

ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES
DEPARTMENT OF BIOCHEMISTRY

Assessment of RBC-folate level and other determinant factors of neural tube defect among first trimester pregnant women who attends antenatal care in Lideta and T/haimanot health centers, Addis Ababa, Ethiopia

Alemu Adela (BSc nurse, MSc)

A thesis submitted to the School of Graduate Studies of Addis Ababa University, Department of Biochemistry in partial fulfillment of the requirements for the Degree of Master of Science in Medical Biochemistry.



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Addis Ababa, Ethiopia

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This is to certify that the dissertation prepared by Alemu Adela, entitled, “**Assessment of RBC-folate level and other determinant factors of neural tube defect among first trimester pregnant women who attends ante natal care in Lideta and T/haimanot health centers, Addis Ababa, Ethiopia,**” and submitted in partial fulfillment of the requirements for the degree “Master of Science in Medical Biochemistry” in the department of Biochemistry complies with regulations of the university and meets the accepted standards with respect to originality and quality.

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ABBREVIATIONS

ANC	Antenatal care
dTMP	Deoxythymidine monophosphate
DTT	Dithiothreitol
dUMP	Deoxyuridine monophosphate
EDTA	Ethylenediaminetetraacetic acid
Ery-folate	Erythrocyte folate
ETB	Ethiopian birr
MCV	Mean corpuscular volume
MMA	Methyl malonic acid
MTHFR	Methylenetetrahydrofolate reductase
NTD	Neural tube defect
OR	Odds ratio
RBC	Red blood cell
SAM	S-adenosylmethionine synthetase
SPSS	Statistical package for the social science
SSA	Sub Saharan Africa
tHcy	Homocysteine
THF (FH ₄)	Tetrahydrofolate
UMP	Uridine-monophosphate
WHO	World health organization
1C	One-carbon

ABSTRACT

Background: Folate is an essential vitamin for de novo DNA synthesis and cell proliferation. Deficiency of this vitamin during first trimester pregnancy is associated with unintended pregnancy and birth outcomes, particularly neural tube defects.

Objective: The aim of this study was to assess folate status, and other determinant factors of neural tube defect among first trimester pregnant women who were attending antenatal care in Lideta and T/haimanot health centers, Addis Ababa, Ethiopia.

Materials and methods: Institutional based cross-sectional study was conducted from August 8, 2017 to January 3, 2018 at Lideta and T/haimanote health centers. For this study, 160 participants were enrolled via convenience sampling method. Red blood cell folate was measured by electrochemiluminescence binding assay method and MCV was measured by Beckman coulter method. Data were entered in to Epi-Data version 3.1 and analyzed by SPSS version 22.0. Descriptive statistics was used to describe demographic characteristics and to determine magnitude of folate deficiency. Logistic regression was used to identify risk factors of folate deficiency. P-value of < 0.05 was considered as statistically significant.

Results: In the present study, 44/160 (27%) participants had RBC folate level $< 400\text{ng/mL}$, insufficient to prevent neural tube defect. The magnitude of macrocytosis was 2.4% among 82 participants. Besides, only 13/160 (8.1%) participants found to have information about folate and five of them correctly know the timing of folic acid supplementation. The prevalence of folate supplementation during the first trimester was also only two/one hundred sixty (1.3%). Bivariate regression shows income as a determinant factor for folate deficiency (p value = 0.01). Regular consumption of fruit (OR: 0.44, CI: 0.21-0.89), vegetable (OR: 0.38, CI: 0.71-2.29) and milk (OR: 0.47, CI: 0.23-.96) were found to lower the possibility of getting folate deficiency by the bivariate regression. But, regular vegetable intake was the only continued determinant factor by the multivariate regression (adjusted OR: 0.41, CI: 0.18-0.93).

Conclusion: This study established that 27.5% of the first trimester pregnant women had a folate status insufficient to prevent neural tube defect. Furthermore, folic acid usage among the first trimester pregnant women was very low. This study also identified that regular consumption of leafy green vegetable as an independent factor to overcome folate deficiency.

Key words: Folate Metabolism, Folate Deficiency, Pregnant Women, Determinant Factors, Neural Tube Defect

3. INTRODUCTION

1.1 Background

Vitamins are a group of vital organic nutrients that cannot be synthesized by the body and must be available in the diet. Vitamins are needed in specific amounts for biochemical reactions. There are 13 different types of vitamins, from which, 4 are fat soluble (A, D, E, and K) and the other 9 are water soluble (8 B vitamins and vitamin C), classified based on their biochemical activity (Mahmood, 2014). The lipid-soluble vitamins can be absorbed efficiently only when there is normal fat absorption. They are transported in the blood in lipoproteins or attached to specific binding proteins and more likely stored in the body for a long time. The water-soluble vitamins are excreted from the body rapidly since they are not stored for a long time. Their function is mainly as enzyme cofactors. Deficiency of a single vitamin is not common, because malnutrition is associated with multiple deficiencies of the B complex vitamins (Bender, 2009)

Some vitamins, such as folate and B₁₂, are vital for the body cell growth and development. Folate is known as vitamin B₉ which has metabolic roles in serine and glycine metabolism, histidine catabolism, thymidylate synthesis, methionine synthesis and purine synthesis (Klee, 2000; Shuvalov *et al.*, 2017). The conversion of homocysteine to methionine through transfer of a methyl group from the co-substrate methyltetrahydrofolate is done by the help of vitamin B₁₂. The enzyme, methionine synthase, links the important pathway of methylation and the pathways of purine and pyrimidine (thymidine) synthesis through synthesis of 5-adenosyl methionine. In this process vitamin B₁₂ in the form of methylcobalamin as a cofactor is used to generate tetrahydrofolate and prevents folate trap (Fig-1). Therefore, folate has vital functions for the de novo synthesis, repair, and methylation of DNA (Emmerson *et al.*, 2017). Moreover, it has an important role in cell division and it is especially needed during infancy and pregnancy when cell proliferation is intense. It is also required by the human body in order to produce healthy red blood cells and to prevent macrocytosis (Mahmood, 2014).

A deficiency of folate leads to a reduction in thymidylate synthesis that produces megaloblastic anemia and birth defects, particularly NTD. Folate deficiencies also cause hyperhomocysteinemia, which can lead to atherosclerosis formation, and disturbances of methylation, leading to effects on the nervous system and other organs (Green, 2011).

Pregnancy is associated with an increased folate demand and in some cases led to overt folate deficiency. The increase in folate requirement during pregnancy is due to the growth of the

fetus, uteroplacental organs (Tamura and Picciano, 2006) and maternal erythropoiesis (Obeid and Herrmann, 2005; Castellanos-Sinco *et al.*, 2015). Therefore, dietary folate intake does not always meet the increased folate needs during pregnancy.

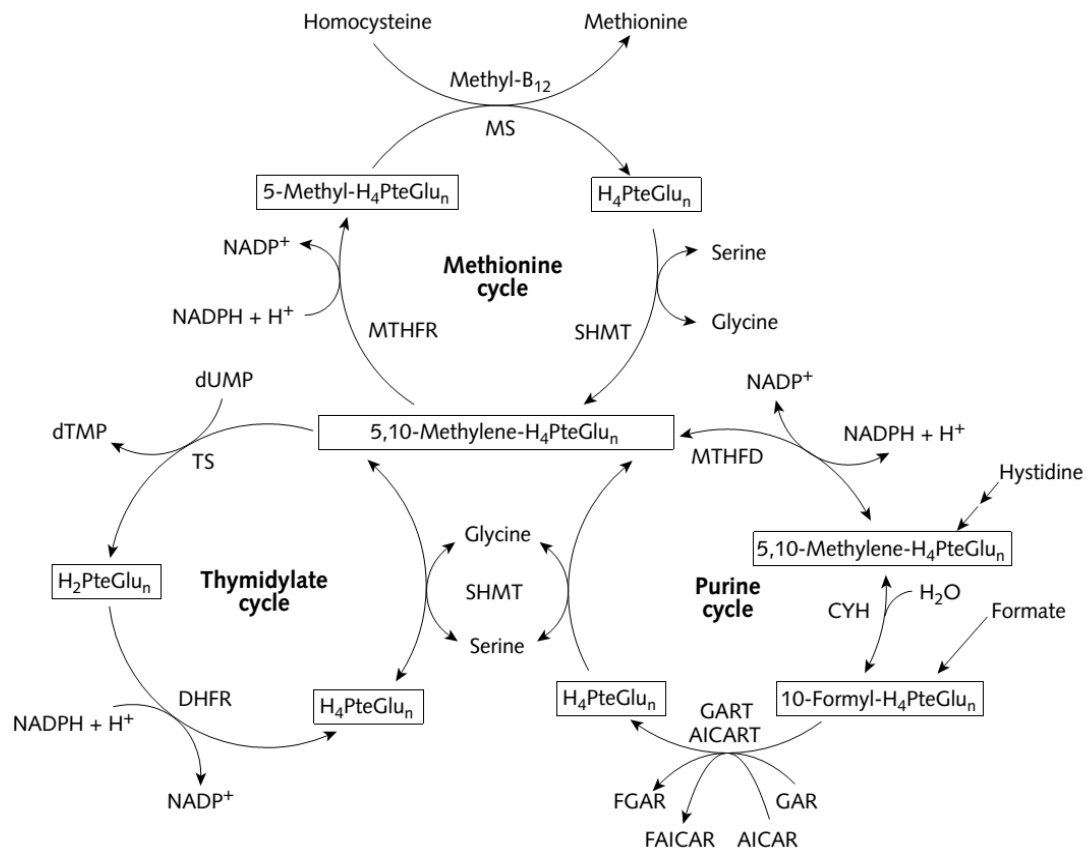


Figure 1: Metabolic cycle of cytosolic one carbon metabolism.

SHMT, serine hydroxymethyltransferase; MTHFR, methylenetetrahydrofolate reductase; MS, methionine synthase; TS, thymidylate synthase; DHFR, dihydrofolate reductase; MTHFD, methylenetetrahydrofolate dehydrogenase; CYH, methenyltetrahydrofolate cyclohydrolase; GART, GAR formyltransferase; AICART, AICAR formyltransferase; dUMP, deoxyuridine monophosphate; dTMP, deoxythymidine monophosphate; FAICAR, formyl-aminoimidazole carboxamide ribonucleotide; AICAR, aminomidazole carboxamide ribonucleotide; GAR, glycinamide ribonucleotide; FGAR, formyl-glycinamide ribonucleotide

Adopted from "Folate and vitamin B12 metabolism" (Shane, 2008)

1.2 Literature review

1.2.1. Folate, its structure and intestinal absorption

Folates are a family of B₉ vitamins which exist in many chemical forms such as dihydrofolate, tetrahydrofolate, 5, 10-methylene-THF and 5, 10-methenyl-THF, and folic acid which is a synthetic form of folates (Fig-2). The coenzyme form that functions in accepting one-carbon groups is tetrahydrofolate polyglutamate, tetrahydrofolate or FH₄. It has three major structural components, a bicyclic pteridine ring that can be reduced or oxidized, paraaminobenzoic acid linker that binds one-carbon units jointly with the pteridine ring, and a polyglutamate tail consisting of several glutamate residues that is important for localization. The 1C group that is accepted by the coenzyme and then transferred to another compound is bound to the 5-position nitrogen atom on the pteridine ring moiety and the 10-position nitrogen atom on the PABA moiety of THF (Michael. *et al.*, 2007)

Folates are synthesized by bacteria (via fermentation) (LeBlanc *et al.*, 2011; Saubade *et al.*, 2017) and higher plants. They are ingested in legumes (beans, nuts, peas, etc.), leafy greens (such as spinach), citrus, some fruits, vegetables (broccoli, cauliflower), liver, and fermented dairy products. Most of the dietary folate derived from natural food sources and in the human body is present in the reduced coenzyme form typically 5-methyl-THF. However, vitamin supplements and fortified foods contain principally the oxidized form of the pteridine ring, folic acid (Delchier *et al.*, 2016).

