factors for anemia and NTDs, adverse effects of NTDs and IFAS guidelines. The IFAS guide includes information on how to take IFAS, when, how to better absorb, side effects of IFAS, and foods rich in iron and folic acid. Educational brochures were also distributed at the end of the education session. The brochure was contained key messages with images and text. After finished the education session, an opportunity was provided for the participant to discuss the education content and clarify any confusion.

Each participants in the interventional arm received two educational health message reminders (Mondays and Thursdays) in local language every week to make sure they were taking the iron folic supplements via mobile during the daytime to avoid making a disturbance. Anyone at home or in the neighborhood who can read is encouraged to read the texts for a woman who cannot read.

The key messages included: “eat a diversified food”, “take one IFAS tablet per day for at least 90 days, “contact your health care provider if you have any complaint related to IFA tablets”, “reach and maintain a healthy weight, “visit health facility before and during pregnancy like ANC and check your health frequently”, avoid toxic substances and environmental contaminants”; avoid living in a stressful or abusive environment; or working with or living around toxic substances” and “Learn your and your partner’s family history” (Table 7.1). The brochure with take-home messages was also distributed to help participants understand what they had learned.

Every month there was a contact with their partners and discussion on the issues. These contacts were also important in reducing drop out and assisting participants with any questions that may arise and increase the involvement of partner in reproductive health.

Table 7.1: Intervention protocol for preconception pictured based nutrition education for women, who had an intention to be pregnant, Dire Dawa city administration, Ethiopia.

Key action (message)	Strategy of intervention	Responsible	Frequency	Adhere to IFAS
-Preconception and prenatal health care service	Home take broacher with key message (in local languages)	Trained health care providers, health extension workers and Principal investigator	Once	Number of intervention participants who received the broacher
-Visit health facility before and during pregnancy like ANC and check your health frequently -Take iron folic acid every day and Consumption of Diversified Diet (The ten food groups) -Life style (stop drinking alcohol, smoking, and using certain drugs, doesn't stay in smoking environment.	Counseling and discussion on uptake of IFAS and risk factors of NTDs and other adverse pregnant and birth outcome as well as methods of prevention		per month	Frequency
-Avoid Toxic Substances and Environmental Contaminants -If you don't do this may be you are at risk giving a child with NTDS and other negative birth outcome like anaemia, low birth weight, and preterm birth.	Reminding SMS text messages via mobile phone		Two message per week	Frequency

The IFAS (30 iron capsules with 60 mg of ferrous sulfate and 0.5 mg of folic acid) was provided to all participants in the intervention and the control groups in every month immediately they removed modern contraception (WHO, 2011a).

All participants received a brief information regarding the proper usage of IFAS when they collected their IFAS by health professional for 5-10 minutes. The information session included on importance of IFAS, duration of the IFAS to be consumed, the possible side effects of the supplement and ways of handing the side effects. Due to absence of preconception services for women who have a plan to pregnancy, the midwifery nurse encouraged the control group to visit the health care facility every month to take the IFAS pills and called when they absent the appointment date. Health professionals did not interfere in any condition with control arm practices, only provision and count of IFAS was performed. After the end of the study similar nutrition information was given to the control group.

7.2.5 Intervention fidelity

The investigators developed criteria to assess the fidelity of the intervention based on the preconception care and dietary guidelines which accordance with WHO recommendations. The criteria included checklists to assess intervention design, training of counselors, counseling process, receipt of intervention. Content validity for the education was assured by sending its content to two experts in nutrition and health educational and behavioral science from academic center. In addition, two expert meetings was held with health professionals in development of materials. To balance the variations equal numbers of participants for the intervention and control groups were taken from eligible women. In addition, to minimize between-educators and counsellors variation the same health extension workers made the all three time repeated visits for each participant. The intervention process was pretested before the implementation of the trial. Besides, each woman received equal numbers and frequencies of counseling, and the lengths of contacts within an intervention group were similar to make the process standardized. Picture based nutrition educator and counsellor’s knowledge and skill were assessed by pre and post-training tests and practical evaluation.

Checklists with counting of the remained IFAS pills were used to measure the adherence of participants to the IFAS intake using interview about their understanding of the core content of the intervention during home-to-home visits in every month.

Study participants, data collectors (worked at gynecology and family planning service), educator and counselors were blinded to the study hypotheses and group allocation. Additionally, the data entry clerk was blinded by labeling the groups with a non-identifiable unique number until analysis was finalized.

7.2.6 Outcomes: A primary outcome of this trial was adherence to IFAS intake and knowledge about IFAS and NTDs.

7.2.7 Sample size

The sample size was calculated using Gpower based on the study considering 80% power, 0.3 effect size and 95% confidence interval with an intervention to control ratio of 1 and margin of error of 0.05. The calculated sample size is 111 and 10% loss to follow up was added. The final sample size for a single arm was 122 in the intervention and control arms each. The total sample size was 244.

7.2.8 Randomization: The study subjects were enrolled when they came to the health facility for removed their modern contraception with an intention of being pregnant or after they have spontaneous abortion when they declare that they want to be pregnant immediately. All the necessary information including full address, mobile phone numbers, and a unique ID number of the study participants were recorded. A list of the ID numbers was compiled and sent to the researcher on a weekly basis. Simple randomization with 1:1 allocation ration was done using ENA for Smart Software. Women randomized to the intervention group only (n=122) received picture based nutrition education and counseling, where participants in both groups would receive IFAS and other service being given by the health facility. The study was considered the Comprehensive Criteria for Reporting Trials (CONSORT) as a guide for study randomization (Figure 7.1).

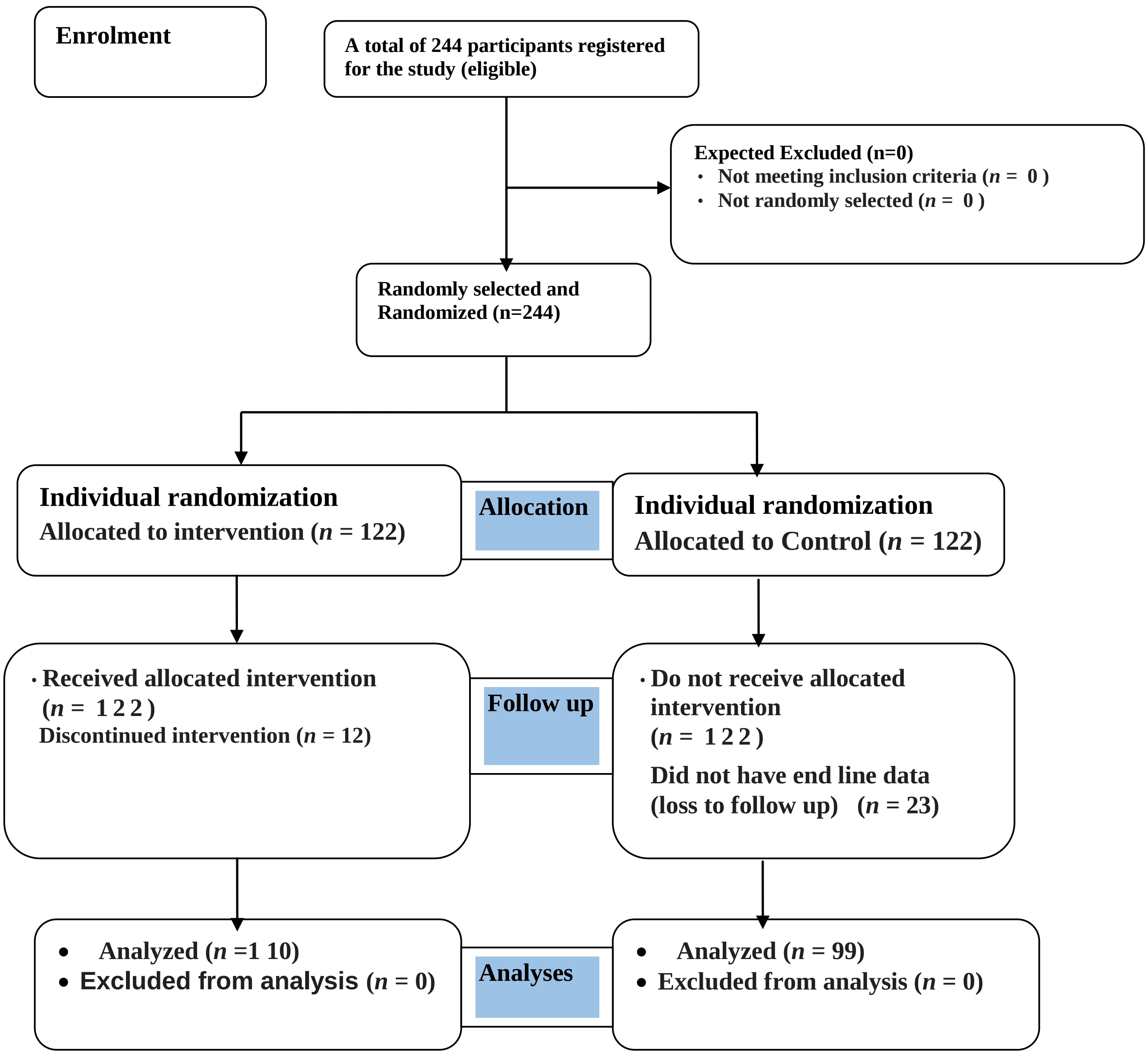

Figure 7.1, Consort flow diagram: Effect of Picture Based Nutrition Education on improving women's Knowledge about and Adhered to Iron Folic acid supplement intake in the Preconception period: a Parallel Randomized Controlled Trial

7.2.9 Data collection tools, procedures and collectors

Details of data collection tool, procedure and collectors are described in **Chapter 2.**

7.2.10 Operational Definition of Terms

Intervention group- Randomly selected women who were given preconception pictured based education and counseling with 60 mg of elemental iron and 400 µg (0.4 mg) of folic acid supplementation for three months beginning with the date of contraceptive removal and pregnancy termination.

