FOLATE DEFICIENCY AMONG PREGNANT WOMEN ATTENDING ANTENATAL CLINIC AT PUMWANI MATERNITY HOSPITAL, NAIROBI COUNTY, KENYA

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Folate deficiency among pregnant women attending antenatal clinic at Pumwani maternity hospital, Nairobi County, Kenya

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

Signature…….. Date……

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This thesis has been submitted for examination with our approval as university supervisors

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DEDICATION

I dedicate this thesis to my beloved husband, Joseph Omolo Saye, My son Mark Ray Saye and my parents Paul Mgamb Orinda and Doris Mgamb. I appreciate their unconditional love and unwavering support all through the period of studies as well as during the period that I was doing this project.

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LIST OF ABBREVIATIONS

ANC	Ante-Natal Care
CDC	Centers for Disease Control and Prevention
CI	Confidence Interval
dTMP	deoxyThymidine Monophosphate
dUMP	deoxyUridine Monophosphate
DHIS	District Health Information System
DFE	Dietary Folate Equivalent
DHF	Dihydrofolate
DHFR	Dihydrofolate Reductase
DNA	Deoxyribonucleic Acid
FAO	Food and Agricultural Organization
FBP	Folate Binding Protein
FNB	Food and Nutrition Board
LMP	Last Menstrual Period
Mcg	Micrograms
MTHF	Methyl Tetra Hydro Folate
NHANES	National Health and Nutrition Examination Survey
NTD	Neural Tube Defects
OR	Odds Ratio
RBC	Red Blood Cell
RCF	Red Cell Folate
RDA	Recommended Dietary Allowance
RR	Relative Risk
SACN	Scientific Advisory Committee on Nutrition
THF	Tetrahydrofolate Reductase
WHO	World Health Organization

5-MTHF 5-Methyltetrahydrofolate

5-MTHF 5-Methyltetrahydrofolate Reductase

DEFINITION OF OPERATIONAL TERMS

Folate Polyglutamate: Refers to the form of folate found in food

Folate Monoglutamate: Refers to the fully oxidized synthetic compound which is mostly used in food fortification and in dietary supplements

Folate deficiency: Serum folate levels of less than 10nmols/L

Borderline Folate Deficiency: Serum folate levels of between 10nmols/L and 15 nmols/L

Normal folate Levels: Serum Folate levels of more than 15nmols/L

Dietary Folate Equivalent (DFE): Refers to the standardized unit for measurement of folate intake

Recommended Dietary Allowance (RDA): Refers to the nutrient intake level considered to be sufficient to meet the needs of almost all healthy people of a given age and gender

Neural Tube Defects: Birth defects of the brain, spine and the spinal cord

Pre-eclampsia: Pre-eclampsia is a pregnancy complication characterized by high blood pressure and signs of damage to another organ system, most often the liver and kidney

ABSTRACT

Folate deficiency is one of the micronutrient deficiencies of greatest public health concern, especially among women of child bearing age due to the potential consequences to the unborn baby. To address micronutrient deficiencies including folate, the Government of Kenya amended the food, drug and chemical substances act to have mandatory fortification of maize and wheat flour with folic acid. This study sought to determine the prevalence of folate deficiency and assess awareness and utilization of folic acid. A cross-sectional study of 247 pregnant women attending antenatal clinic (ANC) at Pumwani Maternity Hospital was conducted. A structured questionnaire was used to interview study participants. Blood samples were collected from all study participants and serum folates analyzed by electrochemiluminescence immunoassay. Of the 247 study participants, 2 (0.8%) had folate deficiency. One hundred and seventy-nine (73.4%) had heard about folic acid supplements while 56 (22.7%) had heard of folic acid fortified flour. Overall, 198 (80.2%) study participants consumed fortified brands of maize flour and 205 (84.4%) consumed fortified brands of wheat flour. In conclusion, the prevalence of folate deficiency was low possibly due to the implementation of mandatory folate fortification of maize and wheat flour. Although there was limited knowledge of fortified flour, utilization was high. The Ministry of Health should reinforce implementation of the legislation on maize flour and wheat flour fortification by all milling industries

CHAPTER ONE

INTRODUCTION

1.1 Background

Folate is a B vitamin (B9) that is essential for normal development and function of the body cells. There are 2 forms of folate: folate polyglutamates (natural folate) and folate monoglutamates (folic acid). Natural folate is the endogenous form of the vitamin which occurs naturally in some foods e.g. green leafy vegetables, beef liver, some fruits, whole grains and legumes. It is heat labile and approximately 50% of natural folate is lost during food preparation (McKillop *et al.,* 2002). Folic acid is the synthetic form of vitamin B9 that is used in dietary supplements and fortified foods. It is more chemically stable than the natural food folate and is 1.7 times more bioavailable than folate (Bailey, 2000).

Folate deficiency refers to lower than normal folate levels in the blood. It is defined by a serum folate level of less than 10 nmol/L (4 ng/mL) or red blood cell (RBC) folate less than 340 nmol/L or 151 ng/mL (Benoist, 2008). The main cause of folate deficiency is inadequate dietary intake of folate. Other causes are: chronic alcohol consumption, mal-absorption syndromes (e.g. celiac sprue) and use of certain medications, such as some anticonvulsants, oral contraceptives, para-amino salicylic acid and pyrimethamine (Allen, 2008). Deficiency occurs more commonly in conditions where the rate of cellular multiplication is increased as is seen in pregnant or lactating women, infants and adolescents. Some of the known consequences of folate deficiency in pregnancy to the unborn child include: prematurity, intra uterine growth retardation, congenital heart defects, oro-facial cleft defects and neural tube defects, (Scholl & Johnson, 2000; Fekete *et al*., 2012).

Neural tube defects result in significant morbidity, mortality and life-long disability including lower limb paralysis, hydrocephalus, bowel and bladder incontinence, and intellectual and learning disabilities (Christianson *et al*., 2006). The cost of treatment and productivity losses associated with neural tube defects is also high yet is preventable by interventions such as folic acid supplementation and consumption of food fortified with folic acid.

The total global prevalence of folate deficiency in the population is unknown due to a lack of data from many parts of the world (McLean *et al*., 2008). Many countries, especially in the developing world, do not routinely assess folate status of the population, and hence have no data on folate deficiency. In countries that do have data on folate status (from national surveys or from small studies), deficiency data vary due to: use of different testing methods, different cut off ranges, differences by race/ethnicity and by region. In Venezuela, the prevalence of folate deficiency among pregnant women was reported to be 36.3% (García-Casal *et al*., 2005) while in Turkey a study found a prevalence of 71.7% (Karaoglu *et al.*, 2010). In Africa, the prevalence was reported to be 18.8% in Benin (Smaı¨la, *et al.*,2011) and 9% in Nigeria (VanderJagt *et al.*, 2007). The prevalence of folate deficiency among the Kenyan population remains unknown, and there is no information regarding folate deficiency status before the implementation of mandatory folic acid fortification of maize and wheat flour.

1.2 Statement of the problem

Folate deficiency results in adverse reproductive outcomes which include: infertility, anemia in pregnancy, intrauterine growth restriction, prematurity, developmental disabilities, neural tube defects, orofacial clefts and congenital heart defects. These conditions lead to significant morbidity, mortality, life-long disability, high economic

costs (including medical and non-medical costs) and psych-social problems, for the family and for the affected child.

In Kenya, the prevalence of folate deficiency among women of child bearing age is unknown. Interventions to improve folate status in Kenya include combined folate and iron supplements and mandatory fortification of maize and wheat flour. Iron-folate supplements are usually given during the first antenatal care visit; most after the trimester. Iron-folate supplements therefore helps in preventing anemia but plays a minimal role in reducing the risk of the adverse events for the baby. This is because organ formation takes place in the first 3 months of pregnancy hence the congenital birth defects would have occurred by then. To increase the dietary intake of folic acid among women of child bearing age, the Government of Kenya amended the Food, Drug and Chemical Substances Act in 2012 through a gazette notice making folic acid fortification of maize and wheat mandatory (Wefwafwa *et al.*, 2012). To create awareness and demand for fortified flour, campaigns were done by the Ministry of Health Nutrition Unit though the media, posters and road shows. However, the level of awareness and the utilization of fortified flour in the country are unknown.

1.3. Study Justification

The prevalence of folate deficiency among pregnant women is unknown. It is important to know the burden of folate deficiency among pregnant women. This is because folate deficiency leads to adverse pregnancy outcomes. Improving maternal folate status will have an impact in reducing the risk of neural tube defects as well as reducing the burden of the other adverse reproductive outcomes associated with folate deficiency. This study has provided baseline data on folate deficiency among pregnant women attending Pumwani hospital as well as the level of awareness regarding fortified products, which will help to determine whether the communication on fortified flour was effective or if

there is a need to repackage the information or use alternative communication media. It has also provided information on the utilization of fortified flour which will help to establish whether demand was created following the communication conveyed through media and helps uncover other issues that potentially hinder the consumption of fortified flour among childbearing aged women. The findings of this study will also be used for reference by other researchers intending to conduct similar studies.

1.5 Objectives of the study

1.5.1 General objectives

To determine the prevalence of folate deficiency and knowledge, attitude and practices on folic acid among pregnant women attending antenatal care clinic at Pumwani Maternity Hospital for the first visit.

1.5.2 Specific objectives

1. To determine the prevalence of folate deficiency among pregnant women attending ANC clinic at Pumwani Maternity Hospital
2. To determine the levels of awareness regarding folic acid fortified flour among women attending the ANC clinic at Pumwani Maternity
3. To determine the reported use of folic acid/folate (dietary, folic acid supplements and folic acid fortified flour) among pregnant women attending ANC clinic at Pumwani Maternity Hospital

CHAPTER TWO

LITERATURE REVIEW

2.1 Physiology and functions of folate

2.1.1 Folate

Folate is a B vitamin (B9) that is essential for normal development and function of body cells. All folate compounds have the same basic molecular structure which consists of three parts: pteridine, para-aminobenzoic acid and L-glutamic acid. Together, the pteridine and para-aminobenzoic acid form a pteroyl group (Hoffbrand, 2001). Folate is a water soluble vitamin hence in the body, it dissolves in water in cells and there is minimal storage in the body, therefore folate is needed on a daily basis (Norwood, 2011). Humans cannot produce folate so it must be acquired through the diet. There are 2 types of folate: folate polyglutamates (natural folate) and folate mono-glutamates (synthetic folic acid).

2.1.1.1. Folate poly-glutamate

Folate polyglutamates (natural folate) refers to the various tetrahydrofolate derivatives naturally present in some foods. They exist as an array of tetrahydrofolate (THF) species which include: 5-methyl-THF, 5-formyl-THF, 10-formyl-THF, 5,10-methylene-THF, and 5,10-methenyl-THF (Gregory, 2012). The main folate forms are 5-methyl-tetrahydrofolate and 10- formyl-tetrahydrofolate (SACN, 2006). Natural folates differ from folic acid in three aspects: (i)They have additional glutamate residues (polyglutamates), (ii) the pteridine ring is fully reduced to tetrahydrofolate or dihydrofolate (DHF) forms and (iii) have additional single carbon units e.g. methyl-CH_3,

formyl-CH_0, methylene-CH_2, methenyl-CH_4 attached to the nitrogen atoms (Hoffbrand & Weir, 2001). About 50% of the natural folates in foods are bioavailable (Caudill, 2010). Up to three quarters of folate polyglutamates activity is lost through harvesting, storage, exposure to light and cooking (McKillop *et al.*, 2002). Liver and green leafy vegetables such as spinach and kales are rich in folates but folates are also found in lower amounts in: whole grains, legumes, egg yolk, dairy products, tuna, salmon, dates, nuts and other vegetables and fruits e.g. broccoli, spring greens, cabbages, avocadoes and oranges.

2.1.1.2 Folate monoglutamate

Folate monoglutamate refers to the fully oxidized synthetic compound which is mostly used in food fortification and in dietary supplements. It consists of para-aminobenzoic acid molecule linked at one end to a pteridine ring and at the other end to one glutamic acid molecule (Hoffbrand & Weir, 2001). The pteridine ring in folic acid is not reduced hence it is very resistant to chemical oxidation. It is more stable than the natural folates and its absorption is also better than that for the natural folates. When consumed without food, folic acid has a bioavailability of ~100%. With food, the bioavailability of folic acid decreases to approximately 85% (Rogers *et al*.,1997).