As dietary folates pass into the duodenum and jejunum, folate conjugates in the brush border of the lumen cleave off glutamate residues to produce the monoglutamate form of folate, by intestinal glutamate carboxypeptidase II (folate hydrolase). Then it can be absorbed primarily within the acid microenvironment at the cell surface. Within the intestinal cells, folate is converted principally to 5-methyl-FH₄, which enters the portal vein and goes to the liver. Small amounts of other forms of folate also follow this route. The liver, which stores half of the body's folate, takes up much of the folate from the portal circulation. Within the liver, FH₄ is re-conjugated to the polyglutamate form before being used in reactions. A small amount of the folate is partially degraded, and the components enter the urine. A relatively large portion of the folate enters the bile and is subsequently reabsorbed (Imbard *et al.*, 2013; Visentin *et al.*, 2014; Delchier *et al.*, 2016).

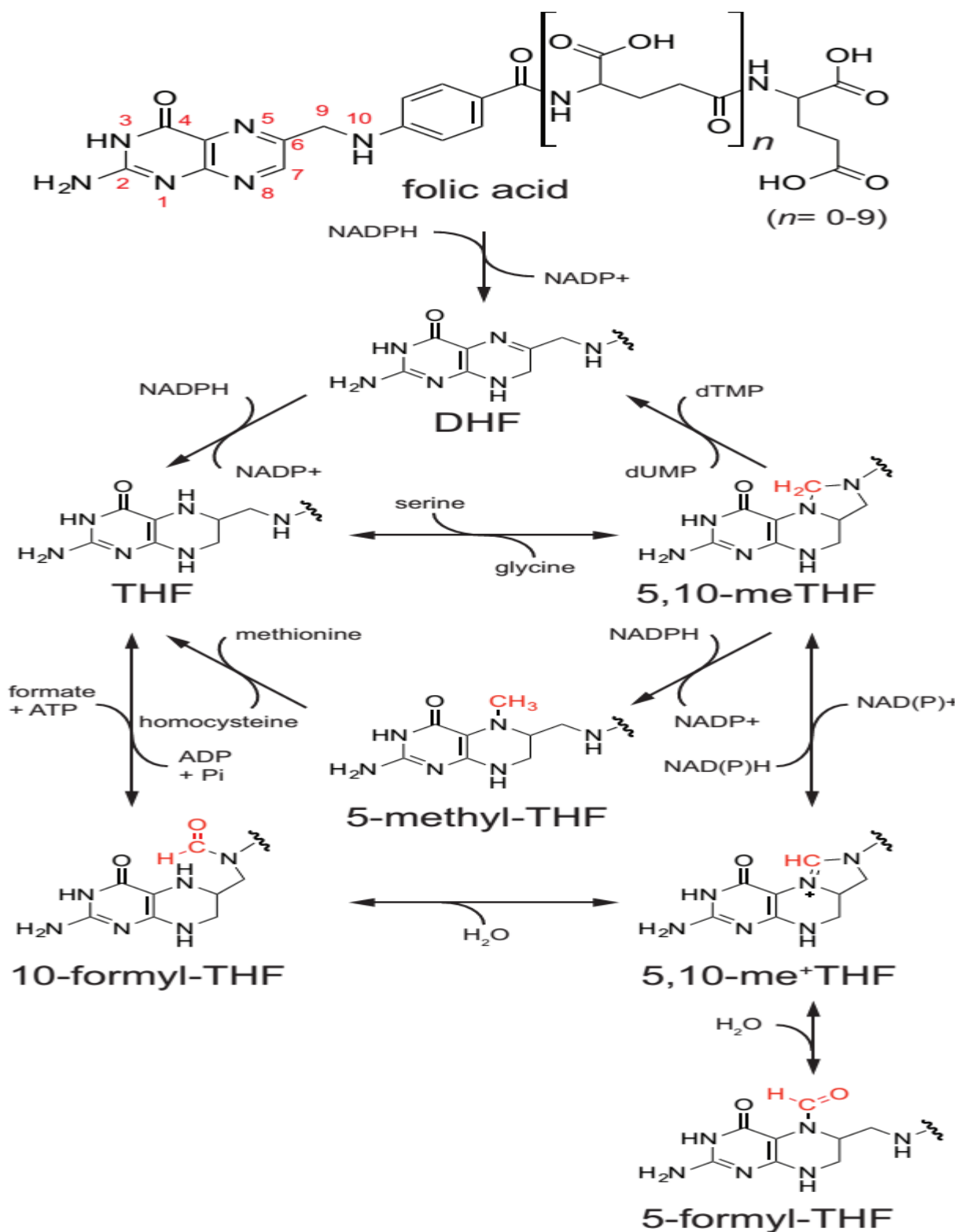


Figure 2: Structures and chemical Transformations of Folates

DHF, dihydrofolate; THF, tetrahydrofolate; 5,10-meTHF, 5,10-methylene-THF; 5,10-me⁺THF, 5,10-methylen-THF

Adopted from “One-Carbon Metabolism in Health and Disease”(Ducker and Rabinowitz, 2017)

1.2.2. Folate metabolism

Folate molecules function as carriers for one-carbon units, allowing them to be used and assembled to support metabolic processes. One-carbon groups transferred by FH_4 are attached either to N^5 or N^{10} , or they form a link between N^5 and N^{10} then these one-carbon units can be oxidized and reduced. Thus, reactions requiring a carbon at a particular oxidation state may use carbon from the one-carbon pool that was donated at a different oxidation state. The most oxidized form is 10-formyl FH_4 . The most reduced form is 5-methyl- FH_4 . Once the methyl group is formed, it is not readily re-oxidized back to 5,10 -methylene- FH_4 , and thus 5-methyl- FH_4 will tend to accumulate in the cell unless its methyl group is transferred to vitamin B_{12} to produce methionine from homocysteine and THF (Shane, 2008; Shuvalov *et al.*, 2017).

Choline, serine, and glycine are the most important dietary sources of folate 1C units. Beyond supporting biosynthetic processes, 1C metabolism plays an essential role in catabolism of these nutrients. Among the various 1C donors, Serine directly transfers its hydroxymethyl group to FH_4 in a reversible reaction in the mitochondria, catalyzed by the enzyme serine hydroxymethyl transferase to produce glycine and 5, 10-methylene- FH_4 (Fig-3). The primary role of folate metabolism here is to generate formate and glycine from the enzymatic cleavage of serine while, cytoplasmic one carbon metabolism is essential for the de novo biosynthesis of nucleotides and methionine dependent cellular methylation reactions (Ducker and Rabinowitz, 2017).

1.2.3. Biochemical function of folate

The one-carbon groups on FH_4 may be oxidized or reduced and then transferred to other compounds. Transfers of this sort are involved in the synthesis of glycine from serine, base thymine required for DNA synthesis and purine bases required for both DNA and RNA synthesis. It is also essential for the transfer of methyl groups to vitamin B_{12} to form methionine from homocysteine (Fig-3). Methionine is important for DNA, protein and lipid methylation (Shuvalov *et al.*, 2017; Ducker and Rabinowitz, 2017).

1.2.3.1. Thymidilate synthesis

The central precursor for generating pyrimidines is uridine-monophosphate (UMP), which does not require the 1C-cofactors for synthesis. UMP through UDP can be converted to either deoxyuridine monophosphate (dUMP) or other nucleotides UTP, CTP and dCTP. Then dUMP produces the nucleotide deoxythymidine monophosphate (dTMP) through methylation by using 5, 10-methylene- FH_4 as a methyl donor. Two hydrogen atoms from FH_4 are used to

reduce the donated carbon to the methyl level. Consequently, dihydrofolate (FH_2) is produced. dihydrofolate reductase enzyme (DHFR) reduces FH_2 to regenerate FH_4 by using reduced nicotinamide adenine dinucleotide phosphate (NADPH) as a hydrogen donor (Fig-3). These reactions contribute to the effect of folate deficiency on DNA synthesis, because dTMP is only required for the synthesis of DNA (Shuvalov *et al.*, 2017).

1.2.3.2. Purine synthesis

During the synthesis of the purine bases, carbons 2 and 8 are obtained from the one-carbon pool, 10-formyl- FH_4 provides both carbons. Folate deficiency would also hinder these reactions, contributing to an inability to replicate DNA because of the lack of precursors (Michael. *et al.*, 2007; Shane, 2008).

1.2.3.3. Methionine synthesis

In the one carbon methabolism, the most reduced form of folate 1C unit, 5-methyl-THF, remethylated homocysteine to form methionine by the action of methionine synthase. 5-methyl-THF is produced by the cytosolic NADPH dependent activity of methylene tetrahydrofolate reductase. After the carbon group carried by FH_4 is reduced to the methyl level, it is transferred to vitamin B_{12} . This is the only reaction through which the methyl group can leave FH_4 . Homocysteine remethylation is a very important physiological reaction because methionine is the substrate for S-adenosylmethionine synthetase. S-adenosylmethionine, the reactive methyl carrier, is the second most common enzymatic cofactor after ATP and plays a major role in epigenetics, biosynthetic processes including phosphatidylcholine, creatine, and polyamine synthesis, and sulfur metabolism. Changes in methionine concentrations lead to changes in the ratio of SAM to S-adenosyl Homocysteine that impact many methylation reactions including DNA & histone methylation (Ducker and Rabinowitz, 2017).