Control group- Randomly selected adults who taken 60 mg of elemental iron and 400 µg (0.4 mg) of folic acid but not given preconception pictured based education and counseling for three months beginning with the date of contraceptive removal and pregnancy termination.

Adherence to IFAS intake:

During the three-month intervention period, the participants were expected to take 90 IFAS pills, on assumption of one tablet of IFAS is taken each day from starting of the removal of modern contraception. The participants were divided into two groups based on their adherence to the IFAS intake. It considered as an adherent to IFAS intake if they were able to take at least 72 and more IFAS pills or 80% from total take period while those were not able to take less than 72 IFAS tablets or less than 80% from total intake period were considered non-adherents to IFAS intake (WHO, 2012, Sendeku et al., 2020)**.** Women who did not attend all counseling sessions were deemed non-adherent to the guidelines. Women who dropped out of the study, on the other hand, were labeled as lost to follow up.

Knowledge: Subjects were graded by asking a series of 10 questions about IFAS and NTDs marking correct answers out of a hundred. Poor knowledge" was defined as an average knowledge score of 50% or less.

Intention to treat (ITT): is calculated all participants who are considering the randomized are included in the statistical analysis and analyzed according to the group they were originally assigned.

Duration of the intervention: The time of picture based health education and counseling was given to the intervention group (three months staring from the second half of May 2020 to the October 2021).

Modern contraception: defined as a medical product including sub-dermal implants, or IUDs or loop which is designed to prevent pregnancy.

7.2.11 Data processing and Stastical analysis

Data processing are presented in **Chapter 2**. Data were presented in the form of descriptive statistics, such as frequency, percentages, mean, and standard deviation. The Kolmogorov-Smirnov test was used to check the normality of the distribution. The effect of the intervention between-group differences were assessed using a chi-square test for categorical variables and an independent t-test for continuous variables with normal distributions. Within-group changes were assessed using a paired t-test after three months. The Cohen's d statistic (effect size) was used to measure the significance of the intervention's effect. It was categorized as follows: < 0.2 was no effect, 0.2-< 0.5 was a small effect, 0.5- <0.8 was a moderate effect, and > 0.8 was a significant effect indicates a strong relationship between the two variables (Cohen, 2013). Adherence to IFAS intake was conducted with the goal of considering the Intention-to-treat analysis (ITT) concept.

Bivarite and multivariable linear regression analyses modelling was performed to detect the most independent variables affecting adherence and knowledge to IFAS by control the possible confounding variables. Analyses are based on valid data available for each (set) of variables analysed and no missing data are imputed. Variables with a p-value of less than 0.05 were considered statistically significant in Bivarite and Multivariable analysis.

7.3 Result

During the recruitment period (May 2021 and finished October 2021), a total of 244 eligible participants who were enrolled in the study (intent-to-treat population,). Each arm contained 122 women. A total of 35(14.3%) participants (n=12 from intervention and n=23 from control arm) were declined from the study due to different reasons. Overall, the mean (±SD) age of the participants was 28.1±5.19 years, with 42% of the participants being between the ages of 26 and 34 years old. All participants were married. There were no statistically significant differences between the socio-demographic characteristics and pregnancy-related information of participants in the intervention group and those participated in the control group in terms of age, educational status of women, partner educational status, history of taken IFAS during pregnancy, (P>0.05). Additionally before the intervention, there were no significant differences between the two groups in terms of history of IFAS or FAS before pregnancy, knowledge on NTDs and preconception of IFAS (P>0.05). However, there were statistically significant difference between two groups in terms of history of visited ANC during pregnancy, history of NTDs affecting pregnancy, and history of spontaneous abortion (P<0.05) (Table 7.2).

Table 7.2: The socio-demographic characteristics and pregnancy-related information of the study participants (n=244)

Variables	**Intervention arm (n=122)**	**Control arm (n=122)**	***P***
	n(%)	**n(%)**	
Age (years)			
18-25	41(33.6)	43(35.2)	0.6
26-34	49(40.2)	53(43.4)	
>34	32(26.2)	26(21.3)	
Mean age (years)	28.1±5.19		
Educational status			
Illiterate	22(18)	24(19.7)	0.74
Literate	100(82)	98(80.3)	
Partner educational status			
Illiterate	17(13.9)	10(8.2)	0.15
Literate	105(86.1)	112(91.8)	`
History of IFAS or FAS before pregnancy			
No	11(97.5)	121(99.2)	0.32
Yes	3(2.5)	1(0.8)	
History of visited ANC during pregnancy			
No	46(37.7)	65(53.3)	0.015
Yes	76(62.3)	57(46.7)	
History of taken IFAS during pregnancy			
No	74(60.7)	86(70.5)	0.1
Yes	48(39.3)	36(29.5)	
History of NTDs affecting pregnancy			
No	110(90.2)	118(96.7)	0.03
Yes	12(9.8)	4(3.3)	
History of spontaneous abortion			
No	113(92.8)	120(98.4)	0.03
Yes	9(7.4)	2(1.6)	

Pearson correlation (Chi square test), significant at P<0.05, IFAS: Iron and Folic Acid supplementation, FAS: folic acid supplementation.

After three months of intervention the proportion of adherence to IFAS was 23% (n=56). With regard to adherence within the groups, 42.6% (n=52) in the intervention group and 3.3% (n=4) in the control group and the intervention group were significantly higher than in control group. In the intervention group the proportion of adherence to IFAS intake among participants increased by 40.1% and there were statistically difference (*P*<0.0001). At the end of the study, the proportion of adherence to IFAS increased slightly in the control group and it was increased by 2.5% in the control group (P=0.31). The difference in difference between the two groups of adherence to IFAS intake was 37.6% and there were a statistical significance (P<0.0001) (Table 7.3). End line data of adherence between the groups did differ from baseline data (P=0.31 Vs P<0.0001).

Table 7.4 presents the changes in knowledge between and within group. Level of knowledge between the groups did differ before and after intervention (P= 0.87 Vs P<0.0001). The mean knowledge scores were 48 (±26 SD) at their highest and 13 (±8.3 SD) at their lower. A comparison of the scores at base line and end of three months follow-up showed that the mean change in knowledge was 17.6 (±28 SD) points in the intervention group and 18.5(±18.8 SD) in the control group. The overall the mean change in knowledge Mean (±SE) between group was 0.9 (±3.04 SE) and there were significant differences between two groups (P<0.001).

Table 7.3: Differences between baseline and end line adherence to IFAS and differences in differences between intervention and control groups

Variable	**Intervention group (n=122)**			*P-value* within group	**Control group (n=122)**			*P-value* within group	**Difference in Difference**	*P-value* between group
	Baseline	End line	Difference (EL-BL)		Baseline	End line	Difference (EL-BL)			
Adherence (%)	2.2	42.6	40.1	<0.0001	0.8	3.3	2.5	0.31	37.6	<0.0001

EL, End line BL, Base line, Significant at *P*-value 0.05

Table 7.4: A comparison between participants' mean scores for knowledge of IFAS

Parameter	**Intervention (n=122)**			**Control (n=122)**				*P-value between groups (before intervention)*	*P-value between groups (after intervention)*	Effect size Eta (Cohen's d statistic)	Label
	Baseline	End line	Difference (BL-EL)[1]	Baseline	End line	Difference (BL-EL)[1]	DID				
	Mean (±SD)	Mean(±SD)	Mean(±SD)	Mean(±SD)	Mean(±SD)	Mean(±SD)	Mean(±SE)				
Knowledge	31 (± 13.6)	48(± 26)	17.6(± 28)	13(± 8.3)	31.5(± 19.5)	18.5(± 18.8)	0.9(± 3.04)	0.87	<0.0001	0.85	Significant effect

Significant at *P*-value 0.05, DID=Difference in Difference

Bivariate and multivariate linear regression analyses were conducted to identify the variables that could independently predict higher adherence rates among the participants of the intervention group following the program. History of NTDs affecting pregnancy, history of spontaneous abortion and knowledge were independently associated with adherence to IFAS (*P*<0.05). The most identified factors that affected adherence to IFAS intake were history of NTDs affecting pregnancy and knowledge levels (*P*<0.001) (Table: 7.5).

Table 7.5: Bivariate and multivariate linear regression analyses for the factors affecting adherence to IFAS.

Parameters	Bivariate		Multivariate	
	β-coefficient (95% CI)	***P***	**β-coefficient (95% CI)**	***P***
Age	0.03(-0.035-0.1)	0.35	0.03(-0.03-0.1)	0.34
Educational status of participants	0.014(-0.13-0.16)	0.85	0.015(-0.12-0.14)	0.82
Partner educational status	0.04 (-0.15-0.28)	0.68	0.04(-0.13-0.2)	0.63
History of visited ANC during pregnancy	-0.045 (-002-0.1)	0.5	-0.03(-0.16-0.1)	0.62
History of taken IFAS during pregnancy	-0.034(-0.17-0.1)	0.6	-0.04(-0.18-0.1)	0.54
History of NTDs affecting pregnancy	0.5(0.2-0.7)	0.001*	0.4(0.21-0.6)	<0.0001*
History of spontaneous abortion	0.36(0.06-0.65)	0.01*	0.32(0.09-0.5)	0.007*
Knowledge	0.006(0.004-0.008)	<0.0001*	0.006(0.004-0.008)	<0.0001*

R^2=0.19; F=14.7; B, unstandardized coefficients; C.I, confidence interval. All variables with p < 0.05 were included in the multivariate analysis. * Statistically significant at p < 0.05.