2.1.2 Folate metabolism

In the gut, the conjugase enzyme found in the mucosal brush borders breaks down folate polyglutamates to the mono-glutamate form to facilitate its absorption in the jejunum. Within the intestinal mucosa, the monoglutamates undergo two successive reductions to form tetrahydrofolate which is the active form of folate. Tetrahydrofolate is methylated within the intestinal mucosa and what enters the portal blood is 5-methyl tetrahydrofolate mono-glutamate (5-MTHF), which is the only form of folate found in

circulation. Folic acid is not conjugated, therefore is more bioavailable than the natural folate and it is reduced to tetrahydrofolate (THF) by the enzyme dihydrofolate reductase (DHFR). THF is methylated in the gut mucosa to 5-MTHF; the form found in the circulation (SACN, 2006). 5-MTHF circulates bound to albumin and is transported into tissues via a membrane carrier or receptor mediated process. For folate to be retained in tissues it must be converted to long chain length polyglutamates forms; 5-MTHF is therefore metabolized to tetrahydrofolate (THF) which is then conjugated into poly-glutamate forms to prevent it from leaving the cell. De-methylated tetrahydrofolate mono-glutamate is released by extra hepatic tissues and is transported bound to a plasma folate binding protein to the liver, where it is either conjugated for storage or methylated to 5-MTHF which is secreted in the bile. There is considerable entero-hepatic circulation of folate, equivalent to approximately a third of the dietary intake. 5-MTHF secreted in the bile is reabsorbed in the jejunum together with food folates (Figure 2.1)

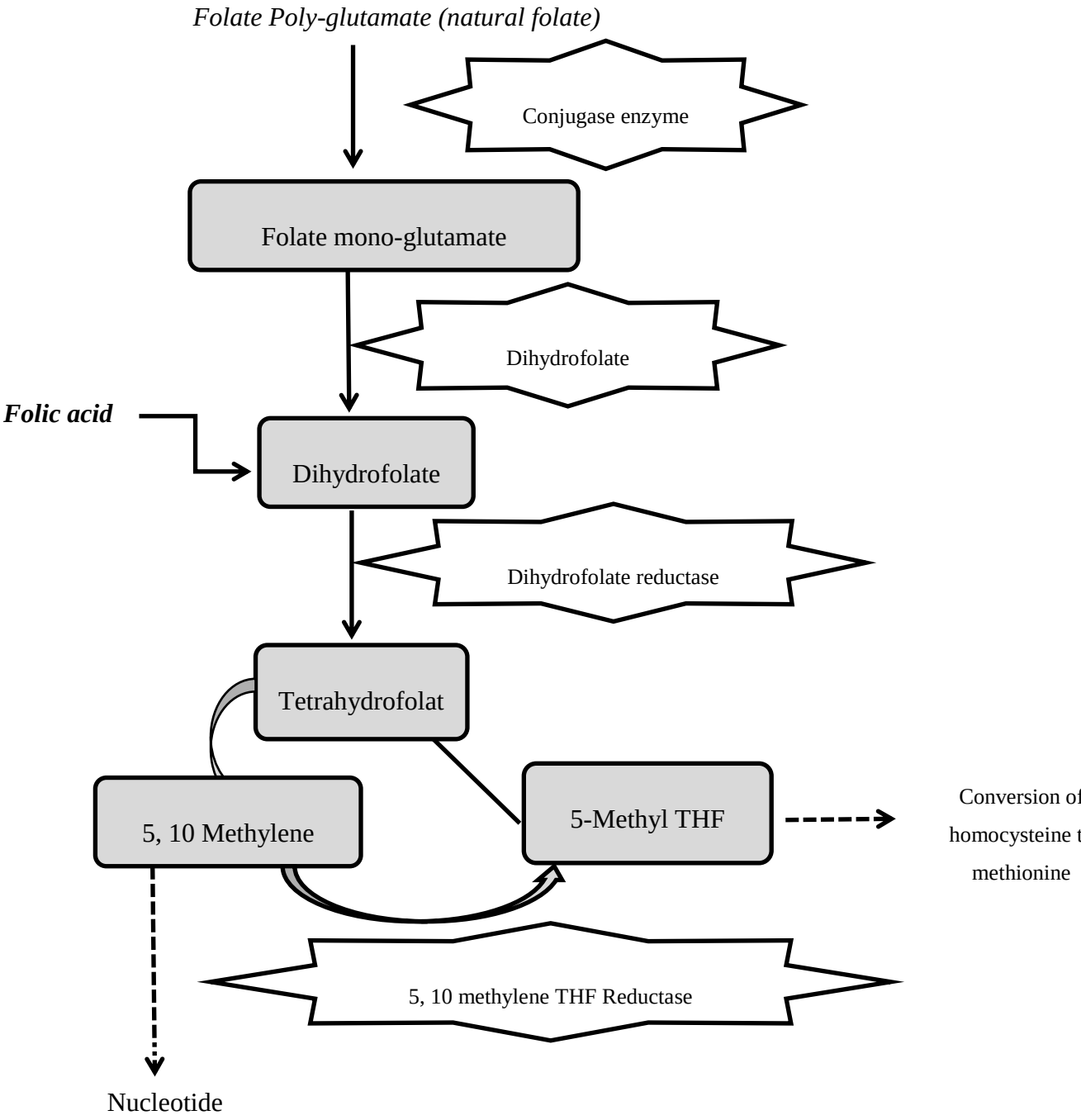

Figure 2.1 Intestinal absorption and metabolism of folate

2.1.3 Functions of folate

Tetrahydrofolate is the active form of folate. It acts as a coenzyme in many reactions that involve the transfer of one carbon units to other compounds (one carbon metabolism). These carbon groups can be carried on N^5, N^{10} or bridged between N^5 and N^{10} (e.g. 5-Methyl tetrahydrofolate carries methyl groups on N^5). There are 5 different forms of tetrahydrofolate which facilitate different metabolic reactions by donating and accepting single carbon units. These include: (i) 5-methyl tetrahydrofolate, (ii) 5, 10-methylene tetrahydrofolate, (iii) 10-formyl tetrahydrofolate, (iv) 5, 10-methenyl tetrahydrofolate and (v) 5-formimino tetrahydrofolate. The one carbon metabolism plays an important role in DNA synthesis and amino acid metabolism (methionine and homocyteine). In one of the carbon transfer reactions, 5, 10-methylene tetrahydrofolate transfers a methyl group to deoxyuridylate (dUMP) to form deoxythymidylate (dTMP) which is one of the four ribonucleotides required for DNA synthesis. Due to its role in DNA synthesis, folate plays an important role in growth and development and in the synthesis of red and white blood cells. DNA plays an important role in cell division which is critical for the formation of new cells (SACN, 2006; Ulrich *et al*., 2008).

Another important carbon transfer reaction involves the metabolism of methionine and homocysteine (amino acids). Folate helps in the conversion of methionine (from meat protein) to homocysteine. It also plays an important role in the conversion of homocysteine to methionine; 5-MTHF donates a methyl group to homocysteine to form methionine and THF. The reaction is catalyzed by the enzyme methionine synthase with B12 acting as a cofactor. This is the only reaction that regenerates tetrahydrofolate from 5-methyl tetrahydrofolate; without it, all the folate will be trapped as 5-MTHF and the other forms of folate will not be available. This reaction helps in preventing the buildup of homocysteine to high levels which are harmful to the body. Folate's role in the control of homocysteine levels, DNA synthesis and methylation reactions are all critical for proper brain function and normal functioning of the nerves (Bailey & Gregory, 1999)

2.1.4 Current recommendations of folate intake

Dietary Folate Equivalent (DFE) is the standardized unit for measurement of folate intake. DFEs consider the higher bioavailability of synthetic folic acid compared to naturally occurring food folate (FNB USA, 1998). Synthetic folic acid taken with food is 85% bioavailable but food folate is only about 50% bioavailable; folic acid taken with food is therefore 85/50 (i.e. 1.7) times more bioavailable (Hoyo *et al.*, 2011).

Recommended Dietary Allowance (RDA) is the nutrient intake level considered to be sufficient to meet the needs of almost all healthy people of a given age and gender. Table 2.1 shows the RDA given in DFEs for adolescents and adults as outlined by the U.S. Food and Nutrition Board.

Table 2.1: Recommended dietary allowances for folate in DFEs (FNB USA, 1998)

Age group	RDA mcg/day(Males)	RDA mcg/day(females)
Adolescents 14-18 years	400	400
Adults 19 years and older	400	400
Pregnancy all ages	-	600
Breast feeding all ages	-	500

2.2 Folate deficiency

2.2.1 Prevalence of folate deficiency among pregnant women

The global prevalence of folate deficiency is unknown due to scarcity of worldwide data on folate deficiency. Studies conducted in different countries on different continents have shown a wide variation in the prevalence of folate deficiency among pregnant women ranging from 9% in Nigeria to 71.7% in Turkey. In South America, a study conducted in Venezuela yielded a prevalence of 36.3% (García-Casal *et al.*, 2005). Data

from the National Health and Nutrition Examination Survey (NHANES) revealed a reduction in the prevalence of low serum folate levels among women of reproductive age in the United States from 20.6% in 1988–1994 (pre-fortification) to 0.8% in 1999–2000 (post-fortification) and has remained low between 2005–2006 (McDowell *et al.*, 2008). In Asia, a high prevalence of 71.7% was reported in Turkey (Karaoglu *et al.*, 2010).

The prevalence of folate deficiency among pregnant women in Africa is varied across different countries due to utilization of different testing methods and different cut off points. The prevalence of folate deficiency among pregnant women was found to be: 18.8% in Benin (Ouédraogo *et al.*, 2012) and 9% in Nigeria (VanderJagt *et al*., 2007). In Ethiopia, a study conducted among women aged 15-19 years yielded a prevalence of 31.3% (Haidar, 2010) and a different study conducted to determine the prevalence of folate deficiency in women of reproductive age in nine of the eleven administrative regions of Ethiopia reported a prevalence of 46% (Haidar, 2010). In South Africa, the prevalence of folate deficiency among women of childbearing age was reported to be 27.6% before fortification and 0% after fortification (Modjadji and Alberts, 2007). A study conducted in Cameroon among Cameroonian women and children reported a prevalence of 17% and 8% in women and children, respectively (Shahab *et al.*, 2014). In Kenya, the prevalence of folate deficiency among pregnant women remains unknown.

2.2.2 Effects of folate deficiency in pregnancy

2.2.2.1 Anemia in pregnancy

Folate deficiency can cause anemia during pregnancy. In the bone marrow, the red blood cell precursor (stem cell) usually divides as DNA is created within the cell. When there is folate deficiency, DNA synthesis is impaired hence cell division is also impaired.

RNA synthesis however goes on. The cells therefore grow larger but cannot divide hence they get stuck in the bone marrow as large immature form (megaloblasts). When they are released into the blood stream they are called macrocytes. The macrocytes cannot function properly hence oxygen transport is impaired. This results into a type of anemia known as megaloblastic or macrocytic anemia (Sharma & Shankar, 2010).

2.2.2.2 Birth defects

Folate deficiency/insufficiency is one of the factors associated with an increased risk of birth defect affected pregnancies (e.g. cleft lip and palate, congenital heart diseases and neural tube defects). Folate is required for DNA synthesis; if it is not present in adequate amounts during the first 28 days of pregnancy, DNA required for neural tube development cannot be formed leading to neural tube defects which include spina bifida, encephalocele and anencephaly. Folate also plays a role in the synthesis of glutathione; an antioxidant that protects against environmental toxins such as arsenic. Deficiency of folate in pregnancy may therefore lead to accumulation of these toxins resulting in birth defects such as cleft lip and palate, heart defects and limb malformations (Finnel *et al*., 2008).