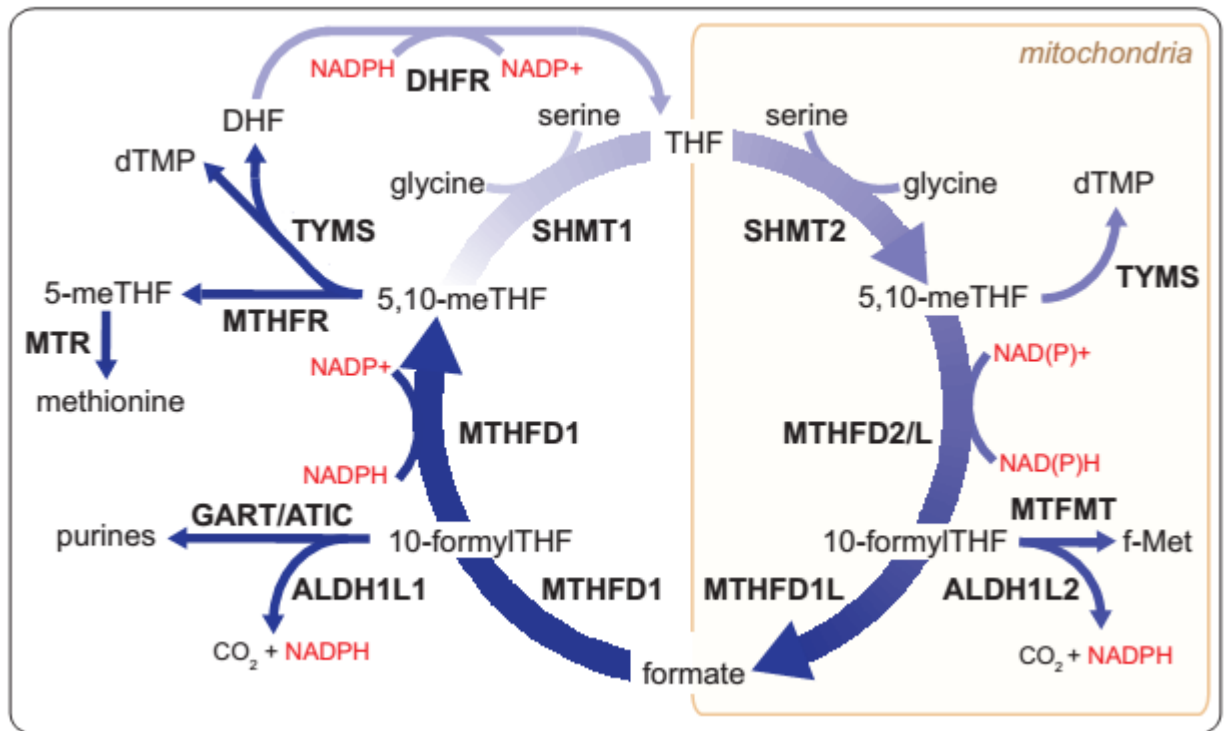


Figure 3: Products and Compartmentalization of Folate-Mediated 1C Metabolism

“Through an interlinked set of mitochondrial and cytosolic reactions, folate metabolism supports 1C anabolic reactions” SHMT1/2, serine hydroxymethyl transferase, cytosolic(1)/ mitochondrial(2); MTHFD1, methylenetetrahydrofolate dehydrogenase, cyclohydrolase, and formyltetrahydrofolate synthetase 1; MTHFD2/L, methylenetetrahydrofolate dehydrogenase 2/ 2-like; MTHFD1L, monofunctional tetrahydrofolate synthase, mitochondrial; MTFMT, mitochondrial methionyl-tRNA formyltransferase; TYMS, thymidylate synthetase; MTHFR, methylenetetrahydrofolate reductase; MTR, methionine synthase; DHFR, dihydrofolate reductase; GART, phosphoribosylglycinamide formyltransferase; ATIC, 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase; ALDH1L1/2, cytosolic (1)/mitochondrial (2) 10-formyltetrahydrofolate dehydrogenase.

Adopted from “One-Carbon Metabolism in Health and Disease” (Ducker and Rabinowitz, 2017)

1.2.4. Folate deficiency and its effect during pregnancy

Poor dietary intake (e.g., low intake of folic acid–fortified foods, dark-green leafy vegetables, legumes, select fruit such as orange juice), excessive alcohol intake, medical conditions with increased cell proliferation or use of certain drugs like anticonvulsants, antituberculosis drugs, oral contraceptives and intestinal malabsorption due to tropical sprue, celiac disease and inflammatory bowel diseases, can lead to folate deficiency. Increased cellular requirements that occur in chronic hemolytic anemias, pregnancy, lactation and rapid growth in adolescence can also exacerbate folate deficiency when the diet is inadequate. Common gene polymorphisms (specially, MTHFR C677T polymorphism) may also have contributions to

folate deficiency. Folate deficiency appears much more rapidly once depletion begins and clinical changes appear within 3 to 4 months because human body can only store about a 3 month's supply of folate (Allen, 2008 ; Bailey *et al.*, 2015; Ni *et al.*, 2016; Hiraoka and Kagawa, 2017).

The essentiality of folate for the synthesis of DNA and homocysteine- methionine metabolism links it with the unsuccessful pregnancy outcome when the mother is deficient in this micronutrient. Folate deficiency, leading to megaloblastic anaemia, is the second most common cause of anaemia during pregnancy. Folate deficiency during pregnancy, especially around the time of conception, is strongly correlated with increased risk of fatal structural abnormalities including neural tube defects and congenital heart disease. It has also a significant association with abruptio placentae, preeclampsia, spontaneous abortion, stillbirth, preterm delivery, low birth weight and small for gestational age (Anne *et al.*, 2008; Greenberg *et al.*, 2011; Chen *et al.*, 2017)

1.2.5. Effects of folate deficiency on neural tube defect

Neural tube defects (NTDs), including anencephaly and spinal bifida, arise due to the failure of closure of the neural tube between the third and fourth weeks of gestation. Neural tube defects are common birth defects with a heterogeneous and multi-factorial etiology with interacting genetic and environmental risk factors. Up to 50-70% of NTDs can be prevented by maternal folic acid supplementation due to the metabolic importance of folate and folate receptors for normal development of the central nervous system and of several neural-crest-derived tissues (Antony, 2007; Safi *et al.*, 2012).

Folate and its related dysmetabolisms influence the process of genomic DNA methylation. Abnormal methylation caused the change of gene expression, induced cellular misbehavior, including apoptosis, proliferation and differentiation, led to the occurrence of NTDs (Beaudin and Stover, 2009; Wang *et al.*, 2015). Thus, there is strong evidence of a causal association between low maternal folate intake or lower folate status and increased risk of NTD (Beaudin and Stover, 2009).

There is also moderately strong evidence of an association between the MTHFR 677C→T gene variant, which affects folate metabolism, and increased risk of NTD. For TT homozygous individuals, the polymorphism results in partial enzyme deficiency and higher folate requirements. But, the distribution of the T allele varies substantially among ethnic groups, with a lower prevalence of the TT genotype among Sub-Saharan Africans (0%–2%)

(Rosemary *et al.*, 2013). However, variants in other folate genes for which no biochemical phenotype have been also reported to influence homocysteine levels and/or disease risk. These includes methionine synthase A3150→G associated with reduced homocysteine levels and a variant in C1 THF synthase (1958G→A) as a risk factor for NTD and for second-trimester pregnancy losses (Shane, 2008).

And, for the protective effect of folic acid supplementation or consumption of fortified foods against NTD, All women, from the moment they begin trying to conceive until 12 weeks of gestation, are advised to take a folic acid supplement (Tamura and Picciano, 2006; Rosemary *et al.*, 2013; WHO, 2015a). In addition evidences show that low maternal vitamin B12 status is associated with an increased risk of NTD independently (Ray *et al.*, 2007).

1.2.6. Folate status during pregnancy

Pregnancy consists of a series of small, continuous physiologic adjustments that affects the metabolism of all nutrients and increases the demand for macro and micro nutrients. The adjustments undoubtedly vary widely from woman to woman depending on her pre pregnancy nutrition, genetic determinants of fetal size, and maternal lifestyle behavior (King, 2000). No matter what, maternal plasma folate concentration is one of the micro nutrient reported by both earliest and recent studies as it steadily decline over the course of pregnancy in pregnant women who have not taken folic acid supplementation (Ek and Magnus, 1981; Obeid and Herrmann, 2005; Hur *et al.*, 2012).

This decline can be due to a physiological response to pregnancy, related to hemodilution, alterations in renal function, and hormonal changes and increased placental and fetal demands (King, 2000; Sande *et al.*, 2013). There is also increased maternal catabolism of folate as pregnancy progresses, coinciding with periods of rapid cell proliferation and equivalent to a turnover of nearly 400 µg per day by the third trimester (McPartlin *et al.*, 1993). Although, the widespread use of prenatal folic acid supplementation normalized folate level during pregnancy in different parts of the world, still it is a potential public health problem of the developing world.

A longitudinal study (Milman *et al.*, 2006) has measured folate and homocysteine status during normal pregnancy and postpartum at 18, 32 and 39 weeks of gestation and 8 weeks postpartum when the women were lactating. The study demonstrated a significant fall in plasma and erythrocytic folate through 18, 32 and 39 weeks of gestation and postpartum. The prevalence of plasma folate deficiency (< 6 nmol/L) was increased from 0.7% to 19.0%

during pregnancy to postpartum ($P < 0.0001$). Ery-folate also displayed a similar significant fall, the prevalence of low Ery-folate $< 0.40\text{nmol/L}$ increased from 0.5% to 17.2% during pregnancy to postpartum ($P < 0.0001$). This study was agreed with another longitudinal study, by which a decrease in maternal Ery – folate concentration by 31% from 20 weeks gestation to 27 weeks postpartum were reported (Hur *et al.*, 2012).

On the contrary, a cross-sectional study done to note the value of serum Vitamin B₁₂, folic acid, and ferritin in normal and high-risk pregnancies reported a significant higher serum folic acid level during pregnancy as compared to non pregnant state. The possible reason for maintained folate indices were folate supplementation of the women (Sharma *et al.*, 2016), which are sufficient to decreasing the risk of folate depletion during pregnancy and possibly increasing folate availability for maternal-fetal transfer. This result was consistent with studies that have been observed in well-nourished women in developed countries (Gernand *et al.*, 2016). In general, folate status decreases through pregnancies and postpartum unless it is normalized by folic acid supplementation and nutritional adjustment.

1.2.7. Prevalence of folate deficiency during pregnancy

There is no good estimate of global folate deficiency exist for pregnant women. The prevalence of folate deficiency in the first trimester in the developed countries is decreased from 55%-1% after the era of folic acid fortification of flour (Brito *et al.*, 2015; Gernand *et al.*, 2016). In turkey, the prevalence of folate deficiency among first trimester pregnancy were found to be 0.5% (Karabulut *et al.*, 2011). But it is still a problem of low income and middle income countries. For example in Belgium 39% of first trimester pregnant women had RBC folate status less than 400 ng/ml that might not be optimal to prevent NTD (Vandevijvere *et al.*, 2012). In Côte d'Ivoire in West Africa, the prevalence of folate deficiency (plasma folate $< 4\text{ ng/mL}$) among women of reproductive age was reported 86% (Rohner *et al.*, 2014). And in eastern Sudan Serum folate deficiency ($< 6.6\text{ ng/mL}$) was reported in 57.7% of pregnant women (Abdelrahim *et al.*, 2009).

In Ethiopia, we found only one study that assessed the status of folate in pregnant women. The study measured the dietary intakes of a convenience sample of Sidama women in late pregnancy who consumed either maize ($n= 68$) or fermented enset (*Enset ventricosum*) ($n= 31$) as their major energy source. The study has analyzed blood samples for a complete blood count, vitamin B-12 and folate status, and for other micronutrients. In this study only 2% low plasma folate ($<6.8\text{ nmol/L}$ (3 ng/mL)) was reported. The authors attributed additional source of folate in foods as a reason for absence of folate deficiency (Gibson *et al.*, 2008).

In contrast, a cross-sectional study in folate deficiency in women of reproductive age in nine administrative regions of Ethiopia reported 46% severe folate deficiency and 21.2% marginal folate deficiency. National survey on micronutrients by EPHI also reported the prevalence of serum folate and RBC folate among women of reproductive age as 17.3% and 32% respectively. The discrepancies among the first and the second two studies can be due to differences in study population and study period, since the two surveys found the lowest prevalence of folate deficiency in the Addis (22.2%/25.14%), Amhara (33%/20.17%) and southern Ethiopia (33.6%/22.56%) regions respectively (Haidar and Melaku, 2010; Zerfu *et al.*, 2016). In general, those studies showed as folate deficiency is a potential public health problem in Ethiopia.

1.2.8. Determinants of folate status in pregnant women

Many determinant factors including, socio-demography, parity, trimester and dietary habit are associated with folate status of pregnant women in various countries. For example in Belgium unplanned pregnancy and smoking during pregnancy were associated with a lower RBC folate concentration for both first and third trimester pregnancy. In addition, a higher education level and the intake of folic acid containing multivitamins were associated with a higher RBC folate concentration in both samples of pregnant women when other factors remained constant. For women in the first trimester of pregnancy, RBC folate concentrations were significantly associated with ethnicity. In addition, null parity was associated with a higher RBC folate concentration but younger age was associated with a lower RBC folate concentration (Vandevijvere *et al.*, 2012).