7.4 Discussion

Many research findings have been published about the knowledge and adherence to IFAS intake among pregnant women. To our knowledge no study is conducted regarding to knowledge about and adherence to IFAS intake among women’s who plan to be pregnant. The current study is the first parallel randomized controlled trial to evaluate the effectiveness of nutrition education on knowledge about and adherence to IFAS intake using an individual education through picture based nutrition education and counselling approach. The major goal of this intervention was to encourage women’s who plan to be pregnant to have a good knowledge about and adhered to IFAS intake to avert the risk of developing NTDs affecting pregnancy and other related negative impacts on women and neonates.

In the present study the adherence with IFAS intake were 2.2% and 42.6% (before and after intervention) within the intervention group ($P<0.0001$) compared to the 0.8% and 3.3% (before and after intervention) within the control group ($P=0.31$) and the average number of supplements taken in the intervention group was significantly higher than in the control group.

This implies that nutrition education intervention package was significantly positive impact in improving women's adhered to preconception of IFAS intake in the intervention group than control group. Therefore, institutionalized based with link to home delivery nutrition education is one of the promising interventions that could improve the pregnant and birth outcome and extend to future generation and community as whole (Mok et al., 2018). Even if, the study participants was different from our study, this study was in agreement with study conducted in Ethiopia (Mengesha et al., 2021) in Bihar, India (Wendt et al., 2014), in Kenya (Matiri et al., 2015), from Jordan, Saudi Arabia, India and Indonesia (Nahrisah et al., 2020, Elsharkawy et al., 2022, Abujilban et al., 2019, Noronha et al., 2013, Permatasari et al., 2021).

The nutrition knowledge scores were significantly higher at end line and three months after the sessions compared to the baseline and there was statistical significant difference between the intervention and control groups. However, the mean scored of knowledge in the control group showed some improvement after intervention. The results of previous studies are in line with our own, as we found that the nutrition education was significant impact in improvement knowledge (Sisay and Tesfaye, 2021, Gitau et al., 2013, Kostanjevec et al., 2012, Ardanjani, 2015). In addition these studied reported that a significant differences in the means between two groups (intervention and control) after intervention only, while no significant mean difference was observed at base line concurrent with our findings. This may be due to the sensitization that occurred during the initial exposure to the questionnaire and the recall of the answers from the baseline question (Barthassat, 2014, Prelock et al., 2008). The significant improvement in knowledge and adherence among the intervention group may also be related to the methods and repetition of the nutrition education intervention package for three months with strictly follow-up (WHO, 2011a). Similarly, home visits encourage participants to practice what they know to improve their health (Brämswig et al., 2009). The repetition education were remained longer in memory according to the information process theory (Shiffrin and Schneider, 1977). However, the proportion of improvement in knowledge and adherence was reported differs between studies (Wang et al., 2015). These differences are related to the duration of the intervention and the method(s) used to deliver the intervention (Gitau et al., 2013).

The study setting could be the second possible justification. This study was conducted on women in urban areas. Urban women were more exposed to different media about the importance of iron folate intake compare to the rural counterparts. Their partners may also be more exposed to various sources of information and more easily understand what the health care workers advices.

Despite the fact that the biomarker test of the participants was not performed, and it is unknown whether the level of red blood cell folate is increased or the same as before intake of IFAS, various laboratory-based researches provide evidence that intake of IFAS over at least 12 weeks before conception by women of child-bearing age increased the level of red blood cell folate, potentially lowering the risk of neural tube defect-affected pregnancy and (Hao et al., 2008, Bationo et al., 2019, Cordero et al., 2015, Crider et al., 2014, Lamers et al., 2006, Organization, 2015, De Bruyn et al., 2014, Brämswig et al., 2009, Norsworthy et al., 2004, Samson et al., 2020, WHO, 2015b, WHO, 2015a). Based on this concrete evidence, we would expect women who adhere to IFAS to be more protective against NTDs if they became pregnant, because folate sufficiency or optimal blood folate concentrations are directly linked to the risk of neural tube defects (Stevens et al., 2013, WHO, 2012).

In general, positive aspects of this intervention include a significant improvement in women's knowledge of nutrition to reduce the risk of NTDs, so the intervention should ideally have a positive impact on both the women themselves and their children. The positive effect will help mothers to form a basis for proper nutrition in subsequent years.

The study has also a significant practical implications for improving IFAS adherence, which will avert the risk of developing NTDs and improve maternal and child health in order to achieve the Sustainable Development Goals (SDG). The findings imply the importance of implementing community-level education through health extension as well as at family planning services and other appropriate departments within the health facility through health extension workers as well as through involving males. Furthermore, this approach has the potential to increase adherence and decrease dropout rate of ANC program in the country. Therefore, our intervention designed in a way that can be applicable anywhere in a health care system. So, the findings of this study also highlight the importance and effectiveness of adapting preconception policy and national guidelines. Our intervention was no interference neither did the authors gave financial incentives for health counselor of either arms. In conclusion, nutrition and health knowledge may not lead to actual behavioral changes, and other behavioral studies need to be done to reach general conclusions.

7.5 Conclusion

In conclusion, picture-based nutrition education intervention approach with follow-up through frequent household visits have a significant impact in knowledge and adherence of preconception of IFAS intake among women plan to pregnant. It helped to improve women's knowledge of anemia, NTDs during pregnancy, raises women's awareness of better dietary practices, reduces neonatal and maternal morbidity and mortality.

This intervention is very short, simple, and cost effective. We strongly recommend that this intervention should be wider implement in the country because of the fertile ground of health care system. We have a recommend for health policy: The first recommendation is to emphasize that preconception picture based nutrition education package program should be endorsed by policy makers for women's who remove the modern contraception for having a plan to be pregnant, focusing on behavioral intake of preconception of IFAS intake on the existing health care system (linking to health facility with health extension program). Additionally, this intervention package will be developed a comprehensive national strategy to prevent adverse pregnancy and birth outcome during pregnancy. Another is to resolve the nauseating effect of iron-folic acid supplement as this was the majority side effect reported by participants in the study so, the health care providers will provide a brief explanation with possible solution. Our third recommendation is for the Ethiopia government to support food fortification that consumed by larger proportion of the population. A large-scale community based study with a larger sample size is required to determine whether this approach is cost-effective.

Chapter 8: General Discussion, Future Research Perspectives, and Conclusion

8.1. Introduction

NTDs are more commonly attributed to neonate morbidity, mortality and disability. In Ethiopia, the neonatal mortality rate was 43 deaths per live births (ICF, 2019), while the mortality attributed to NTDs is not known and does not measure the outcome systematically. The burden of NTDs in different countries were remains underestimated and has been a challenging issue of public health concern.

A crucial first step to avert the risk of developing NTDs among neonates is understanding the burden of NTDs (WHO, 2010; WHO, 2016; Arena *et al*., 2015). Retrieval of a retrospective data helps to know the true burden of NTDs in the country. However, due to poor data management, a few data is missed. Micronutrients play a central part in metabolism and in the maintenance of tissue function (Bailey and Cerda, 1988, ÖZKAN, 2018, Shonibare et al., 2020). Specific nutrients and general nutritional status of the mother may play key roles in altering the development of the placenta, effects that have direct consequences on the fetus and neonate health for a long life (Oliver et al., 2007, Thayer et al., 2020, Moreno-Fernandez et al., 2020). The etiology of NTDs is complex, multifactorial in origin, but mainly dietary factors play a major role (Berhane et al., 2022, Gupta and Hanson, 2022, Crivellenti et al., 2018, Biagi et al., 2019, Carmichael et al., 2010, Groenen et al., 2004, Safi et al., 2012, Scott et al., 1995, Laurence, 1989, Schorah et al., 1980, Group, 1991, Bhide, 2021, de la Fournière et al., 2020). Different metaanalysis and systematic reveiew studies reported that 70-95% NTDs are linked to genes involvement and maternal vitamin intake (MVI) (Mishra et al., 2020). Maternal folate or iron deficiency during gestation impacts both the mother and the developing embryo with elevated risks for NTD well as for the long-term health condition (Sifakis and Pharmakides, 2000, Imdad and Bhutta, 2012, Scholl and Reilly, 2000, Pritchard et al., 1969, Preziosi et al., 1997, Menon et al., 2016, Gao et al., 2016, Castaño et al., 2017, Helmy et al., 2018).

In Ethiopia, different strategies are implemented in the health system, and there has been an improvement in maternal and child health status (FMoH, 2015b, UN, 2015, FMoH, 2015a, Flores *et al*., 2015).

However, the problem related to preconception iron-folic acid supplements to reduce the risk of NTDs has not been properly addressed in developing countries, including Ethiopia (Njamnshi *et al*., 2008, Sharma *et al*., 2018, Morris *et al*., 2018, Gedefaw *et al*., 2018).

NTDs largely preventable public health problem if, a woman gets enough dietary folate and iron or iron-folic acid supplement before and during early pregnancy. Therefore, the absence of preconception folic acid or iron-folic acid is a one of the major concerns in the developing countries including Ethiopia. Therefore, studying the burden, risk factors, dietary practice of women, and exploring the practice, challenges and opportunities of preconception iron-folic acid supplement can help countries to avert the risk and put the intervention in different tier of the community as well in the government sectors that reduce the morbidity, mortality and disability of the future generation.

8.2. Summary of the main findings

The main findings of this research emerge from five consecutive studies: retrospective cohort study, matched case control study and comparative cross sectional study design (Chapter 3-5), qualitative study (Chapter 6) and randomized controlled Trial (Chapter 7). The trend and burden of NTDs, the risk factors, maternal dietary practice on development of NTDs, the practice, challenge and opportunities of preconception folic acid supplementation was investigated in the study area and new preconception pictured-based health education and counseling showed with excellent tool for to promote adhere to iron-folic acid supplement to avert the neonate with developing NTDs.

In chapter 3, the trend and burden of NTDs was evaluated using retrospective cohort study design in the study area. (***Published on Plos-One***; 2022.,38(1):*14.doi:10.1186/s40101-019-0205-2. (https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0264005).*

The determinants for development of NTDs were identified using a matched case-control study in Chapter 4. ***(Accepted on Plos one: 2022*** doi:10.1371/journal. pone,0272554. *https://doi.org/10.1371/journal.pone.0272554*.)