2.2.2.2.1 Folate deficiency and neural tube defects

The neural plate closes between days 21 and 28 post-conception to form what will eventually be the spinal cord and cranium. Spina bifida, encephalocele and anencephaly are neural tube defects which result from improper closure of the neural plate. Spina bifida results from improper closure of the caudal end whereas encephalocele and anencephaly result from improper closure of the cranial end. During pregnancy, the risk of neural tube defects is increased if there is folate deficiency. The risk is increased up to 10-fold as folate status changes from adequate to poor. An inverse dose response

relationship between folate status and risk of neural tube defects has been reported (Daly *et al*., 1997)

Most pregnancies in women whose folate intake is as per the RDA are not affected by neural tube defects, evidence has shown that folate intakes above the RDA early in pregnancy reduces the risk of neural tube defect-affected pregnancies (L B Bailey, 2000). Due to the lower bioavailability and poor stability of the food folate, it is very hard to have optimum folate levels for the prevention of neural tube defects based on diet food folate alone. To improve the maternal folate status, folic acid supplementation and consumption of folic acid fortified food is recommended (Martínez-Frías, 2006).

2.2.2.3 Low birth weight and preterm delivery

Folate plays a critical role in the formation of red blood cells. In folate deficiency in pregnancy, there is impaired red blood cell production which leads to inadequate blood flow hence poor oxygen delivery to the fetus. This leads to fetal growth retardation and preterm birth. An observational study on dietary folate and serum folate levels and their influence on pregnancy outcomes showed that women with low folate intake (<240 micrograms/d) had > 3 times greater preterm deliveries and low birth weight infants than women with folate intake > 240 micrograms per day ($p < 0.05$) (Schall, 1996).

2.2.2.4 Miscarriages and preeclampsia

Folate deficiency results in high homocysteine levels. This is because folate is required for methionine and homocysteine metabolism; a process which is critical for the control of homocysteine levels. High homocyteine levels damage the lining of blood vessels and causes buildup of plaque leading to habitual spontaneous abortion and pregnancy complications (e.g. placental abruption and preeclampsia (Scholl & Johnson, 2000).

2.2.3 Causes of folate deficiency

2.2.3.1 inadequate dietary intake

The main cause of folate deficiency is inadequate dietary intake. Many factors can lead to inadequate dietary intake, including limited consumption of fresh, minimally cooked folate rich foods, limited consumption of folic acid fortified food or due to chronic alcoholism (Allen, 2008).

2.2.3.2 Inadequate folate absorption

Inadequate absorption of folate results from mal-absorption syndromes like celiac disease and drug interactions, such as interaction of folate with some anticonvulsants (phenytoin, phenobarbital and sodium valproate), oral contraceptives, methotrexate, sulfasalazine, pyrimethamine and large doses of non-steroidal anti-inflammatory drugs (Stover, 2008).

2.2.3.3 Increased folate requirements

Increased folate requirements occur in conditions where the rate of cell multiplication is increased e.g. in pregnancy, lactation, infancy, malignancies, increased hematopoiesis (hemolytic anemia and chronic blood loss) and in increased metabolic activities e.g. hyperthyroidism (Norwood, 2011).

2.2.3.4. Inadequate Utilization

Vitamin B12 deficiency, ascorbic acid deficiency, excess dietary amino acids (glycine and methionine) and deficiency of the enzymes (congenital or acquired as seen in

chronic liver disease) required for folate metabolism result in inadequate utilization of folate (Herbert, 1973).

2.2.4 Factors associated with folate deficiency

2.2.4.1 Diet

A study conducted in Australia on folate status and health behaviors in two Australian indigenous populations found that aboriginal men who reported taking two or more servings of vegetables daily were 40% less likely to have low red blood cell folate regardless of age ((Li *et al.*, 2012). Another study conducted in Ethiopia on prevalence of folate deficiency among women of reproductive age reported a lower risk of folate deficiency among those who had meat, milk or eggs more than once a week and those who had vegetables and grains in addition to their staple crop more than once a day ((Haidar, 2010). Low dietary folate intake was also found to be associated with folate deficiency in adolescent girls in Turkey (Oner *et al.*, 2006).

2.2.4.2 Low Income

A study conducted in California USA among women of childbearing age showed that low income women are at a higher risk of folate deficiency compared to high income women (Cena *et al.*, 2008). Women with a lower socioeconomic status were found to have a lower serum and red blood cell folate in a study in southern California (Caudill *et al.* 2001). In Canada, a study on socio-demographic and lifestyle factors associated with folate status among non-supplement-consuming Canadian women of childbearing age, low income was found to be associated with low folate levels (Shi *et al.*, 2014). Among adolescent girls in Turkey, it was observed that low family income was associated with folate deficiency (Oner *et al*., 2006).

2.2.4.3 Cigarette smoking

In a study conducted in Australia, it was observed that female aboriginal smokers had double the risk of low red blood cell folate compared with non-smokers while the male smokers had three times the risk. However, there was no dose effect relationship on the numbers of cigarettes on red blood cell levels. In this study (Li *et al.*, 2012).

2.2.4.4 Ethnicity

In California USA, non-Hispanic women were reported to consume more synthetic folic acid and total folate than white and black women (Cena *et al.*, 2008). Results of the analysis of the National Health and Nutrition Examination Survey (NHANES) dataset done to compare serum and red blood cell folate levels among women of childbearing age from three ethnic groups in the U.S.A (non-Hispanic white, non-Hispanic black and Mexican American) showed that non-Hispanic white women had the highest blood folate values, followed by Hispanic women, and then non-Hispanic black women (CDC 2002).

2.2.4.5 Other Factors

Marital status, parity, oral contraceptive use, level of education and age were found to be associated with folate deficiency in some studies. In Ethiopia; unmarried women, women with higher parity and women who used oral contraceptives were reported to have a higher risk for folate deficiency (Haidar, 2010). Young age of 15 to 19 years and level of education below secondary were found to be associated with low folate levels in Canadian women (Shi *et al*., 2014).

2.2.5 Strategies for improving maternal folate status

2.2.5.1 Folic acid supplementation

Supplementation is the provision of relatively large doses of micronutrients, usually in the form of pills, capsules or syrups. One advantage of supplementation is the ability to supply an optimal amount of a specific nutrient in a highly absorbable form. Periconceptional folic acid supplementation has been shown to be effective in improving maternal folate status and subsequently reducing the prevalence of neural tube defect affected pregnancies (Toriello, 2011).To reduce the risk of neural tube defects, it is recommended that all women, from the moment they begin trying to conceive until 12 weeks of gestation, should consume 400μg/day of folic acid from supplements (WHO, 2007). Many developed countries have implemented the policy on folic acid supplementation; most developing countries including Kenya have not implemented this recommendation. In Kenya, folic acid supplements are given as a pill combined with iron. This is given during the first antenatal care visit to prevent anemia but not for the prevention of neural tube defects since the neural tubes closes by the 28^{th} day of pregnancy. Women who have had a fetus diagnosed with a neural tube defect or have given birth to a baby with a neural tube defect should receive information on the risk of recurrence, be advised of peri-conceptional folic acid supplementation and be offered high-dose supplementation (4000μg/day) to prevent recurrence (WHO, 2007). In Kenya, this is implemented at Kijabe Mission Hospital.

2.2.5.2 Food fortification with folic acid

Food fortification refers to the deliberate addition of one or more micronutrients (vitamins and minerals) to certain foods to increase the intake of these micronutrient(s) in order to correct or prevent a demonstrated deficiency and provide public health benefits. Within the legal context, food fortification can be classified as either voluntary

or mandatory. Voluntary fortification refers to food manufacturers freely choosing to fortify certain foods with specific micronutrients in response to permission given in the national food law, or when encouraged by the government to do so. Mandatory fortification is when the food manufacturers are legally obligated by the government to add specific nutrients in a specific amount to certain foods. Due to the uncertainty about the level of industry uptake of fortification, voluntary fortification is less likely to deliver a favorable outcome in increasing micronutrient intakes of target populations as compared to mandatory fortification. Globally, mandatory regulations are mostly applied to the fortification of food with micronutrients such as iodine, iron, vitamin A, and increasingly folic acid (WHO/FAO, 2006).

Globally, 80 countries have legislation on mandatory fortification of at least one industrially milled cereal grain of which 22 are African countries (Cameroon, Congo Brazzaville, Benin, Egypt, Ghana, Guinea, Cote d'Ivoire, Kenya, Liberia, Mali, Mauritania, Morocco, Niger, Nigeria, Rwanda, Senegal, Sierra Leone, South Africa, Tanzania, Togo and Burkina Faso); 79 countries have legislation on wheat flour fortification and 12 countries have legislation to fortify maize products. All the mandatory countries fortify wheat flour with at least iron and folic acid apart from 4 countries which do not include folic acid and one country that does not include iron ("Global Progress- Flour Fortification Initiative,").

Mandatory fortification of staple foods with folic acid has been shown to improve maternal folate status and has also led to the reduction in prevalence of neural tube defects in several countries. In Chile, mandatory folic acid fortification (started in January 2000) resulted in significant increases in serum folate and red cell folate of 3.8 and 2.4-fold respectively, in women of reproductive age one year after fortification (Hertrampf & Corte, 2004). The rates of spina bifida and anencephaly-affected births in Chile also declined by 51% and 42% respectively post fortification (Lopez-Camelo *et al,*

2005). In the USA and Canada, mandatory fortification started in 1998. Analysis of the data from the National Health and Nutrition Examination Survey (NHANES) showed that large increases in blood folate levels of the U.S. population occurred between 1988–1994 and 1999–2000 (McDowell *et al.*, 2008). In 2001, the prevalence of neural tube defect-affected births had decreased by 19% following fortification (Honein et al., 2001); by 2004 CDC reported a 27% decline in neural tube defect rates following fortification. In Canada, the prevalence of serum folate insufficiency declined from 0.52% to 0.22% post fortification, that of red blood cell folate insufficiency fell from 1.78% to 0.41% (Ray *et al*, 2002) and a 50% reduction in the prevalence of neural tube defects was reported (Mills and Signore, 2004). South Africa began mandatory fortification in 2003 and the prevalence of neural tube defects fell by 30.5% from 1.41 to 0.98 per 1,000 births (RR = 0.69; 95% CI: 0.49-0.98; p= .0379) within five years (Sayed *et al*., 2008). In Kenya, mandatory fortification started in 2012; all manufacturers of maize and wheat flour are required to add 1.5 mg/kg of folic acid to maize and wheat flour (Food, Drugs and Cchemical Substances Act). However, compliance of the industry to the mandate has not been evaluated.

2.3 Measurement of folate status

Serum folate reflects recent dietary intake and is subject to greater fluctuation. It is however the most widely used indicator for measuring folate status at the population level because it is inexpensive and more practical. The WHO technical consultation on folate and vitamin B12 deficiencies acknowledged that there was lack of universally accepted cut off points to define folate and vitamin B12 deficiencies due to the differences in folate levels with different laboratory assessment methods used. Despite this challenge, the cut off points for defining folate deficiency were set at < 10 nmol/L (4ng/mL) for serum folate and < 340 nmol/L (151ng/mL) for red blood cell folate. These cut off points were derived from the United States National Health and Nutrition

Examination Survey (US NHANES III) and were based on the plasma vitamin concentrations below which plasma metabolites (total homocysteine) become elevated (de Benoist, 2008).

Serum folate levels can be measured using microbiological assay (with lactobacillus casei/rhamnosus), radiometric competitive binding assays (radio-assays) or by chemiluminescent immunoassay. Microbiologic assay has been in use for the past 50 years and is the gold standard in the measurement of folate status. Use of radio-assays started 30 years ago. Due to technical difficulties in conducting microbiologic assays, many clinical laboratories switched to radio-assays because of the ease of use (Shane, 2011; Molloy *et al*., 1998). Chemiluminescent immunoassay has also been used in some laboratories. Its sensitivity is comparable to that of radio assays.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study design

A cross-sectional study with an analytic component was conducted at the antenatal care clinic of Pumwani maternity hospital using quantitative methods.

3.2 Study Site

The study was conducted at Pumwani Maternity Hospital which is located in Nairobi County. According to the 2009 Census, Nairobi County has a population of 3,138,369 people (Census, 2009) and an area of 694.9 Sq.km. Pumwani Maternity Hospital is located in the East side of Nairobi in Kamukunji District close to Mathare and Korogocho slums, two of Nairobi's biggest slums. It is a referral facility for complicated obstetric cases and serves a catchment population of about half a million people; most of whom are of low income. It is one of the largest maternity hospitals in Sub-Saharan Africa; approximately 17,000 deliveries are conducted in Pumwani Maternity Hospital annually, which accounts for 22% of all deliveries in Nairobi County. The choice of the study site was based on the following: Pumwani Maternity is the largest maternity hospital in Kenya, it serves approximately 20% of the pregnant women in the county.