This results were confirmed by a study that was conducted to assess the relationship between socio-demographic factors and folate status in non-supplement-consuming Canadian women of childbearing age, in which a significant high prevalence of lower folate status in Young age (15–19 years), White ethnicity, less than secondary education, lowest income adequacy, smoking and high body mass index was reported (Shi *et al.*, 2014). On the other hand, food security and regular vegetable in take were found to be main factors for the prevention of folate deficiency by a large-scale national nutrition survey in Pakistan (Soofi *et al.*, 2017).

A cross-sectional study in folate deficiency in women of reproductive age in nine administrative regions of Ethiopia found higher severe folate deficiency in women who; were unmarried ($p = 0.002$), had parity of 4–6 ($p = 0.001$), had no illnesses ($p = 0.001$), and followed lower plant and animal product food diets (0.001). In addition, the study reported the lowest prevalence of folate deficiency in the Addis, Amhara and southern Ethiopia

regions within the country (Haidar and Melaku, 2010). In general, pregnant women who are smokers, youngsters, multiparous, unmarried, uneducated and malnourished are at a higher risk of folate deficiency.

1.2.9. Folic acid supplementation as a prevention for neural tube defect

Lots of researches are already demonstrated the importance of folic acid supplementation for the prevention of occurrence and recurrence of NTDs as well as other folic acid-sensitive congenital malformations (De Wals *et al.*, 2007). Therefore Peri-conceptual folic acid supplementation is a primary preventive intervention for NTD that can be implemented in primary care settings (Chitayat *et al.*, 2016; Mills, 2017). Thus, supplementation of 0.4- 1.0 gram of folic acid must be given to all women planning pregnancy or capable of pregnancy in a daily basis. However, this recommendation is given to countries which has fortified dietary staple with folic acid like in USA (Chitayat *et al.*, 2016). For that reason, higher dosage of folic acid supplementation might be needed for countries which have no effective folate fortified foods particularly, sub Saharan Africa, especially Ethiopia. Because, citizens live in SSA are suffered by massive vitamin and mineral deficiencies (Addo *et al.*, 2010; FMOH, 2011; Chitayat *et al.*, 2016).

Furthermore, supplementation of folic acid for the privation of NTD in the developing world is very lower as compared to the developed world which has legislated policy for folic acid fortification. Peri-conceptual folic acid usage in Nigeria and India was found to be 2.5% and 4.75% respectively (Lawal and Adeleye, 2014; Saxena *et al.*, 2016). And, in Ethiopia one study showed lower usage of folic acid supplement (1.92%) during NTD protective time (Dessie *et al.*, 2017). On the contrary, survey in Turkey reported folic acid intake by 48.6% first trimester pregnant women and another Italian multicenter survey reported 23.5 % preconception folic acid use (Köken *et al.*, 2013; Nilsen *et al.*, 2016).

This differences may be as a result of women in the developing world has lower awareness and knowledge about folate and its supplementation as compared with women lives in Europe. Earlier systemic review showed lowered peri-conceptual folic acid intake in women with a low level of formal education, immigrant status, young maternal age, lack of a partner and an unplanned pregnancy. The review also reported the positive effect of mass media campaigns on peri-conceptual folic acid use by a factor of 1.7 to 7.2 (Ray *et al.*, 2004). This review is in consistent with other studies (Lawal and Adeleye, 2014; Saxena *et al.*, 2016; Kim *et al.*, 2017). In addition, peri-conceptual folic acid intake was improved in different parts of the world after public health campaign about folic acid (Rofail *et al.*, 2012).

Besides, non compliance to take folic acid tablets due to fear of side effects was also reported as a reason for lower usage of folate supplementation (Birhanu *et al.*, 2018). Generally, women of the developing world are highly susceptible for folate related NTD than the developed world.

1.3 Statement of the problem

Folate deficiency is the second cause of anemia during pregnancy, following iron deficiency, due to increased requirement and dietary restriction. But the global magnitude of the problem is unavailable due to under representation of pregnant women in study surveys (McLean *et al.*, 2008; Gernand *et al.*, 2016). The burden of folate deficiency in different countries is fluctuating. However it is a public health problem of low income and middle income countries (Gernand *et al.*, 2016). The general prevalence of folate deficiency in Canada as well as America is less than 1% (Colapinto *et al.*, 2011; Gernand *et al.*, 2016). In addition, the prevalence of folate deficiency during first trimester pregnancy in the developed countries is decreased from 55%-1% after the era of folic acid fortification of flour (Brito *et al.*, 2015; Gernand *et al.*, 2016).

On the other hand, in Côte d'Ivoire (west Africa), the prevalence of folate deficiency (< 4 ng/mL) among women of reproductive age was 86% (Rohner *et al.*, 2014) and in neighboring eastern Sudan, Serum folate (< 6.6 ng/ml) deficiency was reported in 57.7% of pregnant women (Abdelrahim *et al.*, 2009). A cross-sectional study in women of reproductive age in nine administrative regions of Ethiopia reported 46% severe folate deficiency (≤ 4 ng/mL) and 21.2% ($> 4-6.6$ ng/mL) marginal folate deficiency. In addition, Ethiopian public health institute national survey on micronutrient deficiencies showed RBC-folate deficiencies (< 140 ng/L) on 32% and 27% of non pregnant reproductive age women of Ethiopia in general and Addis Ababa in particular respectively (Haidar and Melaku, 2010; Zerfu *et al.*, 2016).

Folate deficiency in early pregnancy is associated with increased risks for neural tube defects in the developing fetus, intrauterine growth retardation, preeclampsia and early miscarriage (Tamura and Picciano, 2006; Sande *et al.*, 2013). In addition, inappropriate treatment of B12 deficiency with folate can cause irreversible nerve degeneration. Therefore, measuring the level of both vitamins and supplementing accordingly is very important for the prevention of NTD and other congenital abnormalities (WHO, 2015c).

Various contributory factors including maternal nutrition and physiological changes during pregnancy lead to folate deficiency. folate fortified foods are not available in food stuffs of Addis Ababa (FMOH, 2011). And the burden of NTD cases in Ethiopia is significantly higher compared to other SSA countries (Deribe *et al.*, 2012). Furthermore, NTD cases were covered 30.8% of birth defects and increased in Addis Ababa and Amhara Region from 2010 – 2014 (Taye *et al.*, 2016). Due to these reasons the city's population is at higher risk of folate

deficiency that can lead to an increased occurrence of NTD. Unfortunately, no systematic study has been undertaken so far in Addis Ababa to understand whether folate deficiency is a public health problem of first trimester pregnant women to cause neural tube defect to the developing fetus.

1.4 Significance of the study

Prevention as well as treatment of folate deficiency in pregnant women is the most important aspect of antenatal care in order to prevent negative pregnancy outcomes such as neural tube defect and intrauterine growth retardation. Evaluating the magnitude of folate deficiency as well as the predisposing factors that leads to this problem is helpful in the improvement of prevention and treatment strategies.

Therefore, this study is expected to help the health practitioners to predict this vitamin deficiency during first trimester pregnancy and treat accordingly. This study also addresses the policy makers and stake holders to revise guide lines that can improve folate status in first trimester pregnant women of Addis Ababa. It is hoped that the data obtained in this preliminary investigation of pregnant women from some health centers of Addis Ababa, will help the future researchers to continue this study and analysis so that neural tube defect in the developing fetus in Ethiopia will be reduced in the future.

2 OBJECTIVE OF THE STUDY

2.1 General objective

The main objective of this study was to assess erythrocyte folate level, and other determinant factors of neural tube defect among first trimester pregnant women attending ante natal care units at Lideta and T/Haimanot health centers, Addis Ababa, Ethiopia.

2.2 Specific objectives

- To estimate the prevalence of folate deficiency among first trimester pregnant women.
- To evaluate the associated factors of folate deficiency during first trimester pregnancy
- To evaluate the magnitude of folic acid supplementation in first trimester pregnant women.
- To assess the awareness of pregnant women about the timing of folic acid supplementation.

3 MATERIALS AND METHODS

3.1 Study area and period

This study was conducted from August 8 to January 4 at T/haimanote and Lideta health centres, which are found in Lideta sub city, Addis Ababa, Ethiopia. T/Haimanot health centre is located 500m north of Tikur Anbesa Specialised Hospital and Lideta health centre is located behind saint Lideta church. According to the antenatal care registry books of the corresponding HCs, 270 and 500 first trimester pregnant women visited ANC units of T/haimanot and Lideta health centres per year respectively. Both health centres give antenatal care, prevention of mother to child transmission, delivery and referral service for pregnant women in their maternal health department.

3.2 Study design

Institutional based cross-sectional study design was used.

3.3 Population

All pregnant women who were visiting T/haimanot health center and Lideta health center were the source population for the study. The study population for this study was all first trimester pregnant women who were attending ANC units of T/haimanot and Lideta health centers during the study period and meet the eligibility criteria.

3.4 Eligibility criteria

3.4.1 Inclusion criteria

All first trimester pregnant women who were attending antenatal care in the health centres during the study period and voluntary to participate in the study were included.

3.4.2 Exclusion criteria

Conditions that can increase or decrease folate level abnormally such as:

- Women on anti-diabetic, anticancer, anti-tuberculosis, anticonvulsants and anti-malaria drugs
- Women diagnosed with cancer (card review)
- Previous iliac resection surgery
- Any major bleeding and blood transfusion in the previous three months.

3.5 Sample size determination

Single population proportion formula with 95% confidence interval was used to determine the sample size of the study. Previously, a systemic review of data from 2005 to 2015 on micronutrient status of reproductive age and pregnant women in Ethiopia, Kenya, Nigeria and South Africa showed 3–12% folate deficiency during pregnancy (Harika *et al.*, 2017). Based on this the sample size can be calculated as follows;

$$n = \frac{\left(z_{1-\alpha/2}\right)^2 p(1-p)}{d^2}$$

Where n is minimum sample size required; $Z_{1-\alpha/2}$ is the standard normal variable at $(1-\alpha)$ % confidence level and α (level of significance). Usually 95% confidence level is used = 1.96; P is estimate of the prevalence rate of folate (12%) deficiency during pregnancy in the population; d is the margin of sampling error tolerated, assume to be 0.05.

$$n = \frac{1.96^2 \times 0.12 \times 0.88}{0.05^2} \approx 162$$

Therefore, the final estimate sample size of the study was 162.

3.6 Sampling procedure

Five health centers are found in Lideta sub city. Out these, we selected two health centers using random sampling method. The number of study units for each health center was proportionally allocated based on the number of first trimester pregnant women coming per year. Those who were part of the final sample size were selected purposively based on case load till we got 105 and 57 participants in Lideta and T/haimanote HCs respectively. Out of these, 2 participants were excluded from the study due to ineligible blood sample.

Table 1: Sampling techniques used to select study subjects from from lideta and T/haimanot HCs Addis Ababa, Ethiopia.

S.no	Health centres	No. Of first trimester pregnant women visited per year	Proportionally allocated sample
1	Lideta HC	500	105
2	T/ haimanote HC	270	57

3.7 Study variables

3.7.1 Independent variables

- Socio-demographic characteristics; age, marital status, educational level, occupation and economic status
- Gravidity
- Parity
- Number of children
- Birth spacing
- Dietary habit

3.7.2 Dependent variables

- RBC folate concentration
- Mean corpuscular volume.

3.8 Data and Blood sample collection procedures

Socio-demographic characteristics and other data related to pregnancy and life style was collected from the participants via face to face interview using Amharic version structured questionnaire by experienced midwifery professionals. And about 5 ml of fasting venous was collected from 162 study participants, with 160 usable blood samples for RBC folate analysis, by skilled laboratory technologists in both health centres. The blood was collected in EDTA tube. Hemoglobin and MCV were measured before processing it. Aliquots of whole blood were frozen stored at – 80°C until analysis. Safety precautions were taken while handling blood and disposing it.