The prevalence of dietary practices and the association between dietary practice and occurrence of NTDs was determined in the study area in Chapter 5) ***(Published on: Journal of Nutritional Science,*** *https://doi.org/10.1016/jns.2022.02.019 Cambridge University).* The results showed that women who delivered newborns with NTDs hade poor dietary quality.

In chapter 6, the practice, challenge and opportunities of preconception folic acid supplement was explored using qualitative study presented (*submitted*).

In chapter 7, the effect of preconception pictured based health education on knowledge about and adhere to iron-folic acid supplement was using a randomized clinical trial. (***Published on: Journal of Nutritional science, Cambridge University*. *Vol, 11***. *https://www.doi.org/10.1017/jns.2022.51*).

In addition the RCT was registered on 06/04/2021 under the ClinicalTrials.gov with identifier number PACTR202104543567379 and published on clinical Nutrition open science Elsevier. (**Berhane, A**. and Belachew, T., 2022. Effect of preconception pictured-based health education and counseling on adherence to iron-folic acid supplementation to improve maternal pregnancy and birth outcome among women who plan to pregnant:" Randomized Control Trial". *Clinical Nutrition Open Science Volume 42*. *https://doi.org/10.1016/j.nutos.2021.12.002*)

In general, our studies found that the trend and the burden of NTDs was increased year to year in eastern Ethiopia. The finding showed that the burden of NTDs in the study area was 5 times higher than the estimation done by the WHO survey. On the other hand, in study area there were different determinants were found but mainly dietary factors play a major role. Although dietary practices was associated with developing NTDs. Also our study gives a critical clues about the preconception folic acid supplementation service in the existing health service for prevention of NTDs in Ethiopia. The existing health care system and policy is missed to recognize the importance of preconception iron folic acid supplementation for women who intend to become pregnant. Our finding showed that even though, the health care system has a fertile ground to intervene the dynamic increasing of the incidence of NTDs from year to year, there is no space for those women who have a plan to be pregnant. Moreover, the our study found that picture based education is effective in promoting adhere to iron-folic acid supplement to avert the neonate among women who planned to be pregnant and positive contribution to enhance the preventive efforts and it is also a good starting for male involvement in reproductive health with simple, reachable through the existing health care system (linked family planning service with health extension program).

8.3. Implications of the Findings

8.3.1. Implication of the Findings to reducing the risk factors for developing NTDs

Investing in nutrition interventions before pregnancy is increasingly recognized as a potential critical period for targeting interventions to avert negative pregnancy and birth outcomes including NTDs (Ramakrishnan *et al*., 2012). Providing preconception supplements containing iron and folic acid (IFA) and/or dietary approach including food fortification intervention are among the most commonly recommended strategies for improving birth outcomes (Bhutta *et al*., 2013, Engle *et al*., 2007). The study showed that the dietary practices was associated with developing NTDs and has an implication for the future programing of preventives services. However, experimental evidence of the impact of preconception supplementation is scarce although this strategy can give basis for designing preventive measures.

In general, to tackle this situation, effort should be put in the existing health care system including family planning service, such as encouraging women to visit the health care facility if they have a plan to pregnancy with taking preconception iron-folic acid supplement (multivitamins and folic acid pills), provide health education on dietary modification and fortifying certain staple foods. Furthermore, this public health problem should be investigated with scientific evidences and implement effective prevention strategies. This is a critical milestone when we especially think of Ethiopia's plan to be one of lower middle income countries by end of the sustainable development goals in 2025 and reduce the neonate mortality (National Planning Commission of FDRE, 2016). Hence, taking the aforementioned dream of the country into account, this study aimed to update the trend, burden, risk factors and dietary practice, evaluating the current health care system of preconception of iron folic acid supplements with demonstrating the effects of different prevention strategies to reduce the mortality, morbidity and disability due to NTDs.

8.3.2. Implication of the Findings for on interventions for preventing NTDs

Implementation of preconception picture-based health education (Stoneham and Edmunds, 2020), intervention should be consider different problems and obstacles including culture, socioeconomic, accessibility, knowledge and development status is bound in health care provider and users side. As a result, preventive and promote interventions are patchy and not adequately implemented both health care facility and at the community levels. Hence, there is a need for implementing different strategies to reduce the risk of NTDs. There are several reasons as to why Ethiopia should be very vigilant about and have effective prevention strategies for reducing the risk of NTDs before or it is too late.

Increasing driving forces- Ethiopia has seen an increase in both modifiable and non-modifiable risk factors for the past decade and will continue to do so in the years to come given the speed at which it is currently moving. Modifiable risk factors such as maternal malnutrition and exposure to teratogens such as alcohol and tobacco and other known risk factors associated with NTDs include micronutrient deficiencies, maternal diseases such as diabetes, overweight and obesity, and the use of certain medications during pregnancy and rapid urbanization, as well as uncontrollable risk factors such as medical technology advancing; survival rates in babies with birth defects are likely to increase. This requires that countries allocate significant financial resources to accommodate the long-term care of people with disabilities (Agopian et al., 2013b). Some risk factors may not be fully modifiable. Many require behavior change, e.g. spontaneous abortion or termination, abstaining from alcohol or tobacco consumption, changing medication before pregnancy, better disease management or the consumption of folic acid supplements or fortified foods. The non-modifiable of risk factors of NTDs are genetically factors, including age, blood relation marriage, history of spontaneous abortion, NTDs, genetically factors. This could make the situation rather bleak and require organized and evidence-based intervention.

Policy Gap- Every reproductive-aged woman should receive preconception care service before she becomes pregnant (Kasim *et al*., 2016). Preconception care service (PCC) is an important means of improving women's and infants' health outcomes (Floyd *et al*., 2013). To have improved PCC multi-strategic interventions are required (Johnson *et al*., 2006). It needs serious attention from the government and stakeholders. Preconception care is important for the screening, prevention and management of risk factors that affect pregnancy outcomes. Iron Folic acid supplementation before pregnancy and just during postpartum period for at least four weeks helps to protect the occurrence of neural tube defects and other congenital abnormalities (Viswanathan et al., 2017, Tobias et al., 2012, Czeizel et al., 2004). Having awareness about preconception folic acid supplementation is very important for women to start taking folic acid early. Despite this, preconception care has not become part of routine practice in the primary care settings in the study area. Barriers to implementation include the dilemmas whether preconception interventions provided in primary care truly link with improved pregnancy outcomes as well as concerns over the cost-benefit of the interventions. According to the WHO, an explicit health policy can achieve several things: it defines a vision for the future; it outlines priorities and the expected roles of different groups; and builds consensus and informs people (WHO, 2011b). This implies the need to revise the health policy, program to implement the preconception care for women who plan to pregnant to avert the risk factors of developing NTDs and other adverse pregnancy and birth outcome is very crucial. Given the above context, the finding of this PhD. dissertation on preconception folic acid to reduce the risk of developing NTDs (**Chapter 6**) and behavior change communication intervention (**Chapter 7**) for women who had a plan to pregnant to reduce the risk of developing NTDs had practical implemented and a positive effect is seen.

The need for optimal maternal nutrition before and during Pregnancy:-

Nutritional epidemiology studies have risen during last recent years, mostly stimulated by the increasing evidence of the primary role of eating habits on health status (Brambila-Macias *et al*., 2011). During the peri-conceptional period maternal consumption of folate, vegetarian diet, optimum amount of flesh product is lowering of the level of homocysteine and decrease the risk of NTDs (Deb et al., 2018) also maternal dietary practice (Berhane et al., 2022, Gupta and Hanson, 2022), dietary quality (Crivellenti et al., 2018, Biagi et al., 2019, Carmichael et al., 2010, Groenen et al., 2004) is associated with developing of NTDs. Sufficient iron plays an important role in the development of the neural tube and to successful neural tube closure and that deficits in iron might cause some cases of NTDs (Mao et al., 2010, Zohn et al., 2007a, Zohn et al., 2007b).

A woman with history of spontaneous abortions in her immediately preceding pregnancy may have an increased risk of a pregnancy affected by a NTDs (Pei et al., 2019, Mao et al., 2020). An inter-pregnancy interval of less than six months from prior abortion is associated with a number of poor pregnancy out-comes, such as a NTDs (Lu et al., 2011, Talebian et al., 2015, Dolan, 2014, Chen et al., 2014, Campaña et al., 2017, Carmi et al., 1994, Conde-Agudelo and Belizán, 2000). This risk may be further increased when the interval between the end of the previous pregnancy and the beginning of the current pregnancy is short (Campaña et al., 2017, Lu et al., 2011, Todoroff and Shaw, 2000, Ekin et al., 2015).

The current study examined the effect of picture based nutrition education with provision of iron folic acid supplementation among mothers with history of spontaneous abortion, terminated or give birth a stillbirth or died after live birth due to NTDs and who have an intention to have pregnancy on its birth outcome. This is the other compelling reason why there should be a better evidence based responsiveness of the government policies and different sectoral programs in prevention of NTDs. Chapter 7 illustrated the effect picture based education on the incidence of NTDs. This evidence provides an important input designing effective programs for reduction of the risk of NTDs. It will also contribute much to the achievement of sustainable development goals (UN, 2015) and health sector transformation plan of `Ethiopia (FMoH, 2015a).

Increasing Exposure to environmental pollution: Recently negative adverse impact of environmental pollution is significantly increased on the world. In Ethiopia, almost all of farming activities is done using agrochemicals. Agricultural pesticides enter the food chain through animal food sources and crops, including run-off in water bodies (Ben et al., 2019). Agricultural chemicals are used more frequently as part of livelihood activities in the study area.