Figure 3.1: Map of Kenya showing the 47 counties and the study Site

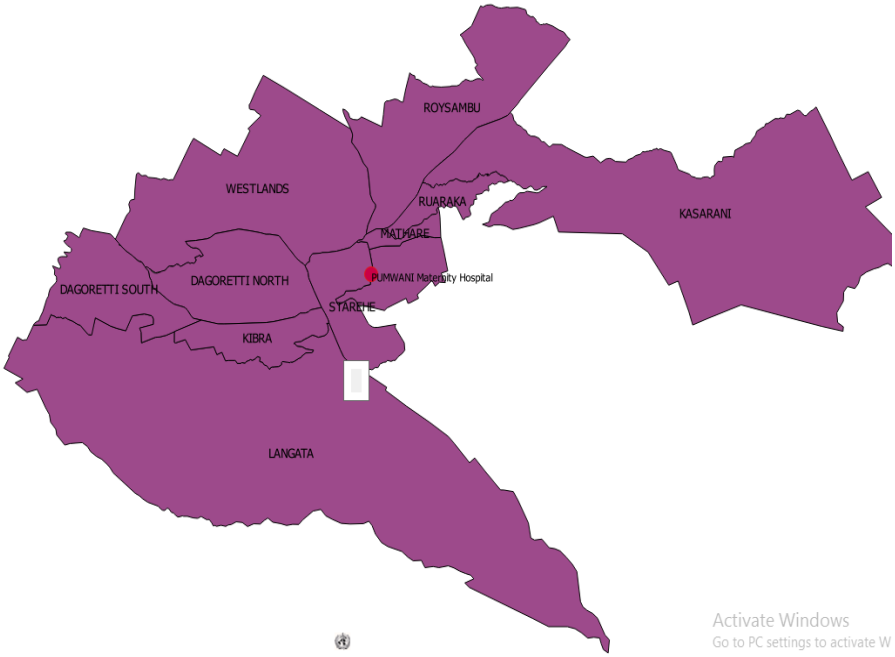

Figure 3.2: Map of Nairobi County showing the sub-counties and the study site

3.3 Study population

The study population was pregnant women (≤24 weeks gestation) seeking antenatal care at Pumwani Maternity for the first visit.

3.3.1 Inclusion criteria

All pregnant women (≤24 weeks gestation) 18 years of age and older who came to Pumwani maternity for the first Antenatal Care (ANC) visit who gave informed consent.

3.3.2 Exclusion criteria

The following were excluded from the study: Those who declined to participate in the study, those who were sick, those who were unable to provide informed consent and those who had a gestation age >24 weeks.

3.4 Sampling and Sample size

3.4.1 Sampling Technique

Systematic random sampling was done. The sampling interval was obtained as shown below:

$$\text{Sampling interval} = \frac{\text{Expected number of pregnant women 18 years and older with a gestational age of } \leq 24 \text{ weeks who will have come to Pumwani maternity hospital within the data collection period (2 months)}}{\text{sample size}}$$

The expected number of pregnant women 18 years and older with a gestational age of ≤24 weeks who will have come to Pumwani maternity hospital for the first visit (the

numerator) was estimated by: Reviewing the antenatal clinic records at Pumwani maternity hospital to obtain the number of women 18 years and older who came to Pumwani maternity hospital for the first visit with a gestational age of ≤24 weeks for the preceding 2 months. After determining the sampling interval, a random number was picked using the computer to identify the first study participant to be included in the study. The subsequent participants were then selected based on the sampling interval. If an eligible person declined to participate in the study, the next eligible person was picked.

3.4.2 Sample Size Determination

The sample size was estimated using the Cochran formula (Naing *et al*., 2006)as follows:

$$n=\frac{z^2pq}{d^2}$$

Where*:*

n=Required Sample Size

z=Confidence level at 95% (Standard value is1.96)

p=Prevalence of folate deficiency among pregnant women, 18% according to a study in Benin (Ouédraogo *et al.,* 2012)

q=1-p

d=Level of precision (5%)

This yielded a minimum sample size of 235

10% was added to cater for non-response=24

Hence sample size=235+24=259

3.5 Data collection

3.5.1: Administration of Questionnaire

A structured questionnaire was designed and piloted. The questionnaires were pretested prior to commencement of the study. Questions that were not well understood were then revised. The questionnaire was administered through face-to-face interviews before the women received ANC services.

Antenatal register was also reviewed to obtain information on the fundal height to compare with the gestational age as per the last menstrual period. In case of discrepancies between the gestational age as per fundal height and that as per the LMP, gestational age as per fundal height was used.

3.5.2 Collection of blood samples

Two milliliters of blood was collected from each participant using a sterile anticoagulant free tube and the cap securely closed. The tubes were clearly labeled with questionnaire number, date and time of sample collection. The samples were then transported to the Lancet Laboratory where they were centrifuged, and serum stored frozen at a temperature of -80^0C. This is because folate has been shown to remain stable in human serum for up to one year at a temperature of -70^0c (Jansen *et al.*, 2012). Analysis of the samples was done in batches on a monthly basis.

3.5.2.1. Folate level determination

The samples were tested for serum folate using chemiluminescent immune assay. The folate assay employed a competitive test principle and used natural folate binding protein (FBP) specific for folate (Eclia E., n.d.) Serum folate levels below <4 ng/mL were indicative of folate deficiency (de Benoist, 2008).

3.6 Study variables

3.6.1 Independent variables

The independent variables of interest were: age, level of education, marital status, employment status, family-size, number of children under five years old, gestation, parity, duration since the last pregnancy, number of preterm births, family-planning method used, type of flour used, reported use of folic acid supplements, awareness regarding flour fortification with folic acid, cigarette smoking and alcohol consumption.

3.6.2 Dependent variable

Serum folate levels was the dependent variable. Serum folate levels of <10nmols/L was classified as folate deficiency while serum folate levels of between 10nmols/L and 15 nmols/L was defined as borderline folate deficiency.

3.7 Data Management and analysis

Data entry and cleaning was done using EPI INFO version 7 while data analysis was done in SPSS version 22.0. Descriptive statistics was done and it included frequencies and proportions for categorical variables and the measures of central tendency (mean,

median and mode) and measures of dispersion (range, interquartile range and standard deviation) were used for continuous variables. Bivariate analysis was to be done but it was not done due to the low prevalence of folate deficiency.

3.8 Ethical considerations

Ethical approval was given by Kenyatta National Hospital Research and Ethics Board. Administrative clearance was obtained from the County Director of Health, Nairobi County and the medical superintendent of Pumwani Maternity Hospital. A written informed consent was obtained from all the study subjects after fully explaining to them the nature of the study. Those who agreed to participate in the study put their signature or thumb print on the written consent papers. The laboratory results were shared with the study participants through the telephone. The results were also shared with the attending clinicians. To ensure confidentiality, the data was stored in a password protected laptop.

CHAPTER FOUR

RESULTS

4.1: Enrollment of Participants

A total of 259 study participants were enrolled into the study. Twelve blood samples were insufficient in quantity and could not be analyzed. Hence, serum folate results were available for only 247 study participants. All the analysis therefore focused on the 247 participants with serum folate results. This constituted 105% of the sample size.

4.2: Characteristics of study participants

4.2.1: Socio-demographic characteristics

Of the 247 study participants, the majority (59.9%) were aged between 20-29 years. The mean age (SD) was 27.2 (5.2) years. Two hundred and twenty (89.1%) were Christians, 174 (70.4%) lived in formal settlements, 113 (45.7%) reported their highest level of education to be secondary school, 109 (44.1%) were self-employed and 196 (79.4%) were married. Ninety-six (38.9%) of the study participants reported the highest level of education of their spouses to be secondary school and 104 (42.1%) reported that their spouses had formal employment (Table 4.1)

Table 4.1: Socio-Demographic Characteristics of pregnant women Attending: Antenatal Clinic at Pumwani Maternity Hospital, October to November 2014

Variable	Categories	Frequency (%) N=247
Age	<20	11 (4.6)
	20-29	144 (59.8)
	30-39	83 (34.4)
	40-49	3 (1.4)
Religion	Christian	221 (89.5)
	Muslim	26 (10.5)
Residence	Informal settlement	73 (29.6)
	Formal settlement	174 (70.4)
Level of education	University	9 (3.7)
	College	55 (22.4)
	Secondary	113 (46.1)
	Primary	66 (26.9)
	No formal education	2 (0.9)
Employment Status	Formal employment	62 (25.3)
	Self employed	109 (44.5)
	Unemployed	74 (30.2)
Marital status	Married	196 (79.4)
	Single	49 (19.8)
	Divorced	2 (0.8)
Spouse's level of Education	University	29 (14.8)
	College	39 (19.9)
	Secondary	93 (47.4)
	Primary	29 (14.8)
	No formal education	1 (0.5)
	Don't Know	5 (2.6)
Spouse's Employment Status	Formal employment	102 (41.3)
	Self employed	92 (37.2)
	Unemployed	2 (0.8)
	Don't Know	51 (20.6)
Total (N)		**247**

4.2.2: Obstetric characteristics

Of the 247 study participants, 84 (34.0%) were in their first trimester. Gravidity ranged from 1 to 7 with 84 (34.0%) of the participants being primigravidas. Two hundred (81.0%) reported ever using family planning methods before and 146 (59.1%) reported that the pregnancy was planned (Table 4.2)

Table 4.2: Obstetric Characteristics of pregnant women Attending Antenatal Clinic at Pumwani Maternity Hospital, October to November 2014

Variable	Categories	Frequency (%) N=247
Gestation in weeks	≤13 weeks	84(34.0)
	13-24 weeks	163(66.0)
Gravidity	1	84(34.0)
	2	72(29.1)
	3	63(25.5)
	4	20(8.1)
	5+	8(3.2)
Ever used any family planning method	No	47(19.0)
	Yes	200(81.0)

Of the 163 participants who reported being pregnant before, 58 (35.6%) reported that their last pregnancy was ≥5 years before, 55 (33.7%) had experienced a miscarriage before, 12 (7.4%) had ever delivered a premature baby before and 9 (5.5%) had delivered a low birth weight baby before (Table 4.3)

Table 4.3: Characteristics of previous pregnancies among pregnant women Attending Antenatal Clinic at Pumwani Maternity Hospital, October to November 2014

Variable	Categories	Frequency %) N=163
Duration since last pregnancy	<1year	24(14.7)
	1-2years	40(24.5)
	3-4years	41(25.2)
	5years or more	58(35.6)
Ever had a miscarriage	No	108(66.3)
	Yes	55(33.7)
Ever Delivered prematurely	No	151(92.6)
	Yes	12(7.4)
Ever had a low birth weight baby	No	154(94.5)
	Yes	9(5.5)

Two hundred study participants reported having used family planning methods before. Of these, 96 (48%) reported having used oral contraceptive pills (Figure 4.1)

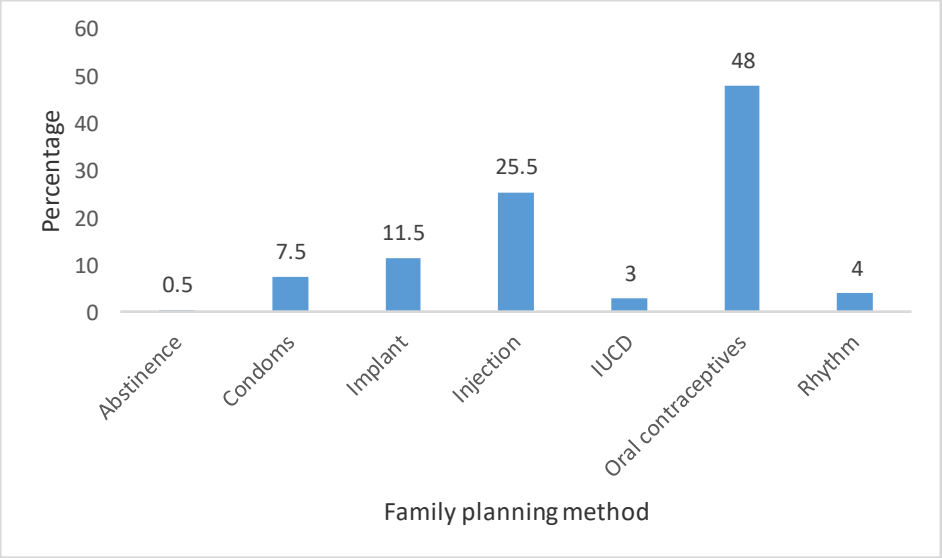

Figure 4.1: Family planning methods used by pregnant women Attending Antenatal Clinic at Pumwani Maternity Hospital, October to November 2014

4.2.3: Clinical characteristics

Twenty-five (10.1%) of the 247 study participants reported suffering from chronic illnesses including HIV, epilepsy, diabetes and hypertension, 19 (7.7%) reported using medication prior to the pregnancy and 46 (18.6%) reported having being ill during the pregnancy. Forty-six (18.6%) of the participants reported having used alcohol before but only 5 (2%) reported that they were still using alcohol. Seven (2.8%) participants reported ever smoking cigarettes, but none reported currently smoking cigarettes. Twenty (8.1%) had a smoker in their house hold (Table 4.4).