3.9 Test principles of the laboratory analytes

3.9.1. Measurement of RBC folate concentration

There are several methods to assay and calculate folate RBC concentration. Some of these methods are used in medical field, and some others in pharmacological studies/investigations (Karmi *et al.*, 2011). In this study, RBC folate was measured by electrochemiluminescence binding assay method using Elecsys Folate RBC assay analyser which employs a competitive test principle using natural folate binding protein (FBP) specific for folate. Folate in the sample competes with the added folate (labeled with biotin) for the binding sites on FBP (Roche-diagnostics, 2014) .

Whole blood treated with anticoagulants (EDTA) was mixed with ascorbic acid solution and incubated for liberation and stabilization of the intracellular folate. The resulting hemolysate sample was 1st incubated with the folate pretreatment reagents; bound folate was released from endogenous folate binding proteins. Then the pretreated sample was incubated with ruthenium labeled folate binding protein, a folate complex is formed. Afterwards, of streptavidin-coated microparticles and folate labeled with biotin were added to form ruthenium labeled folate binding protein-folate biotin complex. Finally, the reaction mixture was aspirated into the measuring cell where the microparticles were magnetically captured onto the surface of the electrode. Application of a voltage to the electrode then induces chemiluminescent emission which was measured by a photomultiplier (Roche-diagnostics, 2014).

Results were determined via a calibration curve which was specifically generated by the instrument by 2-point calibration and a master curve provided via the reagent barcode. And the analyzer automatically calculated the analyte concentration of each sample in ng/mL from the calibration curve. Then, folate concentration in the erythrocyte fraction of the sample (RBC folate) was calculated using the following equation:

$$\text{RBC folate} = \frac{\text{analyzer result}}{\% \text{ hematocrit}} \times 100$$

Note: Hematocrit was measured during the sample collection from fresh whole blood.

The cut off value for the lower limit of normal folate level is still controversial across the literatures. In this study, the concentration suggesting to achieve the greatest reduction of NTDs is based on the information provided by WHO, by which RBC folate <905 nmol/L (<400 ng/mL) is defined as red blood cell folate threshold that can be used as an indicator of folate insufficiency in women of reproductive age at the population level (WHO, 2015b).

3.9.2. Determination of Hb and MCV

To determine complete blood count Beckman Coulter method was used. The method accurately count and size cells by using measurable charges in electrical resistance produced by non conductive particle suspended in an electrolyte.

A suspension of blood cells passes through a small orifice simultaneously with an electric current. A small opening (aperture) between electrodes is the sensing zone through which suspended particles pass. In the sensing zone, each particle displaces its volume of electrolyte. Beckman Coulter measures the displaced volume as a voltage pulse, the height of each pulse

being proportional to the volume of the particle. The quantity of suspension drawn through the aperture is for an exact reproducible volume. This method is independent of particle shape, color, and density. MCV was calculated directly from RBC histogram (Sarma, 1990).

Twenty five μ l of blood was added to 5.0 mL reagent (potassium ferricyanide and potassium cyanide), mixed and left for 3 minutes. The hemoglobin was freed by the lysis of RBCs and combined with potassium cyanide which then formed a cyanmethemoglobin compound. The absorbance was measured at 550nm wavelength in a photoelectric calorimeter against a standard solution (Elwood and Jacobs, 1966).

3.10 Data processing and analysis

Simple descriptive statistics such as; mean, standard deviation, median, frequency, percentiles and percentage were used to present socio-demographic characteristics and magnitude of RBC folate level and MCV. Logistic regression and Chi-square test were used to analyze associated factors of folate deficiency and awareness about folates respectively. The data were entered by using Epi-Data statistical software version 3.1 and then exported to SPSS software version 22.0 for analysis. And a p-value of <0.05 was considered to be statistically significant in all the analyses.

3.11 Data quality control and management

- ✓ The socio-demographic characteristics and related data were collected by nurse professionals.
- ✓ All the laboratory procedures were handled by laboratory technologists.
- ✓ Tools that were used to measure and analyze the test are standardize and automated.
- ✓ Data coding, entering, verifying, and cleaning were performed with a great care.

3.12 Ethical approval

Before starting data collection and preliminary study, ethical clearance letter with reference number SOM/BCHM/146/2009 was obtained from the Departmental Research and Ethics Review Committee, Department of Biochemistry, College of Health Science, Addis Ababa University. And we got collaboration letters in both health centres. The purpose of the study was briefly explained for the study participants and they were informed that their responses will be treated with strict confidentiality. Samples and data had been collected after the study participants gave full consent. Confidentiality, anonymity, neutrality, accountability and academic honesty were maintained throughout the study.

3.13 Operational (working) definitions

- Conversion factor: $\text{nmol/L} \times 0.44 = \text{ng/mL}$, $\text{ng/mL} \times 2.27 = \text{nmol/L}$
- First trimester pregnancy: Time period extending from the **first** day of the last menstrual period through 12 weeks of gestation
- Folate insufficiency for NTD prevention at the population level: RBC folate level $< 400\text{ng/mL}$ or $< 906\text{ng/mL}$
- Gravida: The total number of confirmed pregnancies that a woman has had, regardless of the outcome
- Parity: The number of times that a woman has given birth, alive or dead.
- Regular dietary intake: continues consumption of diet type on daily, weekly or monthly basis.
- RBC folate deficiency: Folate level $< 151\text{ng/mL}$ or $< 340\text{nmol/L}$
- Serum/plasma folate deficiency: Folate level $< 4\text{ng/mL}$ or $< 10\text{nmol/L}$.

4 RESULTS

4.1 Socio demographic characteristics

A total of 162 first trimester pregnant women attending ANC in Lideta and T/haimanot health centers were participated in the present study. From which the blood sample of two participants were not usable and excluded. The mean \pm SD participants' age was 26.5 ± 4 years with a minimum age of 17 years and a maximum age of 42 years. Of which, 79 (49.4%) were between 25-39 years. Among all the participants 149 (93.1 %) were married, 125 (79.1%) were literate and 82 (51.2%) were occupied. Monthly income of 55 (35.7%) participants was between 2,501 to 4,999 birr (Table-2).

Table 2: Socio-demographic characteristics of first trimester pregnant women attending ANC at selected health centers of Addis Ababa, Ethiopia, 2017/18

Variables		Frequency	percent
Age	≤ 24	45	28.1
	25-29	79	49.4
	≥ 30	36	22.5
Marital status	Married	149	93.1
	Not married	11	6.9
Education	Illiterate	35	21.9
	Literate	125	79.1
Occupation	Employed	82	51.2
	Not employed	78	48.8
Monthly income	≤ 2500 ETB	54	35.1
	2501- 4999 ETB	55	35.7
	≥ 5000 ETB	45	29.2

ETB: Ethiopian birr

4.2 Obstetric history

Our study shows as 72/160(45%) participants were primi gravida and the number of pregnancies ranges from 1 to 4. From a total of 83 participants, who had given birth before, 49(59 %) of them wait three or less years between pregnancies (Fig-4).

Table 3: Obstetric history of first trimester pregnant women attending at selected health centers of Addis Ababa, Ethiopia, 2017/18

Variables		Frequency	Percent
Gravida	primi	74	44.6
	other	92	55.4
	Total	166	100.0
Pregnancy interval	≤3years	49	59.0
	>3years	34	41.0
	Total	83	100.0
Number of children	≤1	141	84.9
	≥2	25	15.1
	Total	166	100.0
History of previous neural tube defect	Yes	2	2.3
	No	86	97.7
	Total	88	100.0

4.3 Folic acid usage and level of awareness for NTD prevention

Our study shows as only 13/160 (8.1%) participants had information about folic acid; of which only four/one hundred sixty (2.5 %) of them know at least one dietary source of folate. During the time of data collection only two/one hundred sixty (1.3 %) participants were taking folate supplementation but five/one hundred sixty (3.1%) participants correctly know when to take supplementary folic acid for NTD prevention (Table -3).

Table 4: Folic acid usage and level of awareness for NTD prevention during first trimester pregnancy, Addis Ababa, Ethiopia, 2017/18

Variables		Frequency	Percent
Heard of folic acid	Yes	13	8.1
	No	147	91.9
Source of folic acid	Know	4	2.5
	Don't know	156	97.5
folic acid supplement usage	Yes	2	1.3
	No	158	98.7
Supplementation timing	Correctly-know	5	3.1
	Partially-know	8	5.0
	Don't know	147	91.9

Partially-know indicate respondents who answer as folic acid should be taken either before pregnancy or in the early pregnancy.

4.4 Dietary intake history

A total of 160 participants were asked about their consumption habits on some types of foods; the types of foods asked and the magnitude of participants who reported as they consumed them regularly were fruit-104 (65%), green leafy vegetable-110 (68.8%), grain-160 (100%), milk-94 (58.8%), meat-89 (55.6%) and egg-62(38.7%)(Table-4).

Table 5: Dietary intake history of first trimester pregnant women in first trimester pregnancy, Addis Ababa, Ethiopia, 2017/18

Dietary intake		Variables					
		Fruit	Green leafy vegetable	Grain	Milk	Meat	Egg
		N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Yes	Daily	7(6.7)	12(10.9)	94(58.7)	22(23.4)	4(5.6)	--
	Weekly	92(88.5)	93(84.5)	66(41.3)	71(75.5)	58(81.7)	57(91.9)
	Monthly	5(4.8)	5(4.5)	--	1(1.1)	9(12.7)	5(8.1)
	Total	104(65)	110(68.8)	160(100)	94(58.8)	89(55.6)	62(38.7)
No		56(35)	50(31.2)	--	66(41.2)	71(44.4)	98(61.3)

4.5 Prevalence of micronutrient deficiency

Among 160 participants for whom RBC-folate concentration were measured, 44(27.5%) had folate insufficiency for NTD prevention (<400ng/mL). But only two /eighty two (2.4%) participants had MCV level >100 fL (Table-5).

Table 6: Prevalence of micronutrient deficiency during first trimester pregnancy, Addis Ababa, Ethiopia, 2017/18

	Total no.	Mean± sd	Median	25 th percentile	Deficiency (abnormality) (No/%)
RBC folate	160	--	585	369.6	44(27.5)
MCV	82	91.4±4.9	--	--	2(2.4)

Note: MCV was measured for only 82 participants due to the unavailability of functional hematologic analyzer.

4.6 Association between awareness about folate with formal education

The relationship between formal education and information about folate was statically significant (p value = 0.043) by chi-square test (Fig-5).

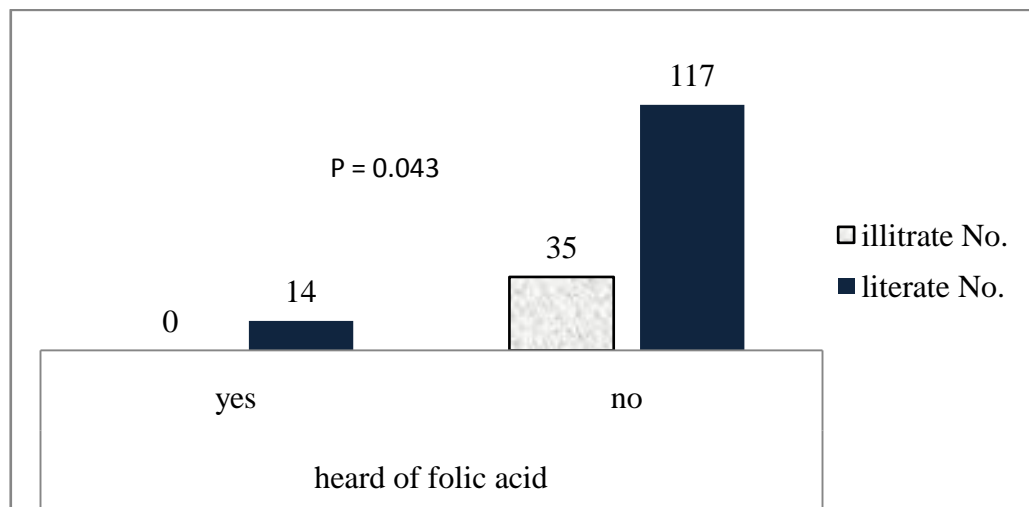


Figure 4: Association between formal education and awerness about folate during first trimester pregnancy, Addis Ababa, Ethiopia. 2017/18

4.7 Maternal characteristics associated with folate deficiency

Bivariate logistic regression shows that, participants who got monthly income less than 2,500ETB has the possibility of having RBC folate level < 400ng/L by a factor of 4.03 compared to those who got monthly income more than 5,000ETB (OR 4.03, CI 1.53-10.6) (Table-6).