Human exposure to pesticides can occur either through occupational exposure, environmental influences, or through food or water ingestion of pesticide residues after the application of these compounds (Kalliora *et al*., 2018). This implies that the need to keep our environment from agrochemical and multifaceted approach to address the risk factors is very crucial. Therefore, it is critical to identify key intervention strategies. Given the above context, the report of this dissertation on the determinants of NTDs (Chapter 4) and behavior change communication (Chapter 7) on NTDs have wider practical implications for abating the negative consequences of risk factors accrued due to contaminated food and water by agricultural or occupational waste.

8.3.2 Methodological Considerations

This study was teaching and referral hospital based with large area; which was intentionally made to as the hospitals have huge case load referred from different areas and covered a wide area. Although the study showed that the study area have a great burden and the trend is increased year to year using case control and comparative cross sectional design could identify the risk factors for developing NTDs. Finally, although the health educational behavior change communication has a significant positive effect on averting the risk of developing NTDs the sustainability of such a behavior change after the cessation of the study was not documented.

8.3.3 Future Research Perspectives

In chapter 3 of this dissertation, hospital based trend and burden of NTDs in the area is investigated. Special teaching Hospital based study on determinants of NTDs are evaluated in the study area which may not be representative of what is prevailing in the community future community based follow up study should be used to investigate the trend, burden and associated factors and the association between dietary practice of women and developing NTDs. As a matter of fact almost 30% of women delivered in their home without support of trained health professionals (ICF, 2019), also this study did not assess the RBC folate concentration of women who developed newborns with and without NTDs and women in a reproductive age. Furthermore, this study did not investigate the environmental interactions between genetic and epigenetic factors (Chapter 4 and 5).

Secondly, although this dissertation showed that there is absence of preconception iron-folic acid supplementation for women who have a plan to be pregnant to reduce risk of developing NTDs (Chapter 6), it is limited in scope to the eastern part of the country. Future research should explore this issue in all regions or in the country. This dissertation has also demonstrated that controlled behavior change communication for three months can have a significant positive effect on adherence to iron-folic acid supplementation for reducing the risk of NTDs **(Chapter 7**). However, the sustainability of such a behavior after the cessation of the study was not assessed. Future study is recommended to address the sustainability of behavior changes and the optimal time for giving a follow up education to sustain the positive behavior. In addition, this study did not measure the level of red blood cell folate and ferritin before and after taking iron folic acid supplementation.

8.4. Conclusions

- ✓ The trend of NTDs increasing significantly in the study area year to year. The burden of NTDs in the study area was relatively higher than the survey conducted by WHO and recorded the second high burden area among regions in the country.
- ✓ NTD was associated with residence area, history of spontaneous abortion, history of severe anemia, history of fever, antibiotics use before or during early pregnancy, and exposure to agrochemicals. Among identified determinants, dietary and nutritional related factors played a significant role in the development of NTDs in the study area.
- ✓ There is poor dietary practice in the study area and dietary intake of folate resources is positively associated with lower prevalence of NTDs. The findings imply the need for considering such a dietary practice as a basis for public health promotion.
- ✓ Good dietary practice as measured by meal frequency, DDS, ASF and FVS. It has negatively associated with NTDs.
- ✓ There is no preconception folic acid supplement for women having a plan to become pregnancy in existing health care system to reduce the risk of NTDs due to absence of preconception care service due to policy, guideline and program of preconception care service in the country.
- ✓ A three months preconception pictured-based health education and counseling has significant positive effect on adherence to iron-folic acid supplementation to reduce the risk of NTDs.

8.5. Recommendations

- ✓ NTDs registration should be required in health care facilities and should be included in the health management information system. Also, ongoing registering data should be monitored, analyzed, and interpreted, and reported to relevant bodies for the purpose of developing a strategy for preventing and controlling the burden of NTDs and other public health problems.
- ✓ Develop and strengthen the capacity of regions and country's to monitor NTDs, as well as develop and strengthen laboratory capacity to measure the amount of folate present in the blood.
- ✓ Efforts should be made to control environmental factors such as agrochemicals that contaminate food and water sources.
- ✓ Maternal dietary practices should be considered as a foundation for public health promotion in the prevention of NTDs.
- ✓ Preconception picture based nutrition education package program including preconception care service should be endorsed by policy makers for women's who remove the modern contraception for having a plan to be pregnant, focusing on behavioral intake of preconception of IFAS intake on the existing health care system (linking to health facility with health extension program).
- ✓ Behavior change interventions based on various strategies, including picture-based health education on preconception folic acid or iron-folic acid supplementation should be implemented at the community, school, and health facility levels should be implemented to curb the emerging burden of NTDs in Ethiopia.
- ✓ In general, mandatory supporting folic acid fortification using common vehicles like flour, salt and oil or practicing good dietary folate food sources or preconception folic acid or iron-folic acid supplementation is required.
- ✓ Engage a global network of partners who are experts in conducting NTD prevention programs to track, monitor blood folate concentrations, and develop intervention programs to increase the amount of folic acid consumed by women of reproductive age.
- ✓ Future research should examine the sustainability of such behavior changes by using a community based study.

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10. ANNEXES

10.1 Curriculum Vitae

A. PERSONAL STATUS

Name**;-Anteneh Berhane Yeyi** Age**;-**38

Sex;-Male Year and Date of Birth:- 13/4/1984 GC

Nationality**;-**Ethiopian Place of Birth**;-** Gewane (Afar) **(Former Harerge)**

Marital Status**;-**Married Current Address: Dire Dawa

Cell phone no **-** +251-915-19 44 54 /+251-931-60 19 19 Office Tel-

B. EDUCATIONAL STATUS

Grade/Class	**Name of School/College**	**Year**	Degrees	**Place/Area**
University	Jimma University, Department of Nutrition and Dietetics	2018-	PhD fellow in Nutrition	Jimma
University	Haramaya University	2013-2015 G.C	MPH in Nutrition	Harer
University	Jimma University	1998-2000 E.C	BSc in Environmental Health	Jimma
Secondary and Preparatory School	Dodola High School	1994-1997 E.C		Dodola (Oromia)
Primary School	Gewane Primary School	1987-1993 E.C		Gewane (Afar)

C. Language Ability

Types of language	**Speaking**	**Reading**	**Writing**
Oromiifa	V.good	Excellent	Excellent
Amharic	Excellent	Excellent	Excellent
English	V.good	Excellent	Excellent
Afaregna	Basic	Good	Good
Somali	Basic	Good	Good

D. OTHER SKILLS;-Computer Application of Software System Such as Word, Excel, Power Point, Epi data, Epi info, SPSS, R-soft ware, WHO Anthros and Anthros plus, ENA for SMART, Food processor…..

E-TOTAL WORK EXPERIENCE; - Greater than 14 year

F-JOB POSITION

- **Expert** of Communicable Disease Prevent & Control ,IDSR (Integrated Disease Surveillance Response), PHEM (Public Health Emergency Management), Environmental health officer and WaSH Officer at different woreda health office.
- **Inspector** of Food Safety & quality, Health Institutes, Health & health related Institutes Ethiopia Food and Drug Authority.
- **Case team coordinator of Eastern Branch Office, Ethiopia Food and Drug Authority.**
- **Emergency Nutrition Technical Advisor at Ethiopia Red crosses Society Dire Dawa Branch.**
- **Emergency Nutrition Coordinator at FSCE**
- **National Nutrition Program Technical Committee Member at Dire Dawa City administration Health Office.**
- **Lecturer and researcher at Dire Dawa University**.

H-HOBBIES

- Reading books, different update materials which related to my profession & other books, newspaper, Watching football game and films.

I-CONTACT PERSON ADDRESS

No.	Name	Position	Qualification	Address	Phone no
1	Professor Dr. Tefera Belachew	Dean School of Graduate Studies, Jimma University Jimma, Ethiopia	MD, MSc, DLSHTM, PhD	Jimma	0917804072
2	Dr. Gudina Egeta (Associate professor)	Lecturer	(MPH, PhD)	Harer	0911641362
3	Ketema Ayele (PhD fellow)	Lecturer	MPH, PhD fellow	Dire Dawa	0937282987

10.2 Questionnaire

English Questionnaire

Part One: Socio-Demographic factors

SECTION A: Socio-Demographic Characteristics			
In this part I am going to ask you about socio-demographic characteristics related you or your household partner			
A1.	Region/zone of participant/ Study site		
A2.	Permanent Residence of participant/ Study site		
A3.	Residence	1. Urban 2. Rural	
A4.	Maternal Marital Status		
A5.	Current Maternal age		
A6.	Maternal age at beginning of the index pregnancy		
A7.	Partner age		
A8.	Educational status		
A9.	Educational status of your partner		
A10.	Occupational Status		
A11.	Partner occupational status		
A12.	Is there a blood relation between you and your partner?	0. No 1. Yes	
A13.	Family size		
A14.	Religious		
A19.	Average Monthly income (wealth Index)		ETB

SECTION C: MATERNAL OBSTETRIC HISTORY		
In this section, I will ask you about your Pregnancy and Obstetric history (Please Confirm from ANC or other card)		
C1.	History of breast feeding above 2 years	0. No 1. Yes
C2.	History of male gender predominance	0. No 1. Yes
C3.	Do you have elective /termination /spontaneous abortion history?	0. No 1. Yes
C5.	Do you have history of still birth baby?	0. No 1. Yes

C6.	Do you have history of low birth weight?	0. No 1. Yes	
C7.	Do you have history of preterm?	0. No 1. Yes	
C8.	Do you have history of both low birth weight and birth to child with Defects at a time	0. No 1. Yes	
C9.	Do you have previous history of birth defect-affected pregnancy	0. No 1. Yes	
C10.	If Yes, Types of birth defect pregnancy out come		
C11.	If yes, Sex of New born	0. Male 1. Female 2. both	