Table 4.4: Clinical characteristics of pregnant women Attending Antenatal Clinic at Pumwani Maternity Hospital, October to November 2014

Variable	Categories	Frequency (%) N=247
Suffering from any chronic illnesses	No	222(89.9)
	Yes	25(10.1)
Medication use prior to pregnancy	No	228(92.3)
	Yes	19(7.7)
Any Illnesses during pregnancy	No	201(81.4)
	Yes	46(18.6)
Ever taken alcohol	No	201(81.4)
	Yes	46(18.6)
Currently takes Alcohol	No	242(98.0)
	Yes	5(2.0)
Ever smoked cigarettes	No	240(97.2)
	Yes	7(2.8)
Currently smoking cigarettes	No	247(100.0)
	Yes	0(0.0)
A smoker in the HH	No	227(91.9)
	Yes	20(8.1)

4.3 Prevalence and characteristics of participants with folate deficiency

4.3.1 Prevalence of folate deficiency

Out of the 247 study participants, 2 (0.8%) had folate deficiency, 3 (1.2%) had borderline folate deficiency and 242 (97%) had normal folate levels. The median folate was 33.7 nmols/L with an inter-quantile range of 15.5 nmols/L (Figure 4.2).

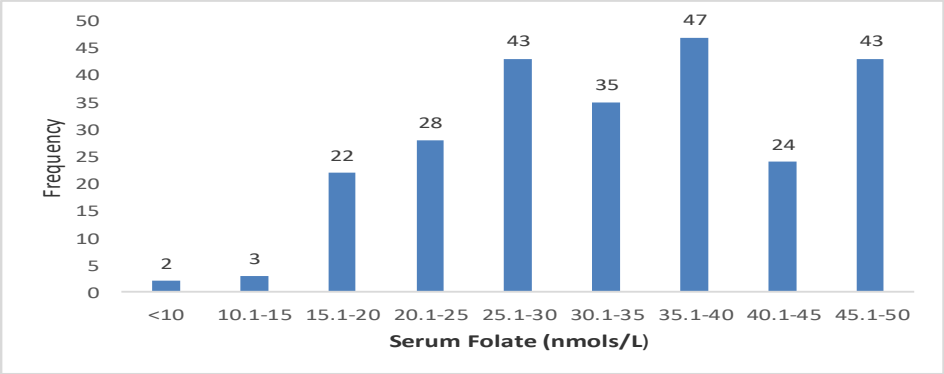

Figure 4.2: Folate level distribution among pregnant women Attending Antenatal Clinic at Pumwani Maternity Hospital, October to November 2014

4.3.2 Characteristics of participants who had folate deficiency

Of the five study participants who had folate deficiency (folate deficiency and borderline folate deficiency), all were aged between 19-35 years, four had education level of secondary school and below, four had a gestation of ≥13 weeks and four reported having used oral contraceptives. Two of the five reported that they had chronic illnesses (epilepsy and HIV) and were on medication. one smoked cigarettes, three used alcohol, four had not used folic acid supplements and four consumed fortified flour (Table 4.5).

Table 4.5: Characteristics of folate deficient pregnant women Attending Antenatal Clinic at Pumwani Maternity Hospital, October to November 2014

Variable	Categories	Frequency
Age	20-29 years	3
	30-39 years	2
Level of Education	Primary	1
	Secondary	3
	College	1
Religion	Muslim	3
	Christian	2
Gestation	<13 weeks	0
	13-24 weeks	5
Gravidity	Primigravida	3
	Gravida 2 plus	2
Ever had a miscarriage	Yes	2
	No	3
Oral Contraceptives use	Yes	4
	No	1
History of Chronic Illness	Yes	2
	No	3
Medication use	Yes	2
	No	3
Ever smoked cigarettes	Yes	1
	No	4
Alcohol use	Yes	3
	No	2
Folic Acid Supplementation	Yes	1
	No	4
Folic Acid Fortified Flour Consumption	Yes	4
	No	1
N		**5**

4.4: Awareness and knowledge on Folic acid (supplements and fortified flour)

4.4.1: Folic acid supplements awareness

Out of the 247 study participants, 179 (73.4%) had heard about folic acid supplements. Of the 179 participants who had heard about folic acid supplements, 137 (76.5%) had heard from the television and 0.6% % heard from neighbours (Figure 4.3)

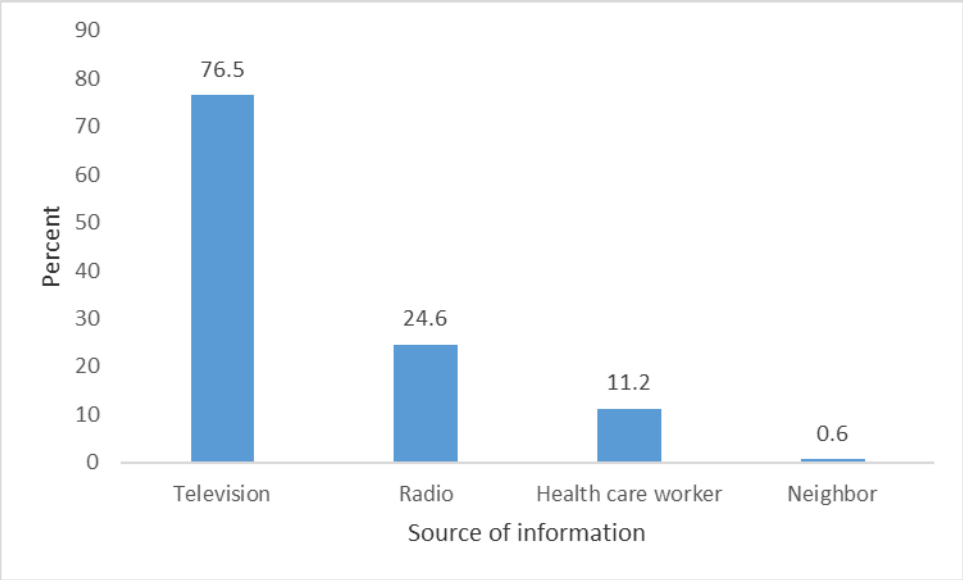

Figure 4.3: Sources of information on folic acid supplements among pregnant women Attending Antenatal Clinic at Pumwani Maternity Hospital, October to November 2014

4.4.2: Folic acid Fortified Flour awareness

Only 56 (23%) of the 247 study participants had heard about folic acid fortified flour. Of these, 42 (75%) knew the meaning of fortified flour while 38(67.9%) knew how to identify fortified flour. Of the 56 study participants who had heard about folic acid fortified flour, 24 (42.8%) heard from the television while the rest got the information from neighbours/friends, radio and health care workers (Figure 4.4)

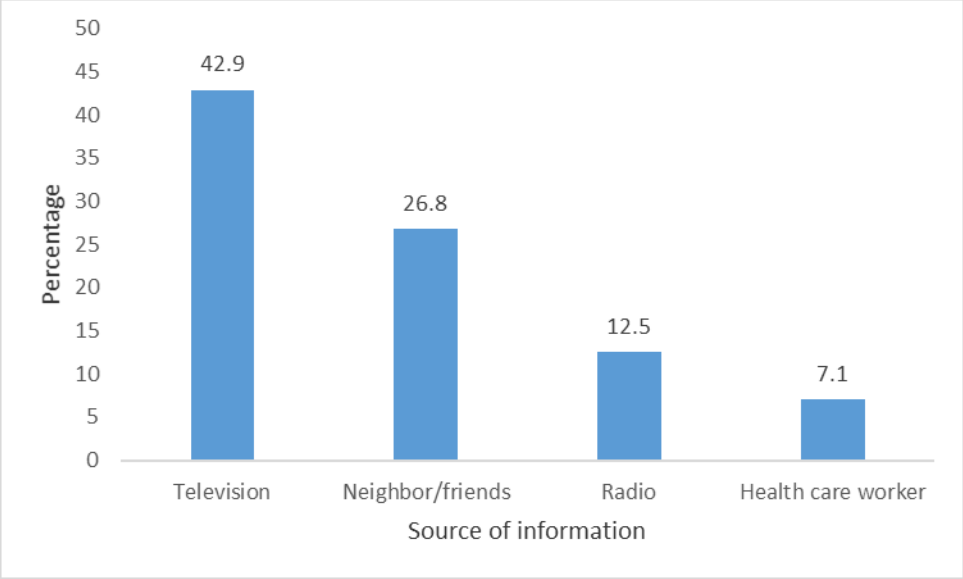

Figure 4.4: Sources of information on Folic acid fortified flour among pregnant women Attending Antenatal Clinic at Pumwani Maternity Hospital, October to November 2014

4.4.3: Folic acid knowledge

Of the 247 study participants, 90 (36.4%) could mention one or more benefits of folic acid to women of reproductive age and pregnant women. Of the 90 who could mention at least one benefit of folic acid, the majority (71.1%) mentioned prevention of anaemia as one of the benefits of folic acid (Figure 4.5)

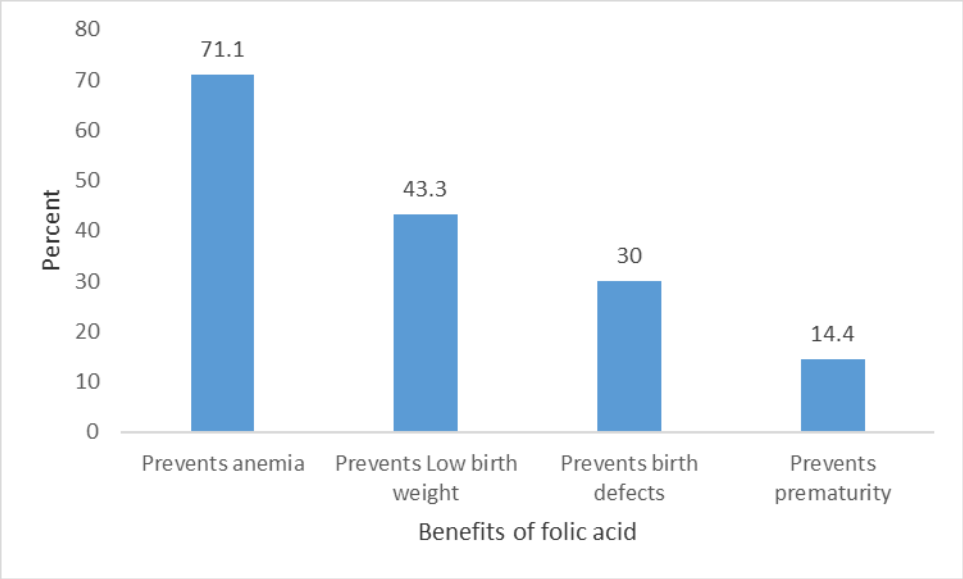

Figure 4.5: Benefits of folic acid mentioned by pregnant women Attending Antenatal Clinic at Pumwani Maternity Hospital, October to November 2014

4.5: Utilization of folate (Dietary, supplements and fortified flour)