Table 7: Factors associated with folate insufficiency during first trimester pregnancy, Addis Ababa, Ethiopia, 2017/18

Characteristic	RBC-folate ≥400ng/mL N (%)	RBC-folate <400ng/mL N (%)	Crude OR (95% CI.)	Adjusted OR (95% CI.)
Age				
≤ 24	34 (29.3)	11 (25)	.84 (.31-2.28)	--
25-29	56 (48.3)	23 (52.3)	1.07 (.45-2.56)	--
≥ 30*	26 (22.4)	10 (22.7)	1	--
Education				
Illiterate	25 (21.6)	10 (22.7)	1.07 (.47-2.46)	--
Literate*	91 (78.4)	34 (77.3)	1	--
Occupation				
Employed	59 (50.9)	23 (52.3)	1.06 (.53-1.12)	--
Not employed*	87 (49.1)	21 (47.7)	1	--
Marital				
Married	108 (93.1)	40 (90.9)	.74 (.21-2.6)	--
Single*	8 (6.9)	4 (9.1)	1	--
Income in ETB				
< 2500	31 (27.9)	23 (53.5)	4.03 (1.53-10.6) [¶]	2.39 (.79-7.28)
2501-4999	42 (37.8)	13 (30.2)	1.68 (.61-4.65)	1.41 (.49-4.09)
5000*	38 (34.2)	7 (16.3)	1	--
Gyny-obes Gravidea				
Primi	53 (45.7)	19 (43.2)	.90 (.45-1.82)	--
Other*	63 (54.3)	25 (56.8)	1	--
Parity				
Once or no	101 (87.1)	37 (84.1)	1.27 (.48 – 3.4)	--
Multipara*	15 (12.9)	7 (15.9)	1	--
No. of children				
≤ 1	100 (86.2)	36 (81.8)	.72 (.28-1.82)	--
≥ 2*	16 (13.8)	8 (18.2)	1	--
Pregnancy interval				
≤ 3 yrs	31 (54.4)	17 (77.3)	2.85 (.93-8.79)	--
>3 yrs*	28 (45.5)	5 (22.7)	1	--

Note: * Reference, ¶: Statistically significant (p < 0.05), OR: Odds Ratio, CI: Confidence Interval, ETB: Ethiopian Birr, yrs: years

4.8 Nutritional habits associated with folate deficiency

Bivariate analysis shows, participants who consumed fruit (OR: 0.44, CI: 0.21-0.89), vegetable (OR: 0.38, CI: 0.18-0.93) and milk (OR: 0.47, CI: 0.23-0.96) were more protected from folate deficiency than who do not consume them frequently. But, by the multivariate analysis, when gravid, income, fruit, vegetable and milk intake are included in the model, vegetable intake (adjusted OR: 0.41, CI: 0.18-0.93) was the only factor associated with folate deficiency (Table-7).

Table 8; Nutritional habits associated with folate deficiency during first trimester pregnancy, Addis Ababa, Ethiopia 2017/18

Dietary intake		RBC-folate ≥400ng/mL N (%)	RBC-folate <400ng/mL N (%)	Crude	Adjusted
				OR (95% CI)	OR (95% CI)
Fruit	Yes	83 (71.6)	23 (52.3)	.44 (.21- .89) [¶]	.60 (.28-1.33)
	No*	33 (28.4)	21 (47.7)	1	1
Meat	Yes	52 (44.8)	19 (43.2)	1.43 (.71 - 2.29)	
	No*	64 (55.2)	25 (56.8)	1	--
Vegetable	Yes	86 (74.1)	23 (52.3)	.38(.19 - .79) [¶]	.41 (.18-.93) [¶]
	No*	30 (25.9)	21 (47.7)	1	1
Milk	Yes	74 (63.8)	20 (45.5)	.47(.23-.98) [¶]	.49 (.22-1.07)
	No*	42 (36.2)	24 (54.5)	1	1

*: Reference, OR: Odds Ratio, CI: Confidence Interval, ¶: statistically significant (p < 0.05)

5. DISCUSSION

Folates are the most important micronutrients for growth and development due to their metabolic role in DNA synthesis and methylation. Folate deficiency during pregnancy is linked with a lot of birth problems, including congenital anomalies, mainly NTD (Anne *et al.*, 2008; Greenberg *et al.*, 2011; Chen *et al.*, 2017). Therefore, evaluating the prevalence of folate deficiency as well as the predisposing factors that leads to this problem during first trimester pregnancy is helpful in the improvement of prevention and treatment strategies.

In this study, 44/160 (27.5%) participants were found to have RBC folate level (<400ng/L) insufficient for the prevention of neural tube defect. This finding was comparable with other finding reported by EPHI (Zerfu *et al.*, 2016) in which 25.3% and 32% folate deficiency (RBC folate <151ng/mL) in reproductive age women of Addis Ababa in particular and Ethiopia in general were reported respectively. Even if the prevalence of the deficiency in our study was comparable with EPHI report, we used the recently recommended cut off value for NTD prevention (RBC folate <400ng/mL) (WHO, 2015b). But, EPHI reported by using RBC folate <151ng/mL as a cut off value, which was previously recommended based on metabolic indicators, that can't indicate the risk of NTD on the general population. Therefore, our finding would be much lower if we were used the cut off value that they were used. Even, with this technical difference, our findings shows lower prevalence of folate insufficiency as compared with the national prevalence they were reported.

Haidar and Melaku (2010) also found 22% and 46% folate deficiency (serum folate \leq 4 ng/mL) in reproductive age women of Addis Ababa and the overall nine administrative regions of Ethiopia respectively. Our study found higher percentage of folate insufficiency compared with the prevalence of that study found in Addis Ababa. But, our finding was lower when compared with the national folate deficiency reported by the above study. However, the prevalence reported by that study was based on serum folate threshold, which is not recommended for prevention of NTDs in women of reproductive age at the population level (WHO, 2015b). The lower folate deficiency (the higher folate level) of women living in Addis Ababa compared to other parts of Ethiopia might be due to easy access of food and health facilities. Since Gibson *et al.* (2008) reported additional source of folate in foods as a reasons for lower prevalence (2%) of folate deficiency (plasma folate < 3 ng/mL) in late pregnancy Sidama women.

Our findings also validates lower folate status in first trimester pregnant women, in comparison to a study conducted in Belgium by Vandevijvere *et al.* (2012) , in which 39% of first trimester pregnant women had RBC folate status less than 400 ng/mL. In addition, Rohner *et al.* (2014) found plasma folate deficiency (< 4ng/mL) in 86% reproductive age women of Côte d'Ivoire (West Africa) and Abdelrahim *et al.* (2009) found serum folate deficiency (< 6.8ng/mL) in 57.7% pregnant women of Eastern Sudan. The lower folate deficiency in our study compared with studies in Belgium, Cote d'Ivoire and Eastern Sudan might be due to the habit of fermented food consumption in Ethiopia since folates are produced through fermentation (LeBlanc *et al.*, 2011; Saubade *et al.*, 2017).

On the contrary, the prevalence of folate deficiency in the first trimester pregnancy in the developed countries is decreased from 55%-1% after the era of folic acid fortification of flour (Brito *et al.*, 2015; Gernand *et al.*, 2016). In addition, in Turkey Karabulut *et al* (2011) found only 0.5% folate deficiency (serum folate <3ng/mL) in first trimester pregnant women. Those findings showed lower prevalence when compared to our study. Thus, the higher prevalence in our study might be due to the unavailability of effective folic acid fortified foods and fortification policy (FMOH, 2011) since Wals *et al.* (2007) showed reduced occurrence of NTD by 46% is in Canada after the year of folic acid fortification, and the prevalence of folate deficiency in USA was more than 50% before the era of folic acid fortification (Gernand *et al.*, 2016). In general, this study defines and establishes that folate deficiency is still a continued public health problem in first trimester pregnant women of Addis Ababa.

Our study also shows two/eighty two (2.4%) participants with macrocytosis (megaloblastic anaemia) (MCV > 100 fr). The prevalence of megaloblastic anaemia was lower compared to the prevalence (27%) of folate insufficiency we found and from those two participants who had megaloblastic anaemia only one had insufficient folate level. This indicates lower functional folate deficiency since folate deficiency is the main cause of megaloblastic anaemia (Ball and Giles, 1964; Castellanos-Sinco *et al.*, 2015). The reasons for this discrepancy could be the cut off value we use to define folate insufficiency, which was not based on metabolic indicators, and the small (82) sample size we used to analyze the prevalence of MCV.

Furthermore, our study shows as only 13/160 (8.1%) of women were heard about folates before the time of data collection and from those only four (2.5%) of them knew at least one food sources of folate that can be supplemented for folate insufficiency in diet. On the contrary, Saxena *et al.* (2016) and Kim *et al.* (2017) showed as 75% and 65.6% of Indian

women and low income Korean women had information about folates respectively. Thus, the prevalence of women who had information about folates in this study was very low compared to those studies. The lesser awareness in this study can be due to limited public health campaign about folic acid and inferior formal education since Rofail *et al.* (2012) showed, improved awareness about folic acid from 6 to 41% following public health campaign. We also found a significant association between formal education and awareness about folic acid/folates, all women who reported as they heard about folates had formal education ($p=0.046$). In line with this finding, the association of formal education and awareness about folates were reported elsewhere (Ray *et al.*, 2004; Lawal and Adeleye, 2014; Saxena *et al.*, 2016).

Folic acid supplementation, at least one month before pregnancy and the entire first trimester, is recommended to prevent congenital anomalies, especially neural tube defect (WHO, 2015a). But, in this study, only five/one hundred sixty (3.1%) of women exactly know the timing of folic acid supplementation and only two/one hundred sixty (1.3%) women were supplemented with folic acid at their first trimester. This finding is in line with a study done by Dessie *et al.*(2017) in Adama, Ethiopia. The study reported as only 1.92% pregnant women were users of folic acid supplement for NTD prevention. This finding is also agreed with the findings in other developing countries, such as findings by Lawal and Adeleye (2014) in Nigeria and Saxena *et al.*(2016) in India.

Conversely, this finding is very low compared with the higher prevalence of peri-conceptual folic acid intake in most parts of the developed countries. Nilsen *et al.* (2016) and Köken *et al.*(2013) found as 23.5% women in Italy and 48.6% women in Turkey were taking folic acid supplement for NTD prevention respectively. The lower prevalence of folic acid supplement usage at the recommended time in this study can be due to lack of information about the importance and timing of folic acid supplementation, and fear of side effects. Since Ray *et al.* (2004) and Rofail *et al.*(2012) showed an improved folate intake after public health compagn in different countries, and (Birhanu *et al.*, 2018) found non-compliance to take folic acid tablets due to fear of some side effects.