SECTION D: MATERNAL HEALTH AND DRUG HISTORY			
In this section, I will ask you about your health (Please Confirm from her ANC or other card)			
D1.	Did you a history of illness during early pregnancy?	0. No 1. Yes	
D2.	If yes what type of illness?		
D3.	Did you have history of chronic hypertension?	0. No 1. Yes	
D4.	Did you have history of diabetes?	0. No 1. Yes	
D5.	Did you have a history preeclampsia?	0. No 1. Yes	
D6.	Did you have a history eclampsia?	0. No 1. Yes	
D7.	Did you have a history of anaemia?	0. No 1. Yes	
D8.	Did you have an experienced/ suffered a stress before/ during pregnancy?	0. No 1. Yes	
D9.	Did you have a history of malaria?	0. No 1. Yes	
D10.	Are you live with HIV/AIDS?	0. No 1. Yes	
D11.	Did you have a history of any type of TB before/during pregnancy?	0. No 1. Yes	
D12.	Did you have a history of fever (hyperthermine)/cold during /before pregnancy?	0. No 1. Yes	
D13.	Did you have history of Celiac disease (digestive disorder that damages the small intestineand triggered by eating foods containing gluten) during this pregnancy?	0. No 1. Yes	
D14.	Did you suffered from viral infection during pregnancy?	0. No 1. Yes	
D15.	Did you Suffered from parasitic infection (Helminthes (intestinal warm & schistosomiasis) and amebiasis infection during pregnancy?	0. No 1. Yes	

D16.	if yes, Did you take Antibiotic (penicilin, amoxicilin, cephalexin, erythromycin, clarithromycin, azithromycin, cipro, levofloxacin, co-trimoxazole, trimethoprim,	0. No 1. Yes	
D17.	History of herbal medicine usage during pregnancy?	0. No 1. Yes	
D18.	Did you have history of taking Anticonvulsant (Antiepileptic drugs (AEDs), (especially phenytoin and carbamazepine, valproic acid,(for epileptic seizures, prevent migraines and treat other brain disorders)during this pregnancy?	0. No 1. Yes	
D19.	Did you have history of taking sedatives (such as Benzodiazepines, Opioids, Morphine, Meperidine, Clonazepam, Diazepam (drug for anxiety, sleep disorders and as general anesthetics) during this pregnancy?	0. No 1. Yes	
D20.	Did you have history of taking antipyretic drug i.e. ibuprofen, analgesics (pain relievers) paracetamol, aspirin, aminopyrine, phenacetin during pregnancy?	0. No 1. Yes	
D21.	Did you have a history of take Fertility enhancing drug (lomiphene citrate, metformin, letrozole, bromocriptine) during this pregnancy?	0. No 1. Yes	
D22.	Did you take Contraceptive pills use around conception during this pregnancy?	0. No 1. Yes	
D23.	Did you have history of taking Methotrexate? (Drug used in the treatment of cancer and rheumatoid arthritis.)	0. No 1. Yes	
D24.	Did you have history of Gastrointestinal disorder (constipation,irritable bowl syndrom, hemorrhoids, anal fissures, perianal infection, diverticular disease, colitis, colon polyps and cancer?	0. No 1. Yes	
D25.	Did you have history of gastric diseases during this pregnancy?	0. No 1. Yes	

SECTION E: ENVIRONMENTAL FACTORS OF PREGNANT MOTHERS AND PARTNERS			
In this section, I will ask you about you're practiced. Do you practice the following?			
E1.	Daily exposure to passive cigarette smoke	0. No 1. Yes	
E2.	Inadequate ventilation during heating	0. No 1. Yes	
E3.	Exposure to radiation (child mother)	0. No 1. Yes	
E4.	Child father exposed to radiation	0. No 1. Yes	
E5.	Use of coal stove for heating	0. No 1. Yes	
E6.	Kitchen in living quarters	0. No 1. Yes	
E7.	Exposure of heavy metals (father)	0. No 1. Yes	
E8.	Exposure of heavy metals(Mother)	0. No 1. Yes	
E9.	Exposure of organic solvents (father)(spray organophosphate chemicals for chat)	0. No 1. Yes	
E10.	Exposure of organic solvents (Mother)(spray organophosphate chemicals for chat)	0. No 1. Yes	

E11.	Exposed to chemicals) (spray organophosphate chemicals for chat)	0. No 1. Yes	

SECTION F: KNOWLEDGE ABOUT NTD, FOLIC ACID, IFA AND VITAMIN B12			
Now, I am going to ask you about your knowledge related to NTD, folic acid, and IFA			
Which of the following correctly describes NTDs?			
F1.	Do you know about NTD?	0. No 1. Yes	
F2.	If yes, what is/are the factors for NTDs?		
F3.	Does NTD a disease acquired by pregnant women?		
F4.	Does NTD can affect baby development in the womb?	0. No 1. Yes	
F5.	Does NTD can be transmitted by contact with affected individuals or parents?	0. No 1. Yes	
F6.	Does most NTDs are preventable?	0. No 1. Yes	
F7.	Does most NTDs can be treated or managed medically?	0. No 1. Yes	
F8.	Do deformities from NTDs can be reduced through physiotherapy?	0. No 1. Yes	
F9.	Does alcohol consumption during pregnancy increase your risk of giving birth to a child with NTDs?	0. No 1. Yes	
F10.	Does the use of some un-prescribed medications increase your risk of giving birth to a child with NTDs?	0. No 1. Yes	
F11.	Does smoking before and during pregnancy increase your risk of giving birth to a child with NTDs?	0. No 1. Yes	
F12.	Does advance maternal age (>40 years) increase the risk of giving birth to a child with NTDs?	0. No 1. Yes	
F13.	Does obesity increase your risk of giving birth to a child with NTDs?	0. No 1. Yes	
F14.	Does DM increase your risk of giving birth to a child with NTDs?	0. No 1. Yes	
F15.	Does consumption of folic acid pre pregnancy and during pregnancy reduce your chances of giving birth to a child with NTDs?	0. No 1. Yes	
F16.	Does consumption of animal source of foods pre pregnancy and during pregnancy reduce your chances of giving birth to a child with NTDs?	0. No 1. Yes	
F17.	Does consumption of food with aflatoxin increase your risk of giving birth to a child with NTDs?	0. No 1. Yes	
F18.	Does consumption of iodaized salt during pregnancy reduce your chances of giving birth to a child with NTDs?	0. No 1. Yes	
F19.	Does a regular checkup throughout the pregnancy period reduce your chances of giving birth to a child with NTDs?	0. No 1. Yes	
F20.	Do you know folic acid?		0. No 1. Yes

F21.	If yes, What is the benefit of using of folic acid supplements?	0. No 1. Yes
F22.	From where did you get this awareness of folic acid?	0. No 1. Yes
F23.	In pre-pregnancy, there is a need of folic acid supplementation?	0. No 1. Yes
F24.	If yes could you mention it please?	
F27.	Do you know about folic acid rich food sources?	0. No 1. Yes
F28.	If yes please mention some sources	
F29.	Does excessive cooking decline food nutritional value?	0. No 1. Yes
F30.	If yes How?	
F31.	Does folic acid deficiency leads to abnormality in new born?	0. No 1. Yes
F32.	If yes could you mention some of the abnormalities please?	
F33.	Do you know IFA?	0. No 1. Yes
F34.	If yes, What is the benefit of using of IFA supplements?	
F35.	From where did you get this awareness of IFA?	
F36.	In pre-pregnancy, there is a need of IFA supplementation?	0. No 1. Yes
F37.	If yes could you mention it please?	
F38.	Does IFA deficiency leads to abnormality in new born?	0. No 1. Yes
F39.	If yes could you mention it please?	

Part three: Nutritional factors

SECTION G: DIETARY ASSESSMENT DATA (FOOD FREQUENCY QUESTIONNAIRE)			
Remembering your experience of the last six months (first two trimesters) pregnancy of the index child, how frequently did you consume the following food items in a typical day/week/month/year?			
Grains and grain products			
	Injera		Per day/times

			Per week/times
			Per month/times
			Per year/times
	Rice		Per day/times
			Per week/times
			Per month/times
			Per year/times
	Spaghetti pasta		Per day/times
			Per week/times
			Per month/times
			Per year/times
	Macaroni		Per day/times
			Per week/times
			Per month/times
			Per year/times
	Soup		Per day/times
			Per week/time
			Per month/times
			Per year/times
	Porridge		Per day/times
			Per week/times
			Per month/times
			Per year/times
	White Bread (Refined Cereals)		Per day/times
			Per week/times
			Per month/times
			Per year/times
	Wheat bread (Homemade Bread) Whole grain		Per day/times
			Per week/times
			Per month/times
			Per year/times
	False banana (Kocho, Bula)		Per day/times
			Per week/times
			Per month/times
			Per year/times
	Chechebsa		Per day/times
			Per week/times
			Per month/times
			Per year/times
	Fetira		Per day/times
			Per week/times
			Per month/times
			Per year/times
Vegetables and vegetable products			
	Carrot		Per day/times
			Per week/times
			Per month/times
			Per year/times
	Tomato		Per day/times
			Per week/times
			Per month/times
			Per year/times
	Pepper (Kariya)		Per day/times
			Per week/times

			Per month/times
			Per year/times
	Chili (Mitimita)		Per day/times
			Per week/times
			Per month/times
			Per year/times
	Green Cabbage (Ye abesha gomen)		Per day/times
			Per week/times
			Per month/times
			Per year/times
	Kale (Kosita)		Per day/times
			Per week/times
			Per month/times
			Per year/times
	Cannonball cabbage (tikil gomen)		Per day/times
			Per week/times
			Per month/times
			Per year/times
	Cucumber (ujure)		Per day/times
			Per week/times
			Per month/times
			Per year/times
	Eggplant (vijijer)		Per day/times
			Per week/times
			Per month/times
			Per year/times
	Snap bean (Fosolia)		Per day/times
			Per week/times
			Per month/times
			Per year/times
	Pump kin (Duba) (Dabaaqula)		Per day/times
			Per week/times
			Per month/times
			Per year/times
	Beetroot (Key sir)		Per day/times
			Per week/times
			Per month/times
			Per year/times
	Potato		Per day/times
			Per week/times
			Per month/times
			Per year/times
	Sweet Potato		Per day/times
			Per week/times
			Per month/times
			Per year/times
	Sprouted potato consumption (growing or green potato)		Per day/times
			Per week/times
			Per month/times
			Per year/times
	Chibis		Per day/times
			Per week/times
			Per month/times