4.5.1: Assessment of Dietary intake of folate

4.5.1.1: 24 hours' recall

The majority of the participants reported having consumed at least one serving of fruits (73.3%), ugali (68.8%), wheat containing foods (63.2%) and vegetables (61.5%) the previous day (Table 4.6)

Table 4.6: Foods consumed by pregnant women Attending Antenatal Clinic at Pumwani Maternity Hospital, 24 hours prior to questionnaire administration

Variable	Frequency	Percentage
Fruit	181	73.3
Ugali	170	68.8
Wheat containing foods	156	63.2
Vegetable	152	61.5
Legume	99	40.1
Meat	65	26.3
Roots and tubers	54	21.9
Eggs	34	13.8
Milk	33	13.4
Fish	14	5.7
Liver	9	3.6
Chicken	7	2.8

4.5.1.2: Food Frequency

The most frequently consumed food was bananas, ugali and green vegetables. These three foods were consumed daily by 139, 119 and 109 of the study participants respectively (Table 4.7)

Table 4.7: Frequency of consumption of the folate rich foods by pregnant women Attending Antenatal Clinic at Pumwani Maternity Hospital, October to November 2014

Food	Daily	4-6 days a week	1-3 days a week	Once in 2 weeks	Rarely	Never
Bananas	139	8	54	1	16	15
Ugali	119	41	75	0	3	0
Green vegetables	109	27	90	0	13	1
Oranges	72	11	54	2	74	27
Avocadoes	56	7	68	4	70	0
Pawpaw	30	9	39	4	92	63
Beans	20	9	134	9	50	18
Chapatis	17	11	102	42	60	0
Liver	4	2	61	14	96	58
Alcoholic beverages	0	0	1	0	6	231

4.5.2: Folic acid supplements utilization

Only 33 (13.4%) of the study participants reported that they were using folic acid supplements. Of the 33 participants who had started using folic acid supplements, 25 (75.8%) started the supplements in the first trimester and only 3 (9.1%) started before conception (Table 4.6 shows the period that the participants started using folic acid supplements).

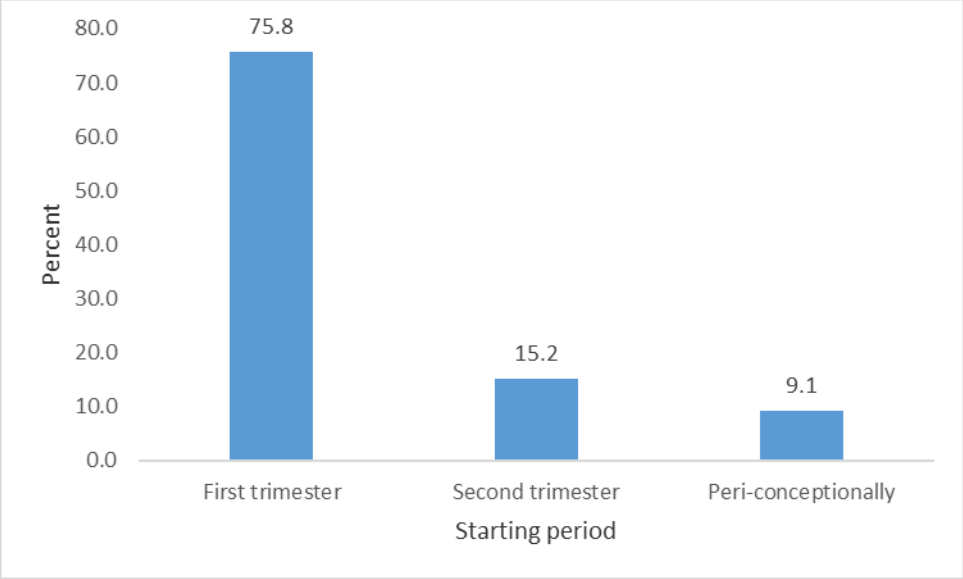

Figure 4.6: Folic acid supplements start period

4.5.3: Folic acid fortified flour utilization

4.5.3.1: Fortified Maize flour Utilization

The majority of study participants (87.7%), consumed maize flour produced from large-scale commercial milling industries while 30 (12.3%) obtained their maize flour from small-scale local mills. One hundred and ninety-eight participants (80.2 %) used folic acid fortified maize flour. Of the 217 participants who purchased maize flour produced by large scale millers, the majority (67.7%)purchased specific brands of maize flour because of taste. Only 4 (1.8%) purchased specific brands of maize flour because they were fortified (Figure 4.7).

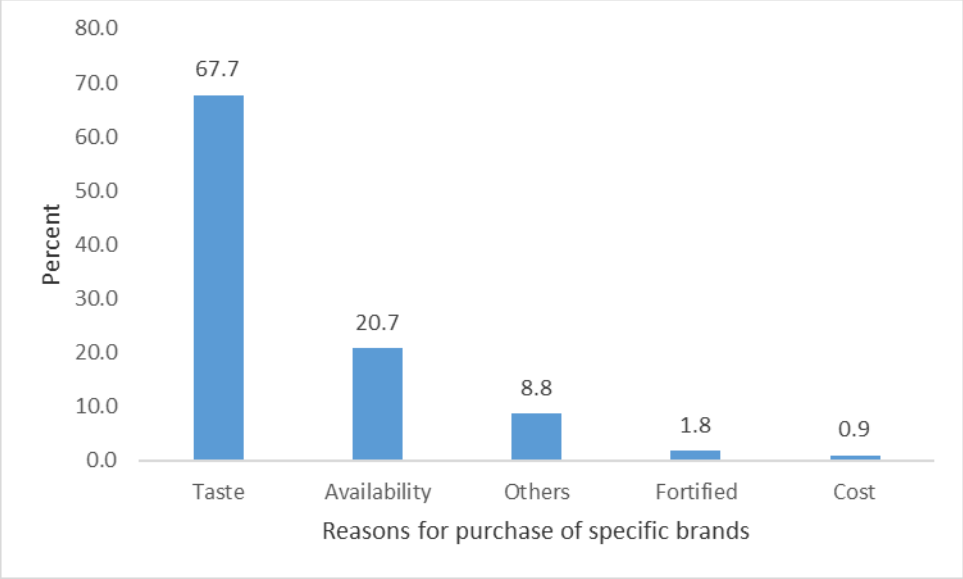

Figure 4.7: Reasons for purchasing specific brands of maize flour by pregnant women Attending Antenatal Clinic at Pumwani Maternity Hospital, October to November 2014

4.5.3.2: Fortified Wheat flour Utilization

The main source of wheat flour for all the participants was from purchase of flour produced from milling industries. Two hundred and five (84.4%) participants used folic acid fortified wheat flour. Two hundred and five study participants (82.9%) purchased specific brands of wheat flour because of their taste rather than fortification status (Figure 4.8)

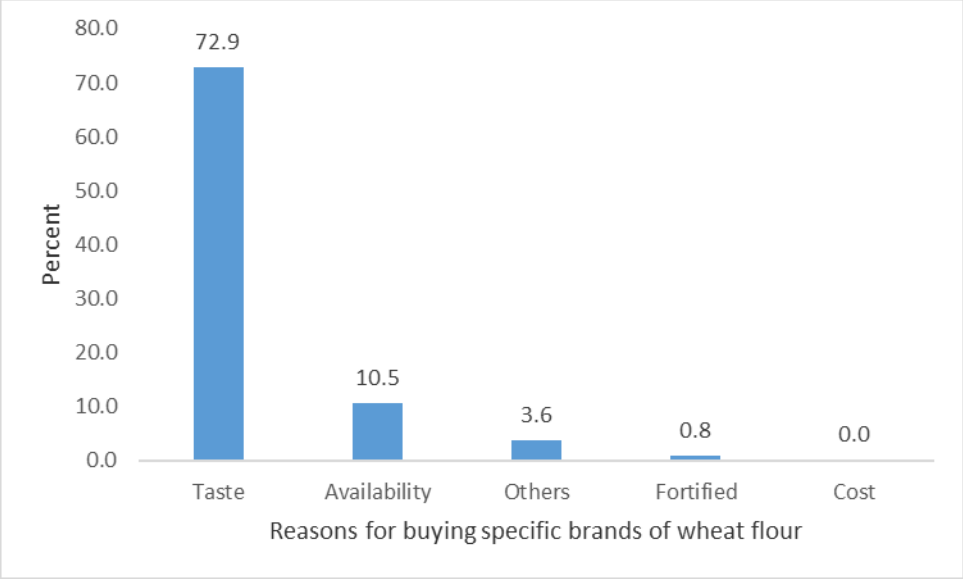

Figure 4.8: Reasons for buying specific brands of wheat flour by pregnant women Attending Antenatal Clinic at Pumwani Maternity Hospital, October to November 2014

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

5.1.1 Prevalence of folate deficiency

The study found the prevalence of folate deficiency among pregnant women attending Pumwani Maternity hospital to be low. The low prevalence of folate deficiency in our study was similar to findings from studies conducted in South Africa and Canada. In South Africa, the prevalence of folate deficiency among non-pregnant rural women of child bearing age was found to have reduced from 27.9% to 0% nine months after mandatory fortification of maize and wheat flour (Modjadji *et al*., 2008) whereas in Canada, a national survey conducted in the post mandatory fortification period reported a prevalence of 1% among the Canadian population (Colapinto *et al*., 2011). Our findings however are contrary to the findings of a community based study in Ethiopia among women of child bearing age and those of a study in Benin among HIV negative pregnant women at the time of first antenatal visit prior to any intervention (Ouédraogo *et al.*, 2012). These studies found prevalence of folate deficiency of 46% and 31.3% respectively (Haidar, 2010 ; Ouédraogo *et al*., 2012). A study conducted in Eastern Sudan among pregnant women also reported a much higher prevalence of 57.7% (Abdelrahim *et al.*, 2009). The three studies were conducted before the implementation of mandatory fortification in these countries while this study was conducted two years after the Government of Kenya amended the food, drug and chemical substances act to have mandatory fortification of maize flour and wheat flour with folic acid and other nutrients. The low folate prevalence of folate deficiency in this study could be attributed to the implementation of mandatory fortification of maize and wheat flour. In addition,

our study also found that all the participants were exposed to either fortified wheat flour or maize flour and this could also explain the low prevalence, additionally, the 24 hours recall and the food frequency information collected also showed that most of the study participants also consumed folate rich foods.

5.1.2 Folic acid awareness

The study also found that awareness on folic acid was high whereas knowledge on folic acid fortified flour was low. The high awareness on folic acid is similar to findings of studies conducted in Europe, United States of America and Nigeria among women of child bearing age. A large European survey found folic acid awareness to be 70% (Bitzer & Bannemerschult, 2013) and a national survey on trends in folic acid awareness and behavior in United States reported an increase in awareness from 52% in 1995 to 84% in the year 2005(Green-Raleigh, Carter, Mulinare, Prue, & Petrini, 2006). In Nigeria, folic acid awareness was found to be 64.6% (Anzaku, 2013). The findings are however contrary to those of studies conducted in Chile and China which found low levels of awareness on folic acid of 47% and 36% respectively(Pardo *et al.*, 2007 ; Ren *et al.*, 2006) and those of studies conducted in Kansas, Australia and Israel which reported higher levels of awareness of 88%, 89% and 85% respectively (Sharp *et al.*, 2009 ; Oddy *et al.*, 2007 ; Amitai *et al.*, 2004). The high awareness on folic acid could be due to media advertisements of iron folic supplements which were ongoing during the study period. The main source of information on folic acid was found to be the television. The proportion of those who heard of folic acid from health care workers was low. These findings are partly similar to those a national survey in United States where 26% of those who had heard about folic acid got the information from health care workers (Green-Raleigh *et al.*, 2006). The findings differ from those of a study conducted in Chile where the majority (54%) of those who had heard about folic acid got the information from midwives and doctors (Pardo *et al.*, 2007) and those of a study conducted in Nigeria where 64.4% of those who had heard about folic acid got the

information from health workers (Anzaku, 2013). The findings are also contrary to the findings of a study in Qatar where 63.4% of those who had heard about folic acid got the information from physicians (Bener *et al*., 2006).