Assured that low folic acid level is an evident environmental risk factor of NTD and it can be prevented by folate supplementation (Antony, 2007; Safi *et al.*, 2012). The high prevalence of folate insufficiency and lower prevalence of folic acid supplementation at the recommended time could be the reasons for high burden of NTD in Ethiopia. Hence, Deribe *et al.* (2013) found a significantly higher occurrence of NTD cases in Ethiopia with compared to other SSA

countries and Taye *et al.* (2016) found as NTD covered 30.8% of birth defects that was increased from 2010- 2014 in Addis Ababa and Amhara regions of Ethiopia.

In the present study logistic regression was done to identify the associated risk factors of folate deficiency. Other studies identified characteristics such as multipara, youngster, unmarried, illiterate, low income and malnourished as the main risk factors of lower RBC/serum folate level (Haidar and Melaku, 2010; Vandevijvere *et al.*, 2012; Shi *et al.*, 2014). In contrast to other studies, we didn't identify parity, age, marital status and educational status as main determinants of RBC folate deficiency/insufficiency. However, we identified income less than 2500 ETB compared to more than 5000 ETB (OR 3.07, CI 1.02-9.28) as significant risk factors of folate deficiency by the bivariate analysis. Similar to this finding, Vandevijvere *et al.* (2012) and Shi *et al.* (2014) also found low income as a main determinant factor of folate deficiency.

The possible reason for this finding could be the consequence of low income, which leads to malnutrition, because women with low income may face challenges to get enough and balanced nutrition since malnutrition is an already identified evident risk factor of folate deficiency (Allen, 2008 , Ni *et al.*, 2016). Therefore, one of the reasons leading to the higher odds of folate deficiency could be dietary habits such as low intake of fresh foods and raw vegetables by those pregnant women as a result of low income. The insignificant effect of other variables on folate deficiency in our study can be due to the small sample size we have examined.

We also examined the effect of frequent nutritional intake on folate deficiency using logistic regression. Bivariate analysis shows as folate deficiency was significantly lower in pregnant women who regularly take fruit (OR: 0.44, CI: 0.21-0.89), vegetable (OR: 0.38, CI: 0.71-2.29) and milk (OR: 0.47, CI: 0.23-.96). But, by multivariate regression only frequent vegetable intake was the determinant factor of folate deficiency (adjusted OR: 0.41, CI: 0.18-0.93). Similar to our finding, Soofi *et al.* (2017) also reported dietary habits such as lower intake of green leafy vegetables as the main determinant factor of folate deficiency. In addition, Haidar and Melaku, (2010) found an association between folate deficiency and lower intake of plant product-containing diet (Haidar and Melaku, 2010).

These findings can be explained by the fact that, folates are found in green leafy vegetables, citrus fruits, liver of cattle and fermented dairy products (Delchier *et al.*, 2016). Therefore, frequent intake of vegetables may lower the likelihood of folate deficiency among pregnant women and frequent consumption of milk also have the possibility to lower risk of folate

deficiency since folates are produced by bacterial fermentation. Milk and its products are some of the fermented foods frequently taken by Ethiopians. But we did not find a significant association between frequent meat consumption with folate deficiency. This might be due to the type of meat consumed by the respondents since organ meat is the best source of folate rather than other meat types. In addition, fruit and milk intake were not the main determinant factor for folate deficiency by the multivariate analysis (table-6). This could be due to the quantity and type of fruit consumption (Delchier *et al.*, 2016) and the small sample size we studied.

6. CONCLUSION

This study establishes that 27.5% of the first trimester pregnant women had a folate level <400ng/mL, which is insufficient to prevent NTD. But, megaloblastic anaemia was not evidently high. The percentage of women who had information about folic acid and food sources of folate was generally small. Lower information was associated with inferior formal education. The prevalence of folic acid intake among first trimester pregnant women was very low. Women who got monthly income less than 2500 ETB compared with more than 5000 ETB were more prone to folate deficiency. This study identifies that women who regularly consume fruit, green leafy vegetables and milk were more protected from folate deficiency than women who didn't consume them and vegetable consumption was the main factor for the prevention of folate deficiency.

Therefore, this study validates as first trimester pregnant women in Lideta sub city are at risk of folate insufficiency that can lead to NTD on the developing fetus.

7. STRENGTH AND LIMITATION OF THE STUDY

As strength, the target population of this study was centered on first trimester pregnant women that allowed us to investigate the right risk population on the occurrence of NTD. We also used the recently recommended cut of value (RBC folate <400ng/L) and biomarker (RBC folate) to identify the risk of NTD on the general population.

The limitations of this study were:

- ✓ It didn't include other clinical biomarkers that are important to investigate risk of NTD such as vitamin B₁₂ and homocysteine level due to the unavailability of resources including reagents.
- ✓ There also could have been recall bias in the face to face interview as in other cross-sectional studies.
- ✓ The quality and quantity of food consumption habit was not taken under consideration.
- ✓ The study design was also another limitation of our study, which didn't allow us to follow and see our participants and their post-natal baby.

8. RECOMMENDATION

Based on our results we suggest the following recommendations

- Health professionals should inform their clients about the source of folates and the right timing of supplementation.
- The policy makers shall implement folic acid fortification policy and strengthen media and public campaign about folate its food sources and timing of folic acid supplementation.
- We also believe as it is important if researchers perform studies longitudinally to identify the extent of the effect of folate and other micronutrient deficiencies on neural tube defect.

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Annex 1: Subject information sheet (English version)

Principal Investigator: **Alemu Adela**

College of health science

Department of medical biochemistry

Dear participant! Here, I the undersigned, at Addis Ababa University College of Health Science, Department of medical biochemistry. Graduate Study Program, currently I will be undertaking research on a topic entitled as Assessment of RBC-folate level and other determinant factors of neural tube defect among first trimester pregnant women who attends antenatal care in Lideta and T/haimanot health centers, Addis Ababa, Ethiopia. For this study, you will be selected as a participant and before getting your consent, you need to know all necessary information related to the study which will be detailed as follows.

Introduction

Privacy is the state of being free from intrusion, and in the context of health care it concerns the responsibility of a care provider to protect a clients from any disclosure (i.e., discovery by others), even unintentional, of personal health data, by providing security to the patient and the patient's records. Confidentiality, in contrast, is the limiting of information to only those for whom it is appropriate. Therefore this information sheet briefly provides the necessary guide to be considered during the study.

Objective the main aim of this study is to assess RBC-folate level and other determinant factors of neural tube defect among first trimester pregnant women who attends antenatal care in Lideta and T/haimanot health centers, Addis Ababa, Ethiopia

Participants to be included: all pregnant women sampled by purposive sampling will be included in the study

Risks and discomfort: Participant in this project will not cause more discomfort and no need of extra sample other than sample taken for diagnostic purpose. But, there could be minor pain and challenge in color of your skin following the blood drawing. The amount of blood taken from each volunteer throughout the study period is 05ml which will not affect your health. There is no major risk in participating in this research, as the whole procedure is carried out by physician and /or health professionals following the standard good clinical practice.

Benefits:-There is no immediate benefit in participating in this study. However you will have the chance to know your RBC-folate level and hematologic characteristics from the laboratory result. And if your result reveals any incidental health problems that need immediate treatment, you will be referred to an appropriate health facility. In addition your participation will contribute in improving the health delivery system for pregnant women affected by folate deficiency. Cost for blood collection and laboratory analysis will be covered by the project.

Incentive:-There is no financial or material incentive in participating in this study.

Confidentiality: The information that we will collect from this research project will be kept confidential. Information about you that will be collected from the study will be stored in a file, which will not have your name on it, but a code number assigned to it. Which number belongs to which name will be kept under lock and key, and it will not be revealed to anyone except the principal investigator.

Participant Rights

Your participation is entirely voluntary and up to you to decide. There is no penalty if you do not agree to participate. Also you have the right not to answer any questions you do not want to. You may also withdraw from the study at any time. If in the middle you decide to stop filling questions and no longer participate, you can stop without worry.

Persons to contact:

If you have any question, you can ask at any time. If you have additional questions about the study, you can contact the:

- Principal investigator: Alemu Adela, cell phone-0949 018410, E-mail alemua143@gmail.com

Thank you for your cooperation.

If you are voluntary to participate in the study we kindly request you to provide your response for the questionnaire in the next page.

Annex 2: Consent form (English version)

Department of medical Biochemistry, School of graduate studies, College of Health Sciences, Addis Ababa University, Consent form for the participation of the study participants in the research project

Name of the study participant

Code number.....

I have clearly been informed about the research project that it aims to assess RBC-folate and status and determinant factors neural tube defect in first trimester pregnant women. I have understood that participation in this study is entirely voluntarily. I have been told that my answers to the questions will not be given to anyone else and no reports of this study ever identify me in any way. I have also been informed that my participation or non-participation or my refusal to answer questions will have no effect on me. I understood that participation in this study does not involve risks. I understood that Alemu Adela is the contact person if I have questions about the study or about my rights as a study participant, I will be contacting the principal investigator.

Respondent's signature _____

Interviewer name _____ signature _____ date _____

QUESTIONNAIRE

Part I: socio- demography⁷

Subject code number.....

1. Age (in years).....
2. Ethnicity.....
3. Educational status
 - a. Can't read or write
 - b. Only read and write
 - c. Primary school
 - d. Secondary school
 - e. Higher education(college or university)
4. Marital Status
 - a. Single
 - b. Married
 - c. Divorced
 - d. Widowed
5. Occupation
 - a. House wife
 - b. Civil servant
 - c. Privet worker
 - d. Merchant
 - e. other
6. Monthly income (in Birr)_____

Part II: pregnancy related questions

Know I want to ask you questions related with your pregnancy?

7. Number of pregnancy_____
8. How many children do you have? _____
9. When was the last time you gave birth? Day_____ month_____ year_____
10. History of previous pregnancy complicated with NTDs
 - a. Yes
 - b. No

11. Do you have any health problem or medical condition not related with pregnancy?

a. yes

b. No

If yes specify _____

12. Have you taking any drug?

a. Yes

b. No

If yes specify _____

Part III: questions on dietary diversity.

Now I would like to ask you about liquids or foods that you eaten in **your home or outside home** since yesterday during the day or night, I am interested in whether you had the item I mention, even if it was combined with other foods. For example, if you ate injera with stew made with mixed vegetable, you should reply yes to any food I ask about that was an ingredient in the injera/stew.

13. Bread, rice, pasta, or other foods made from grains, including thick grain based porridge.

For example, oats, maize, barley, wheat, sorghum, millet or other grains?

a. Yes

b. No

If yes how often you consume those grains

a. Every day

b. Weekly

c. Monthly

d. Don't know

14. Any dark green leafy vegetables?

a. Yes

b. No

If yes how often you consume green leafy vegetables

a. Every day

b. Weekly

c. Monthly

d. Don't know

15. Any fruits or vegetables like bananas, apples, green beans, avocados, pawpaw, tomatoes, oranges, pineapples, passion fruit?

- a. Yes
- b. No

If yes, how often do you eat fruits?

- a. Every day
- b. Weekly
- c. Monthly
- d. Don't know

16. Any meat such as beef, lamb, goat, chicken?

- a. Yes
- b. No

If yes, how often do you eat meat?

- a. Every day
- b. Weekly
- c. Monthly
- d. Don't know

17. Eggs?

- a. Yes
- b. No

If yes how often do you eat eggs?

- a. Every day
- b. Weekly
- c. Monthly
- d. Don't know

18. Milk, cheese, yoghurt?

- a. Yes
- b. No

If yes, how often do you consume milk?