			Per year/times
Fruits and fruit products			
	Banana		Per day/times
			Per week/times
			Per month/times
			Per year/times
	Apple		Per day/times
			Per week/times
			Per month/times
			Per year/times
	Orange		Per day/times
			Per week/times
			Per month/times
			Per year/times
	Papaya		Per day/times
			Per week/times
			Per month/times
			Per year/times
	Mango		Per day/times
			Per week/times
			Per month/times
			Per year/times
	Grape (Weyin)		Per day/times
			Per week/times
			Per month/times
			Per year/times
	Watermelon (habihab)		Per day/times
			Per week/times
			Per month/times
			Per year/times
	Guava (Zeytoni)		Per day/times
			Per week/times
			Per month/times
			Per year/times
	Gishita		Per day/times
			Per week/times
			Per month/times
			Per year/times
	Ambeshok		Per day/times
			Per week/times
			Per month/times
			Per year/time
	Abuka		Per day/times
			Per week/times
			Per month/times
			Per year/times
	Avokado		Per day/times
			Per week/times
			Per month/times
			Per year/times
	Peach (Kok)		Per day/times
			Per week/times
			Per month/times
			Per year/times

	Strawberry (Injori)		Per day/times
			Per week/times
			Per month/times
			Per year/times
	Pineapple (Ananas)		Per day/times
			Per week/times
			Per month/times
			Per year/times
Nuts and seeds			
	Beans (all types)		Per day/times
			Per week/times
			Per month/times
			Per year/times
	Kidney Beans (Boloke/Adenguare)		Per day/times
			Per week/times
			Per month/times
			Per year/times
	Peanuts		Per day/times
			Per week/times
			Per month/times
			Per year/times
	Lentils (Misir)		Per day/times
			Per week/times
			Per month/times
			Per year/times
Meat and meat products			
	Meat		Per day/times
			Per week/times
			Per month/times
			Per year/times
	Poultry (Doro wet)		Per day/times
			Per week/times
			Per month/times
			Per year/times
	Pork, meat		Per day/times
			Per week/times
			Per month/times
			Per year/times
	Liver (Kidney)		Per day/times
			Per week/times
Fish and sea food products			
	Fish		Per day/times
			Per week/times
			Per month/times
			Per year/times
Eggs and egg products			
	Egg,		Per day/times
			Per week/times
			Per month/times
			Per year/times
Milk, Milk Products and milk substitutes			
	Milk,		Per day/times
			Per week/times
			Per month/times

			Per year/times
	Cow milk		Per day/times
			Per week/times
			Per month/times
			Per year/times
	Goat milk		Per day/times
			Per week/times
			Per month/times
			Per year/times
	Sheep milk		Per day/times
			Per week/times
			Per month/times
			Per year/times
	Camel milk		Per day/times
			Per week/times
			Per month/times
			Per year/times
	Cheese		Per day/times
			Per week/times
			Per month/times
			Per year/times
	Yogurt		Per day/times
			Per week/times
			Per month/times
			Per year/times
Fat and oil			
	Butter		Per day/times
			Per week/times
			Per month/times
			Per year/times
	Margarine		Per day/times
			Per week/times
			Per month/times
			Per year/times
	Mayonnaise		Per day/times
			Per week/times
			Per month/times
			Per year/times
	Palm oil		Per day/times
			Per week/times
			Per month/times
			Per year/times
Sugars and sweets			
	Mushebek		Per day/times
			Per week/times
			Per month/times
			Per year/times
	Bakilaba		Per day/times
			Per week/times
			Per month/times
			Per year/times
	Halawa		Per day/times

			Per week/times
			Per month/times
			Per year/times
	Marmalade		Per day/times
			Per week/times
			Per month/times
			Per year/times
	Honey		Per day/times
			Per week/times
			Per month/times
			Per year/times
Beverages (non-milk)			
	Coffee (yejebena)		Per day/times
			Per week/times
			Per month/times
			Per year/times
	Tea		Per day/times
			Per week/times
			Per month/times
			Per year/times
	Soft drink		Per day/times
			Per week/times
			Per month/times
			Per year/times
	Tela		Per day/times
			Per week/times
			Per month/times
			Per year/times
	Ber		Per day/times
			Per week/times
			Per month/times
			Per year/times
	Wine		Per day/times
			Per week/times
			Per month/times
			Per year/times
	Areke		Per day/times
			Per week/times
			Per month/times
			Per year/times
	Tej		Per day/times
			Per week/times
			Per month/times
			Per year/times
	Keneto		Per day/times
			Per week/times
			Per month/times
			Per year/times
Miscellaneous			
	Chewed chat before pregnancy		Per day/times
			Per week/times
			Per month/times
			Per year/times
	Chewed during pregnancy		Per day/times

			Per week/times
			Per month/times
			Per year/times
	Chewed Chat with pea nut before pregnancy		Per day/times
			Per week/times
			Per month/times
			Per year/times
	Chewed chat with pea nut during pregnancy		Per day/times
			Per week/times
			Per month/times
			Per year/times
	Take Alcohol before pregnancy?		Per day/times
			Per week/times
			Per month/times
			Per year/times
	Take Alcohol during pregnancy?		Per day/times
			Per week/times
			Per month/times
			Per year/times
	Consume any food contaminated by Aflatoxin? (cereals or peanut)		Per day/times
			Per week/times
			Per month/times
			Per year/times
	Iodized salt		

SECTION H: STATUS OF NEWBORN			
Now, I am going to ask you about Status of New Born and related issues			
H1.	Gestational age of the identified neural tube defects (NTDs)	1. Extremely preterm (<28 weeks)	
		2. Very preterm(28-31 week)	
		3. Moderate preterm (32-36 weeks)	
		4. Early term(37-38 weeks)	
		5. Full-term(39-40 weeks)	
		6 Post-term(40 weeks)	
H2.	Mode of NTD diagnosis	1. Ultrasound before delivery 2. Identified after delivery/expulsion	
H3.	Date of diagnosis of NTDs	____________________**dd/mm/yyy**	
H4.	Mode of delivery	1. Spontaneous delivery 2. C/S 3. Vacuum 4. Forceps	
H5.	Date of birth	**dd/mm/yyy**	
H6.	Gender of Neonate	0. Male 1. Female	
H7.	Pregnancy outcome	1. Live birth 2. Still birth 3. Elective/ termination of pregnancy with fetal anomaly 4. Spontaneous abortion	
H8.	If the answer is choice number 1 for question , Pregnancy outcome at discharge	1. Alive 2. Dead	
H9.	If dead,	1. Death within few minutes of delivery and before referral to NICU 2. Death after referral to NICU/another hospital	

H10.	If dead, specify date of death (dd/mm/yy)		
H11.	If the neonate was alive, Weight of Neonate	gm / kg	
H12.	Head Circumstance	Cm	
H13.	Type of NTD identified	1. Myelomeningocele 2. Anencephaly 3. Encephalocoeles 4. Meninmyelomeningocele 5. Spinal Bifidia	
H14.	Associated congenital anomaly		0. No 1. Yes
H15.	If yes,	1. Hydrocephalus	
		2. Club foot	
		3.GI anomalies	
		4. Cleft lip + palate	
		5. Others(Specify)	
H16.	Weight of mothers		
H17.	MUAC/RUAC of mothers		
H18.	Height of Mothers		

SECTION J: FUTURE CONTACTS

We are almost finished now. We thank you for your participation in this study. We would like to come back and interview you again in the next six months or so.

J1.	Can you give me the names and addresses of 3 people I can contact who will know where you are?
	1.
	2.
	3.

Retrospective study design questionnaire

Part One: Perinatal outcome of mothers whose pregnancy was complicated with NTDs in Eastern Ethiopia

S. No	Characteristics	Choice	Code	Skip
101	Gender of Neonate	0.Male 1.Female		
102	Date of birth	**dd/mm/yyy**		
103	Pregnancy outcome	1.Live birth 2.Still birth 3.Elective/ termination of pregnancy with fetal anomaly		
104	If the answer is choice number 1 for question 303, Pregnancy outcome at discharge	1. Alive 2.Dead		
105	If dead,	1.Death within few minutes of delivery and before referral to NICU 2.Death after referral to NICU/another hospital		

106	If the neonate was alive, Weight of Neonate	___________________ kg		

Part Two: NTD type and associated anomalies identified in mothers whose pregnancy outcome was complicated with NTDs

S. No	Characteristics	Choice	Code	Skip
201	Type of NTDs identified	1. Myelomeningocele 2. Anencephaly 3. Encephalocele 4. Meningeocele 5. Spina bifidia		
202	Associated congenital anomaly	0. No 1. Yes		
203	If yes,	1. Hydrocephalus 2. Club foot 3.GI anomalies 4. Cleft lip 99. Others(Specify)____________		

10.3 Consent form

Section II. Consent form for mothers who deliver in hospitals (English Version)

I undersigned have been informed about the purpose of this particular research project. I have been informed that I am going to respond to this question by answering what I know concerning the issue. I have been informed that the information I give was used only for the purpose of this study and my identity as well as the information I give was treated confidentially. I have also been informed that I can refuse to participate in the study or not to respond to questions if I am not interested. Furthermore I have been informed that I can stop responding to the questions at any time in the process. Based on the above information I agree to participate in this research voluntarily.

Signature: ____________________ Date: ____________________

NB: 1. if the study subject is voluntary to participate in the study, start the interview.