5.1.3 Utilization of folic acid

Utilization of folic acid supplements (both peri-conceptionally and during pregnancy) was low while the utilization of folic acid fortified flour was high. There was low utilization of folic acid supplements which is similar to findings of studies in Kansas, Qatar and China which reported utilization of 25%, 20.3% and 15% respectively (Sharp *et al*., 2009 ; Bener *et al*., 2006 ; Ren *et al.*, 2006). This is contrary to the findings of a study conducted in a referral hospital in central Kenya where the utilization of folic acid supplements was found to be 51.2% (Maina *et al*., 2013) and those of a study in Senegal, Sudan and Zimbabwe where utilization of 97%, 92.1% and 83% respectively were reported (Fiedler, 2015 *;* Abdullahi *et al*, 2014 ; Matare *et al*., 2015). The low utilization of folic acid supplements could be due to recruitment of the study participants during their first ANC visit.

There were low levels of awareness on folic acid fortified flour and a low proportion of study participants who bought specific brands of maize and wheat flour due to fortification and yet utilization of folic acid fortified flour was high. This is because most of the study participants consumed maize and wheat flour from large scale millers, most of which produced fortified flour following the legislation on mandatory fortification of wheat flour. This highlights the importance of mandatory fortification programmes in improving the folate status of the population.

5.2 Conclusion

1. The prevalence of folate deficiency among pregnant women attending Pumwani Maternity Hospital was low possibly because of the implementation of mandatory fortification of maize and wheat flour.
2. There were low levels of knowledge on fortified flour while knowledge on folic acid supplements was high with low contribution of health care workers as a source of information
3. Utilization of folic acid fortified flour was high while the utilization of folic acid supplements was low

5.3 Recommendations

1. There is need to reinforce the implementation of the legislation on maize flour and wheat flour fortification

2. There is need to enhance health education in health facilities with inclusion of importance of folic acid consumption and sources of folic acid in the communication package

REFERENCES

Abdelrahim, I. I., Adam, G. K., Mohmmed, A. A., Salih, M. M., Ali, N. I., Elbashier, M. I., & Adam, I. (2009). Anaemia, folate and vitamin B12 deficiency among pregnant women in an area of unstable malaria transmission in eastern Sudan. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *103*(5), 493–6.

Abdullahi, H., Gasim, G. I., Saeed, A., Imam, A. M., & Adam, I. (2014). Antenatal iron and folic acid supplementation use by pregnant women in Khartoum, Sudan. *BMC Research Notes*, *7*(1), 498.

Allen, L. H. (2008). Causes of vitamin B12 and folate deficiency. *Food and Nutrition Bulletin*, *29*(2 Suppl), S20-34–7.

Amitai, Y., Fisher, N., Haringman, M., Meiraz, H., Baram, N., & Leventhal, A. (2004). Increased awareness, knowledge and utilization of preconceptional folic acid in Israel following a national campaign. *Preventive Medicine*, *39*(4), 731–7.

Anzaku, A. (2013). Assessing Folic Acid Awareness and its Usage for the Prevention of Neural Tube Defects Among Pregnant Women in Jos, Nigeria. *Journal of Basic and Clinical Reproductive Sciences*. Nigerian Medical Association, Enugu State.

Bailey, L. B. (2000). New standard for dietary folate intake in pregnant women. *The American Journal of Clinical Nutrition*, *71*(5 Suppl), 1304S–7S.

Bailey, L. B., & Gregory, J. F. (1999). Recent Advances in Nutritional Science Folate Metabolism and. *The Journal of Nutrition*, *129*, 779–782.

Bender, D. (2003). *Nutritional Biochemistry of the vitamins* (Second Edi). London: Cambridge University Press.

Bener, A., Al Maadid, M. G. A., Al-Bast, D. A. E., & Al-Marri, S. (2006). Maternal knowledge, attitude and practice on folic acid intake among Arabian Qatari women. *Reproductive Toxicology*, *21*(1), 21–25.

Bitzer, J., & Bannemerschult, R. (2013). Women ' s awareness and periconceptional use of folic acid : data from a large European survey. *International Journal of Women's Health, 5*, 201–213.

Castillo-Lancellotti, C., Tur, J. a, & Uauy, R. (2013). Impact of folic acid fortification of flour on neural tube defects: a systematic review. *Public Health Nutrition, 16*(5), 901–11.

Caudill, M. A. (2010). Folate bioavailability : implications for establishing dietary recommendations and optimizing status 1 – 4. *American Journal of Clinical Nutrition*, *91*(1), 1455–1460.

Cena, E. R., Joy, A. B., Heneman, K., & Zidenberg-Cherr, S. (2008). Low-income women in California may be at risk of inadequate folate intake. *California Agriculture*, *61*(2), 85–89.

Christianson, A., Howson, C. P., & Modell, B. (2006). March of dimes-global report on birth defects, the hidden toll of dying and disabled children. New york.

Colapinto, C. K., O'Connor, D. L., & Tremblay, M. S. (2011). Folate status of the population in the Canadian Health Measures Survey. *CMAJ : Canadian Medical Association Journal = Journal de l'Association Medicale Canadienne*, *183*(2), E100–E106.

Daly, S., Mills, J. L., Molloy, A. M., Conley, M., Lee, Y. J., Kirke, P. N., … Scott, J. M. (1997). Early reports Minimum effective dose of folic acid for food fortification to prevent neural-tube defects. *The Lancet, 350*, 6–9.

de Benoist, B. (2008). Conclusions of a WHO Technical Consultation on folate and vitamin B12 deficiencies. *Food and Nutrition Bulletin*, *29*(2 Suppl), S238-44.

Fekete, K., Berti, C., Trovato, M., Lohner, S., Dullemeijer, C., Souverein, O. W., … Decsi, T. (2012). Effect of folate intake on health outcomes in pregnancy: a systematic review and meta-analysis on birth weight, placental weight and length of

gestation. *Nutrition Journal*, *11*(1), 75.

Fiedler, J. L. . D. A. S. (2015). A rapid initial assessment of the distribution and consumption of iron-folic acid tablets through antenatal care in Senegal.

García-Casal, M. N., Osorio, C., Landaeta, M., Leets, I., Matus, P., Fazzino, F., & Marcos, E. (2005). High prevalence of folic acid and vitamin B12 deficiencies in infants, children, adolescents and pregnant women in Venezuela. *European Journal of Clinical Nutrition*, *59*(9), 1064–1070.

Global Progress- Flour Fortification Initiative. (n.d.).

Green-Raleigh, K., Carter, H., Mulinare, J., Prue, C., & Petrini, J. (2006). Trends in folic Acid awareness and behavior in the United States: the Gallup Organization for the March of Dimes Foundation surveys, 1995-2005. *Maternal and Child Health Journal*, *10*(5 Suppl), S177-82.

Gregory, J. F. (2012). Accounting for differences in the bioactivity and bioavailability of vitamers. *Food and Nutrition Research*, *56*, 1–11.

Haidar, J. (2010). Prevalence of anaemia, deficiencies of iron and folic acid and their determinants in ethiopian women. *Journal of Health, Population and Nutrition*, *28*(4), 359–368.

Haidar J, M. U. and P. R. (2010). Folate deficiency in women of reproductive age in nine administrative regions of Ethiopia : an emerging public health problem. *African Journal of Clinical Nutrition*, *23*(July 2005), 132–137.

Hema, G., & Pyush, G. (2004). Review Article Neural Tube Defects and Folic Acid. *Indian Paediatric Journal*, *41*, 577–586.

Herbert, V. (1973). The flve possible causes illustrated by deflciencies and folic acid1 ’ 2 ’ 3 of all nutrient deFiciency : of vitamin. *American Journal of Clinical Nutrition*, *26*, 77–86.

Hertrampf, E., & Corte, F. (2004). Folic Acid Fortification of Wheat Flour : Chile.

Nutrition Reviews, *62*(6), S44–S48.

Hoffbrand, A. (2001). Folate absorption. *Journal of Clinical Pathology*, *24*(5), 66–76.

Hoffbrand, A., & Weir, D. (2001). Historical Review of folic acid. *Scandinavian Journal of Nutrition*, *43*, 138–146.

Honein, M. a, Paulozzi, L. J., Mathews, T. J., Erickson, J. D., & Wong, L. Y. (2001). Impact of folic acid fortification of the US food supply on the occurrence of neural tube defects. *JAMA : The Journal of the American Medical Association*, *285*(23), 2981–2986.

Hoyo, C., Murtha, A. P., Schildkraut, J. M., Forman, M. R., Calingaert, B., Demark-Wahnefried, W., … Murphy, S. K. (2011). Folic acid supplementation before and during pregnancy in the Newborn Epigenetics STudy (NEST). *BMC Public Health*, *11*(1), 46.

Jansen, E. H. J. M., Beekhof, P. K., Cremers, J. W. J. M., & Schenk, E. (2012). Long-term (in)stability of folate and vitamin B12 in human serum. *Clinical Chemistry and Laboratory Medicine*, *50*(10), 1761–1763.

Karaoglu, L., Pehlivan, E., Egri, M., Deprem, C., Gunes, G., Genc, M. F., & Temel, I. (2010). The prevalence of nutritional anemia in pregnancy in an east Anatolian province, Turkey. *BMC Public Health*, *10*, 329.

Li, M., McDermott, R., D'Onise, K., & Leonard, D. (2012). Folate status and health behaviours in two Australian Indigenous populations in north Queensland. *Public Health Nutrition*, *15*(10), 1959–1965.

Lorenzo, B., Cynthia, M., Muin, K., & David, E. (1999). Features. *New England Journal of Medicine*, *321*(20), 1509–1519.

Maina-Gathigi, L., Omolo, J., Wanzala, P., Lindan, C., & Makokha, A. (2013). Utilization of folic acid and iron supplementation services by pregnant women attending an antenatal clinic at a regional referral hospital in Kenya. *Maternal and*

Child Health Journal, *17*(7), 1236–42.

Martínez-Frías, M. L. (2006). Folic acid: a public-health challenge. *Lancet*, *367*, 2057.

Matare, C., Mbuya, M., Dickin, K., Humphrey, J., & Stoltzfus, R. (2015). Social Support and Depressive Symptoms Predict Adherence To Iron And Folic Acid Supplements Among Pregnant Women In Rural Zimbabwe. *FASEB J*, *29*(1_Supplement), 729.18-.

McDowell, M. a, Lacher, D. a, Pfeiffer, C. M., Mulinare, J., Picciano, M. F., Rader, J. I., … Johnson, C. L. (2008). Blood folate levels: the latest NHANES results. *NCHS Data Brief*, (6), 1–8.

McKillop, D. J., Pentieva, K., Daly, D., McPartlin, J. M., Hughes, J., Strain, J. J., … McNulty, H. (2002). The effect of different cooking methods on folate retention in various foods that are amongst the major contributors to folate intake in the UK diet. *The British Journal of Nutrition*, *88*(6), 681–688.

McLean, E., de Benoist, B., & Allen, L. H. (2008). Review of the magnitude of folate and vitamin B12 deficiencies worldwide. *Food and Nutrition Bulletin*, *29*(2 SUPPL.), 38–51.

Mills, J. L., & Signore, C. (2004). Neural tube defect rates before and after food fortification with folic acid. *Birth Defects Research Part A - Clinical and Molecular Teratology*, *70*(11), 844–845.

Modjadji, S. E. P., Alberts, M., & Mamabolo, R. L. (2008). Folate and iron status of South African non-pregnanat rural women of childbearing age, before and after fortification of foods. *South African Journal of Clinical Nutrition*, *20*(3), 89–95.

Molloy, A. M., Mills, J. L., Kirke, P. N., Whitehead, A. S., Weir, D. G., & Scott, J. M. (1998). Whole-Blood Folate Values in Subjects with Different Methylenetetrahydrofolate Reductase Genotypes: Differences Between the Radioassay and Microbiological Assays. *Clin. Chem.*, *44*(1), 186a–188.

Muga, R. (2009). 1* , 2 . 3. *African Journal of Food, Agriculture, Nutrition and Development*, *9*(3), 814–829.

Norwood, D. (2011). Folic Acid Deficiency. *Nucleus Medical Media*, 4–7.

Oddy, W. H., Miller, M., Payne, J. M., Serna, P., & Bower, C. I. (2007). Awareness and consumption of folate-fortified foods by women of childbearing age in Western Australia. *Public Health Nutrition*, *10*(10), 989–95.