- a. Every day
- b. Weekly
- c. Monthly
- d. Don't know

19. Alcoholic beverages or local alcohol Example Tela, Areke, Borde...

- a. Yes
- b. No

If yes how often and how much per week or per month _____

20. Is there anyone who smokes in your home?

- a. Yes
- b. No

21. Do you Smoke?

- a. Yes
- b. No

If yes how often and how much per week? _____

Part four IV: Knowledge assessment questions

Know I would like to ask you about the vitamin folic acid.

22. Have you heard of folic acid?

- a. Yes
- b. No

If yes, which of the following foods contain high levels of folic acid? (Please mark all that apply)

- a. Apple juice
- b. Green, leafy vegetables
- c. Butter
- d. Beef steak
- e. Offal, especially liver
- f. Breakfast cereals (e.g. cornflakes)
- g. I don't know

23. Do you take vitamin supplements?

- a. Yes
- b. No

If yes, how often do you take it?

- a. Daily
- b. Three times a week
- c. Once a week
- d. Other, specify _____

24. In the context of pregnancy, extra folic acid should be taken
- a. Never
 - b. Before getting pregnant
 - c. In early pregnancy
 - d. Before and in the early weeks of a pregnancy
 - e. I don't know

Annex 3: Subject information sheet Amharic version:

የተሳታፊዎች የፈቃደኝነትና መተማመኛ መረጃ መስጫ ቅፅ

በአዲስ አበባ ዩኒቨርሲቲ የጤና ሳይንስ ኮሌጅ የሕክምና ባዮኬሚስትሪ ትምህርት ክፍል:

እኔ አለሙ አደላ በአዲስ አበባ ዩኒቨርሲቲ ጤና ሳይንስ ኮሌጅ የሕክምና ባዮኬሚስትሪ የድህረ ምረቃ ተማሪ ስሆን የመመረቂያ ጽሁፌን በጥቁር አንበሳ ስፔሻላይዝድ ሆስፒታል የእርግዝና ክትትል ክፍል ውስጥ Assessment of RBC-folate level and other determinant factors of neural tube defect among first trimester pregnant women who attends antenatal care in Lideta and T/haimanot health centers, Addis Ababa, Ethiopia በሚል ርዕስ በመስራት ላይ ነኝ። ለዚህ ጥናት ደግሞ እርስዎ የተመረጡ ስለሆነ ከዚህ ቀጥሎ የሚገኘውን መረጃ አንበበዉ በጥናቱ ላይ መስማማትዎን ወይም አለመስማማትዎን እንዲያረጋግጡ በትህትና እጠይቃለሁ።

መግቢያ:- ጥናቱ ከእርሶ የሚወስዳቸዉ ማንኛዉም መረጃወች ሚስጥራዊነት ሙሉ በሙሉ የተጠበቀ ሲሆን እርሶ በጥናቱ አለመሳተፍዎ ሆነ በማንኛዉም ሰአት ተሳትፎዎን ማቀራጠጥ ይችላሉ።

የጥናቱ አላማ:- የጥናቱ ዋና አላማ በተክለ ሐይማኖት እና ልደታ ጤና ጣቤያዎች ለእርግዝና ክትትል የሚመጡ እናቶችን የደም ፎሌት መጠን መለካት እና ሌሎች ከጽንሰ የአዕምሮ እድገት ጋር ተዛማጅነት ያላቸዉ ነገሮችን ማጥናጠት ነዉ። የጥናቱ ዉጤት ለእናቶች እና ህጻናት ጤና እንክብካቤ የሚጠቅም ሲሆን ከዚህም በተጨማሪ እርስዎ የቫታሚን መጠንዎን እንዲአዉቁ እና ተገቢዉን እርምጃ እንዲወስዱ ይረዳዎታል። በጥናቱ ላይ የሚያደርጉት ተሳትፎ ሙሉ በሙሉ በእርሶ በጎ ፈካደኝነት ላይ የተመሰረተ ነዉ።

በጥናቱ ለመሳተፍ ፈቃደኛ ሲሆኑ ለናሙና ይሆን ዘንድ አምስት ሚሊ ሊትር ያህል ደም በሆስፒታሉ የጤና ባለሙያዎች አማካኝነት የሚሰጡ ሲሆን ናሙና በሚሰጡበት ጊዜ ሁልጊዜ ለምርመራ ከሚሰጡት የተለየ ህመም እና አለመመቻት የለዉም።

ከጥናቱ ጋር በተያያዘ ጥያቄ ቢኖርዎ ወይም ችግር ቢያጋጥም በማንኛዉም ሰአት በሞባይል ቁጥር **0949018410** አለሙ ብለዉ ይደዉሉ ወይም በኢሜል አድራሻ alemua143@gmail.com መላክ ይችላሉ።

በጥናቱ ለመሳተፍ ፈቃደኛ ከሆኑ እባክዎ ከዚህ ቀጥሎ ባለዉ የስምምነት ቅጽ ላይ በመፈረም ይተባበሩ።

እናመሰግናለን!!!

Annex 4: Informed consent (Amharic version)

የተሳታፊዎች ስምምነት ማረጋገጫ ቅጽ

የሚሰጥር ቁጥር -----

አኔ ----- የእናቶችን የደም ፎሌት መጠን ለመለካት እና ሌሎች ከጽንሰ የአዕምሮ እድገት ጋር ተዛማጅነት ያላቸው ነገሮችን ለማወቅ ስለሚሰራው ጥናት እና ለጥናቱ በሚኖረኝ ተሳተፎ ዙሪያ በቂ መረጃ ያገኘሁ ሲሆን በጥናቱ ለመሳተፍ ያለኝን ፈቃደኝነት በፊርማዬ አረጋግጣለሁ፡፡

የተሳታፊ ፊርማ ----- ቀን -----

ክፍል ሶስት ፡- አመጋገብን በተመለከተ ተዘጋጀ ቃለ መጠይቅ

አሁን ደግሞ እስከ ትናንት ድረስ ስለተጠቀሙአቸው የሚበሉ ወይም የሚጠጡ ምግቦች ልጠይቅዎ ነው። ከምጠቅሱልዎ የምግብ አይነቶች አንዱንም ቢሆን ከሌላ ምግብ ጋር ወይም ለብቻው ተመግበው ከሆነ አወ ይሉኛል። ለምሳሌ፡ በየአይነት ተመግበው ከሆነ እንጀራው እንዲሁም ወጡ የተዘጋጀባቸውን ሁሉንም የምግብ (ዕህሎች) አይነቶች ስጠራልዎ አዎ ይሉኛል።

13. ፍራፍሬዎች ማለትም፡ ሙዝ፣ አፕል፣ እሽት(የባቄላ)፣አቦካዶ፣ ፓፓያ፣ ቲማቲም፣ ብረቱካን እና የመሳሰሉትን አዘውትረው ይመገባሉ?

ሀ. አወ ለ. የለም

አወ ካሉ፤ ፍራፍሬ እና የፍራፍሬ ውጤቶችን ለምን ያህል ጊዜ ይመገባሉ?

ሀ. በየቀኑ ለ. በሳምንት
መ. በወር ሠ. አላወቅም

14. ቅጠላማ አትክልቶችን አዘውትረው ይመገባሉ?

ሀ. አወ ለ. የለም

አወ ካሉ፤ አትክልት እና የአትክልት ውጤቶችን ስንት ጊዜ ይመገባሉ

ሀ. በየቀኑ ለ. በሳምንት
መ. በወር ሠ. አላወቅም

15. ጥራጥሬ ማለትም፤ ዳቦ፣ ሩዝ፣ ፓስታ፣ ወይም ሌሎች ከጥራጥሬ ለምሳሌ ከማሽላ፣ ከቆሎካገብስ፣ ከስንዴ እና ከሌሎች ጥራጥሬዎች የሚዘጋጁ ምግቦችን ይመገባሉ?

ሀ. አወ ለ. የለም

አወ ካሉ፤ጥራጥሬ እና የጥራጥሬ ውጤቶችን ስንት ጊዜ ይመገባሉ?

ሀ. በየቀኑ ለ. በሳምንት
መ. በወር ሠ. አላወቅም

16. ወተት እና እርጎ አዘውትረው ይጠቀማሉ?

ሀ. አወ ለ. የለም

አወ ካሉ፤ ወተት እና የወተት ተዋጽኦዎችን ስንት ጊዜ ይጠቀማሉ?

ሀ. በየቀኑ ለ. በሳምንት
መ. በወር ሠ. አላወቅም

17. ስጋ፣ የቦሬ፣ የፍየል፣ የበግ፣ የዶሮ፤ ይመገባሉ?

ሀ. አወ ለ. የለም

አወ ካሉ፣ ስጋ እና የስጋ ውጤቶችን ስንት ጊዜ ይመገባሉ?

ሀ. በየቀኑ ለ. በሳምንት
መ. በወር ሠ. አላወቅም

18. እንቁላል አካውትረው ይመጣሉ?

ሀ. አወ ለ. የለም

አወ ካሉ፣ እንቁላል እና የእንቁላል ተዋጽኦ የላቸው ምግቦችን ስንት ጊዜ ይመጣሉ?

ሀ. በየቀኑ ለ. በሳምንት
መ. በወር ሠ. አላውቅም
ሠ. አላውቅም

19. አልኮሆል ይጠጣሉ?

ሀ. አወ ለ. አልጠጣም

ከጠጡ በሳምንት ወይም በወር ስንት ጊዜ እና ምን ያህል ነው _____

20. በትዎ ውስጥ ሲጋራ የሚያጨስ ሰው አለ?

ሀ. አወ ለ. የለም

21. እርስዎ ሲጋራ ያጨሳሉ?

ሀ. አወ ለ. አላጨሰም

ካጨሱ በሳምንት ስንት ጊዜ እና ምን ያህል? _____

ክፍል አራት፡- ፎሊክ አሲድን በተመለከተ፤

22. ፎሊክ አሲድ ሲባል ስምተው ያውቃሉ?

ሀ. አወ ለ. የለም

23. አወ ካሉ፣ ከሚከተሉት ውስጥ ከፍተኛ የፎሊክ አሲድ መጠን ያላቸው ማግቦች የትኞቹ ናቸው?(እባክዎ መልስ የሆኑትን ሁሉ ይምረጡ)

ሀ. የአፕል ጁስ ለ. አረንጉዴ እና ቅጠላማ አትክልቶች
መ. ቅቤ ሠ. የከብት ስጋ
ረ. ኩላሊት፣ ልብ በተለይ ጉበት ሰ. ጥራጥሬ (ለምሳሌ ቆሎ)
ሸ. አላውቅም

24. እየወሰዱት ያለ የፎሊክ አሲድ እንክብል ወይም ተጨማሪ ምግብ አለ?

ሀ. አወ ለ. የለም

አወ ካሉ እንዴት ነው የሚወስዱት?

ሀ. በየቀኑ ለ. በሳምንት ሶስት ጊዜ
መ. በሳምንት አንድ ጊዜ ሠ. አንድአንድ ጊዜ

25. ከእርግዝና ጋር በተያያዘ ተጨማሪ ፎሊክ አሲድ መቸ ነው መወሰድ አለበት

ሀ. መወሰድ የለበትም ለ. ከእርግዝና በፊት
መ. በመጀመሪያዎቹ የእርግዝና ወራት ሠ. ከእርግዝና በፊት እና በመጀመሪያዎቹ የእርግዝና ወራት
ረ. አላውቅም

Annex 5: Declaration

I declare that this research paper entitled Assessment of RBC-folate level and other determinant factors of neural tube defect among first trimester pregnant women who attends antenatal care in Lideta and T/haimanot health centers, Addis Ababa, Ethiopia is my original work and has not been presented for any degree in any other university. All sources of materials used for the research have duly been acknowledged.

Alemu Adela

Signature_____

Date_____