2. Interviewer signature certifying that informed consent has been given verbally by the respondent.

Name __________________ Date ___________________

Tele __________________ Signature _______________

3. If there are things that require clarification please don't hesitate to ask the Interviewer for clarification.

Address
Anteneh Berhane (PhD student at Jimma University)
Jimma University, Faculty of Public Health, Department of Nutrition and Dietetics
Mobile: 09-15-19-44-54
Email: antishaction@gmail.com Dire Dawa

10.4 Information Sheet

Jimma University

Faculty of Medicine and Health Sciences, Department of Nutrition and Dietetics

Information sheet and Consent form for study population

Section I. Information sheet

1. Name of the study hospital _____2. Questionnaire identification number _______

INTRODUCTION: Good morning/afternoon? My name is ____________________. In this Study which is undertaken by Jimma University, Faculty of Public Health, Department of Nutrition and Dietetics, you and I would have a short discussion of about 20-30 minutes only and I am asking you to help us. Before we go to our discussion, I will request you to listen carefully to what I am going to read to you about the purpose and general condition of the study and you will tell me whether you agree or disagree to participate in this study at the end.

The purpose of this study is to for Neural Tube Defect: Burden, Determinants, Dietary Practice, and Effect of Picture Based Health Education of Women on Their Adherence to Preconception Iron-Folic Acid Supplementation in Eastern Ethiopia. The study will be conducted through interviews. The results of the study will inform design of the nutrition education intervention strategies targeting mothers due to their importance in reproductive and productive roles in the society. I would like to assure you that privacy was maintained strictly throughout. A code number will identify every participant and name will not use. Your responses to any of the questions will not be given to anyone else and no reports of the study will ever identify you. If a report of results is published, only information about the total group will appear. You will not exclude from health packages you get from normal ANC follow-up.

The interview is voluntary and your participation / non-participation, or refusal to respond or stop responding to the questions will have no effect now or in the future on services that you or any member of your family may receive from the service providers.

Are you willing to participate in this study?

1. [ ] Yes. 2. [ ] No

Thank you!!!

NB: 1. if the study subjects agree to participate in the study, go to consent form

2. No need of enforcing the clients to be included in the study

10.5 Broacher (Education Material)

Do you have a plan for next pregnancy/intention to pregnancy?

Make a Plan and Take Action

I. What do you do during preconception and prenatal health care service?

1. Visit health facility before and during pregnancy like ANC and check your health frequently

- ✓ If you currently have any medical conditions, be sure they are under control and being treated.
- ✓ Talk with health professional if you smoke, drink alcohol, or use certain drugs; live in a stressful or abusive environment; or work with or live around toxic substances.
- ✓ Don’t take a drug without prescribe your Doctor maternal use of certain anticonvulsant (antiseizure) medications and any other medication.
- ✓ Take some vaccinations which are recommended before you become pregnant, during pregnancy, or right after delivery.

2. Eat a diet rich in food daily, keep food safety and hygiene and exercise before and during pregnancy

Before and after doing anything please wash your hand washing and keep personal hygiene

3. Take Iron Folic Acid Every Day

Until one month before trying to become pregnant or even if you are not planning to become pregnant, for pregnant women.

Take one tablet per day for at least 90 days, starting as early as possible in pregnancy.

IFA will not harm the mother or the baby in any way.

Common side effects are black stools, stomach upset, constipation and diarrhea. These effects are not
serious and should subside in a few days.

If they do not, take IFA at night or with food or with fruit juice or fruit if possible.

Avoid or limit tea and coffee one hour before and after taking IFA.

4. Stop Drinking Alcohol, Smoking, and Using Certain Drugs and doesn’t stay in smoking environment.

5. Avoid Toxic Substances and Environmental Contaminants

Avoid harmful chemicals, environmental contaminants, and other toxic substances such as synthetic chemicals, metals, fertilizer, bug spray, and cat or rodent feces around the home and in the workplace.

6. Reach and Maintain a Healthy Weight

If you are overweight or obese have a higher risk for many serious conditions, including complications during pregnancy, heart disease, type 2 Diabetes, and certain cancers (endometrial, breast, and colon).

7. Get Help for Violence

Violence can lead to injury and death among women at any stage of life, including during pregnancy.

8. Learn your and your partner family history

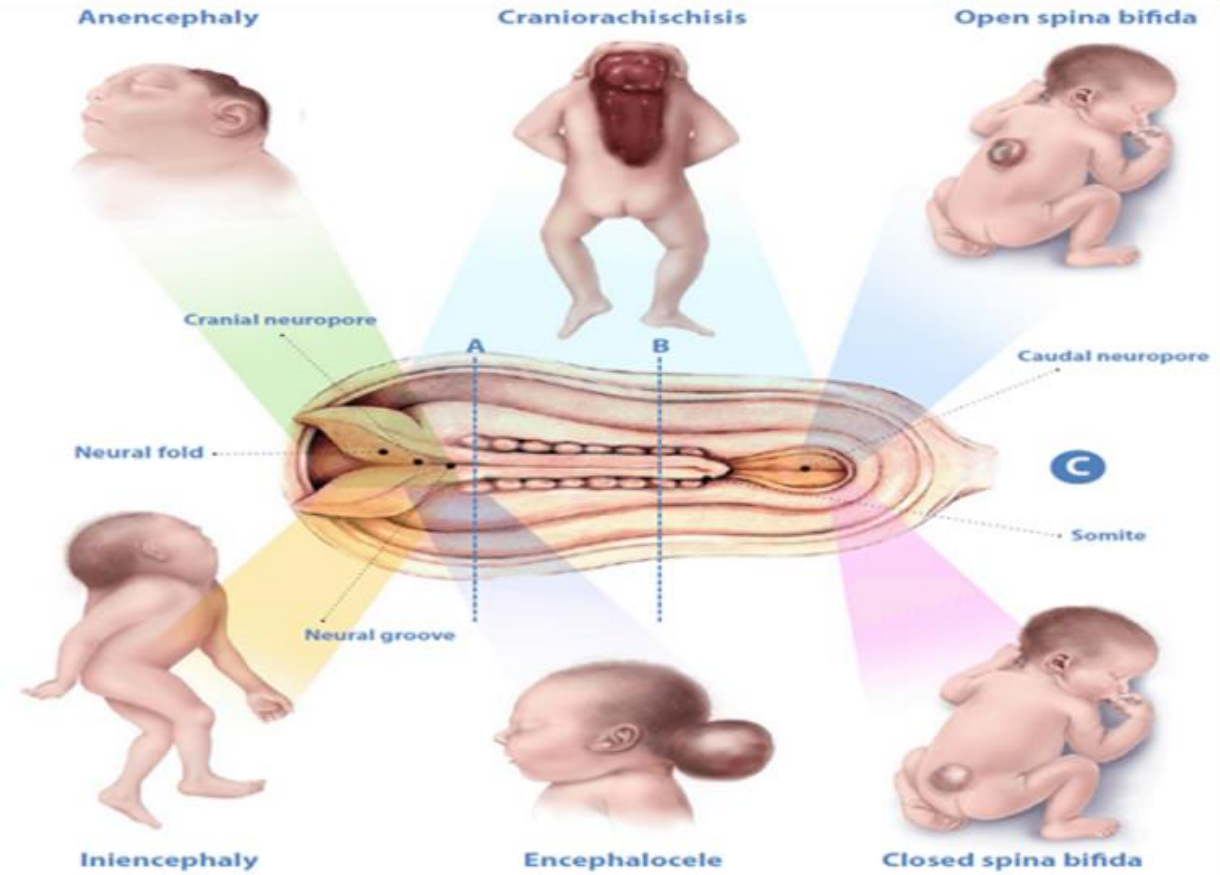
Collecting you and your partner family’s health history can be important for your child’s health.

If either you or the baby’s father

9. Get Mentally Healthy

To be at your best, you need to feel good about your life and value yourself. Everyone feels worried, anxious, sad, or stressed sometimes.

II. If you don't do this may be you are at risk giving a child with neural tube defect and other negative birth outcome like maternal anaemia, puerperal sepsis, low birth weight, and preterm birth.



Jimma University Institute of Health

Institutional Review Board

Ref.No: IHRPGY/738/20

Date: 26/08/2020

To: Anteneh Berhane

Subject: Ethical Approval of Research Protocol

The IRB of Institute of Health has reviewed your research project **"Neural tube defects: determinants, community's experience and effect of picture based education on its birth outcome"**

Thus, this is to notify that this research protocol as presented to the IRB meets the ethical and scientific standards outlined in national and international guidelines. Hence, we are pleased to inform you that your research protocol is ethically cleared.

We strongly recommend that any significant deviation from the methodological details indicated in the approved protocol must be communicated to the IRB before it has been implemented.

With Regards!

Netsanet Workneh (MD, DTM&H)
IRB chairperson
Tel: +251 917762109
E-mail: konetsanet@gmail.com

20 April 2021

To Whom It May Concern:

RE: Neural Tube Defect: Determinants, Community experiences, Effect of Picture Based education on its birth outcome

As project manager for the Pan African Clinical Trial Registry (www.pactr.org) database, it is my pleasure to inform you that your application to our registry has been accepted. Your unique identification number for the registry is **PACTR202104543567379**.

Please be advised that you are responsible for updating your trial, or for informing us of changes to your trial.

Additionally, please provide us with copies of your ethical clearance letters as we must have these on file (via email or post or by uploading online) at your earliest convenience if you have not already done so.

Please do not hesitate to contact us at +27 21 938 0835 or email epienaar@mrc.ac.za should you have any questions.

Yours faithfully,

Elizabeth D Pienaar
www.pactr.org Project Manager
+27 021 938 0835

PACTR
PAN AFRICAN CLINICAL TRIALS REGISTRY

The South African Medical Research Council
Cochrane South Africa | PO Box 19070, Tygerberg, 7505