Oner, N., Vatansever, U., Karasalihoğlu, S., Ekuklu, G., Celtik, C., & Biner, B. (2006). The prevalence of folic acid deficiency among adolescent girls living in Edirne, Turkey. *The Journal of Adolescent Health : Official Publication of the Society for Adolescent Medicine*, *38*(5), 599–606.

Ouédraogo, S., Koura, G. K., Accrombessi, M. M. K., Bodeau-Livinec, F., Massougbodji, A., & Cot, M. (2012). Maternal anemia at first antenatal visit: Prevalence and risk factors in a malaria-endemic area in Benin. *American Journal of Tropical Medicine and Hygiene*, *87*(3), 418–424.

Pardo V, R. A., Lay-Son R, G., Aranda Ch, W., Dib M, M., Espina M, P., Munoz K, M. J., … Tenhamm T, T. (2007). [Awareness and knowledge of the use of folic acid in the prevention of neural tube defects: a survey of women living in Santiago, Chile]. *Revista Medica de Chile*, *135*, 1551–7 ST–[Awareness and knowledge of the use o.

Pardo V, R. A., Lay-Son R, G., Aranda Ch, W., Dib M, M., Espina M, P., Muñoz K, M. J., … Tenhamm T, T. (2007). [Awareness and knowledge of the use of folic acid in the prevention of neural tube defects: a survey of women living in Santiago, Chile]. *Revista médica de Chile*, *135*(12), 1551–7.

Ray, J. G., Vermeulen, M. J., Boss, S. C., & Cole, D. E. C. (2002). Declining rate of folate insufficiency among adults following increased folic acid food fortification in Canada. *Canadian Journal of Public Health = Revue Canadienne de Santé Publique*, *93*(4), 249–53.

Ren, A., Zhang, L., Li, Z., Hao, L., Tian, Y., & Li, Z. (2006). Awareness and use of folic acid, and blood folate concentrations among pregnant women in northern China--an area with a high prevalence of neural tube defects. *Reproductive Toxicology (Elmsford, N.Y.)*, *22*(3), 431–6.

Richard, F., Gari, S., Edward, L., & Rosenquist Thomas. (2008). Gene-nutrient interactions: importance of folic acid and vitamin B12 during early embryogenesis | Read by QxMD. *Food and Nutrition Bulletin*, *29*(2), S86-100.

Rogers, M., Bailey, B., & Gregory, F. (1997). Absorption of folate from fortified cereal-grain products and of supplemental folate consumed with or without food determined by using a dual-label. *American Journal of Clinincal Nutrition*, *66*, 1388–1397.

Sacn. (2006). Folate and Disease Prevention, *retrived from https://assets.publishing.service.uk/government/uploads/attachment_data/file338892/SACN_folate and disease prevention_report.pdf*

S E P Modjadji and M Alberts. (2007). Folate and iron status of South African non-pregnant rural women of childbearing age , before and after fortification of foods. *South African Journal of Clinical Nutrition*, *20*(3), 89–93.

Sayed, A.-R., Bourne, D., Pattinson, R., Nixon, J., & Henderson, B. (2008). Decline in the prevalence of neural tube defects following folic acid fortification and its cost-benefit in South Africa. *Birth Defects Research. Part A, Clinical and Molecular Teratology*, *82*(4), 211–216.

Schall, I. (1996). Dietary and serum folate : of. *American Journal of Clinical Nutrition*, *63*, 520–525.

Scholl, T. O., & Johnson, W. G. (2000). Folic acid: influence on the outcome of pregnancy. *The American Journal of Clinical Nutrition*, *71*(5 Suppl), 1295S–303S.

Shahab-Ferdows, S., Engle-Stone, R., Hampel, D., Ndjebayi, A., Nankap, M., Brown,

K., & Allen, L. (2014). Risk factors for folate deficiency differ from those for vitamin B12 deficiency in Cameroonian women and children (119.7). *FASEB J*, *28*(1_Supplement), 119.7-.

Shane, B. (2011). Folate status assessment history : implications for measurement of. *American Journal of Clinical Nutrition*, *10*, 1S–6S.

Sharma, J. B., & Shankar, M. (2010). Anemia in Pregnancy . *Indian Journal of Medical Research*, *23*(4), 253–260.

Sharp, G. F., Naylor, L. A., Cai, J., Hyder, M. L., Chandra, P., & Guillory, V. J. (2009). Assessing awareness, knowledge and use of folic acid in Kansas women between the ages of 18 and 44 years. *Maternal and Child Health Journal*, *13*(6), 814–21.

Shi, Y., Groh, M. de, & MacFarlane, A. J. (2014). Socio-demographic and lifestyle factors associated with folate status among non-supplement-consuming Canadian women of childbearing age. *Can J Public Health*, *105*(3), e166–e171.

Snow, C. F. (1999). Laboratory Diagnosis of Vitamin B12 and Folate Deficiency. *Archives of Internal Medicine*, *159*(12), 1289.

Stover, B. S. P. (2008). Folate and vitamin B12 deficiencies. Proceedings of a WHO technical consultation held 18-21 October, 2005, in Geneva, Switzerland. *Food and Nutrition Bulletin*, *29*(2 Suppl).

Terry Wefwafwa, Lucy Gathigi , Grainne Mairead Moloney, R. S. and E. (2012). Fortification of wheat flour, maize flour and oil goes mandatory. *retrieved from https://www.humanitarianresponse.info/files/documents/files/April-June 2012/kenya_nutrition_Bulletin/volume 2.pdf*

Toriello, H. V. (2011). Policy statement on folic acid and neural tube defects. *Genetics in Medicine : Official Journal of the American College of Medical Genetics*, *13*(6), 593–596.

Toriello, H. V, Practice, P., & Committee, G. (2005). Folic acid and neural tube defects.

American College of Medical Genetics, *7*(4), 283–284.

Ulrich, C. M., Reed, M. C., & Nijhout, H. F. (2008). Modeling folate, one-carbon metabolism, and DNA methylation. *Nutrition Reviews*, *66*(SUPPL.1), 27–30.

VanderJagt, D. J., Brock, H. S., Melah, G. S., El-Nafaty, A. U., Crossey, M. J., & Glew, R. H. (2007). Nutritional Factors Associated with Anaemia in Pregnant Women in Northern Nigeria, *25*(1), 75–81.

WHO/FAO. (2006). Guidelines on food fortification with micronutrients. *www.who.int/nutrition/publications/guide_food_fortification_micronutrients.pdf*, accessed May 2018

WHO. (2007). Integrated Management of Pregnancy and Childbirth. *www.who.int_child_adolescent/maternal/en/,* accessed May 2018

APPENDICES

APPENDIX 1: INFORMED CONSENT

English Version

INFORMED CONSENT FORM TO PARTICIPATE IN STUDY – "Factors associated with folate deficiency among pregnant women attending antenatal clinic at Pumwani Maternity Hospital"

Please read the information provided below or have it read to you carefully before signing this form. In case of any questions, kindly ask before signing the consent form.

Purpose

We are conducting a study to measure blood folate levels in pregnant women. The purpose of this study is to identify possible factors associated with folate deficiency. As part of the study we are taking a blood sample of pregnant women during their first antenatal care visit. This sample will be used only to measure folate levels. This will help to make recommendations which will help in the reduction of the magnitude of the problem.

It is important to understand that: your participation is totally voluntary, you are free to make inquiries so as to fully understand the study and you have the freedom to decide to participate in the study or not to participate in the study.

In case of any further questions, contact: Elizabeth Adhiambo Mgamb, mobile phone number: 0723 350894, email: elizabethmgamb@yahoo.com

In case you would like to ask someone other than the researcher, you are encouraged to contact the following:

The Director, Institute of Tropical Medicine and Infectious Diseases (ITROMID)

Jomo Kenyatta University of Agriculture and Technology (JKUAT)

P.O. box 62000- 00200 Nairobi

Tel: 067-52711

Email: itromid@nairobi.mimcom.net

Or

Prof. A. N. Guantai
Chairperson, Kenyatta National Hospital/ University of Nairobi- Ethical Review Committee
P.O. box 20723- 00202 Nairobi, Kenya
Tel: +254 20 726300-9 or +254 20 726300 Ext 44355
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Expectations during participation

I will ask you a few questions about yourself, your spouse, your family, your current and previous births and pregnancies and your lifestyle and will take a blood sample from you for the analysis of folate levels in the laboratory.

Harm and/or risks and/or discomforts

We do not anticipate any harm or risk for you but you will experience some pain during the collection of blood samples; to make it easier for you we will collect the sample when the clinician will be collecting the blood samples for antenatal profile tests so as to avoid pricking you twice. We would therefore like to encourage you to participate without any fears.

Benefits

It will cost you nothing to participate in the study. You and the clinic will be given feedback on the laboratory results so that if your folate levels are low you will be able to receive treatment. You will also be given more information about folic acid and flour fortification. You will be contributing valuable information that will help the government implement services to prevent folate deficiency.

Privacy of records

Confidentiality will be maintained throughout the study; you will be interviewed privately and your name will be recorded so as to give you feedback on the results of the test but your name will not appear anywhere in our final report. All information

collected will not be shared and will be stored in a way that cannot be accessed by other people. Any personal information from the interview will not be released unless with your written permission.

Declaration of participant

I Miss/Mrs.……do hereby give consent/ assent to Mrs. Elizabeth Mgamb to include me in the proposed study entitled 'Factors associated with folate deficiency among pregnant women attending antenatal clinic for the first visit at Pumwani Maternity Hospital, Kenya'. I have read the information sheet and have understood the purpose of the study and what will be required on my part if I agree to take part in the study. Any questions I have concerning my involvement in the study have been adequately clarified. I understand that I can discontinue from the study at any stage without any consequences. I also understand that I will be interviewed, examined and my blood sample will be taken to the laboratory for measuring folate levels. I therefore consent voluntarily to participate in the study.

Respondent's signature (left thumb print) ……

Date……

Name of person taking consent……

Signature……*Date*……

Kiswahili Version

IDHINI YA KUSHIRIKI KATIKA UTAFITI – "Nini husababisha upungufu wa madini ya "folate" mwilini kwa wanawake wajawazito wanakuja katika cliniki ya wajawazito katika hospitali ya Pumwani Maternity"

Tafadhali soma habari ifuatayo kwa makini au uhakikishe umesomewa na ukaelewa kabla ya kutia sahihi kutoa idhini ya kushiriki katika utafiti huu. Ukiwa na maswali yoyote, muulize mtafiti kabla ya kutia sahihi.

Madhumuni ya utafiti huu.

Madhumuni ya utafiti huu ni kupeleleza ni nini kinachosababisha upungufu wa madini ya "folate" mwilini kwa wanawake wajawazito wanaokuja katika cliniki ya wajawazito katika hospitali ya Pumwani Maternity kwa mara ya kwanza. Katika utafiti huu, tutachukua damu kutoka kwa watakaoshiriki katika utafiti huu ili kupima kiwango cha madini ya "folate" kwenye damu. Tutapima tu kiwango cha folate pekee. Matokeo ya utafiti huu yatasaidia katika kupendekeza sera za kupunguza shida hii.

Unajulishwa kuwa utashiriki kwa hiari yako na ikiwa kuna masuala ambayo hujaelewa ni vyema kuuliza kabla ya kukubali. Tena una uhuru wa kukataa kushiriki au kukomesha ushiriki wakati wowote utakaoamua bila ya kuchukuliwa hatua yoyote.

Kwa maswali yoyote muulize: Elizabeth Adhiambo Mgamb.

Nambari ya simu ya rununu: 0723 350894, Barua pepe: elizabethmgam@yahoo.com

Ukiwa na suala lolote kuhusu utafiti huu na ungependa kumuuliza mtu mwingine tofauti na anayefanya utafiti, unahimizwa kupata ushauri kutoka kwa:

Director, Institute of Tropical Medicine and Infectious Diseases (ITROMID)

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Au

Prof. A. N. Guantai

Mwenyekiti, kamati ya kushugulikia maadili ya utafiti ya hospitali kuu ya kitaifa ya Kenyatta.

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Matarajio

Nitakuuliza maswali machache kukuhusu, kuhusu mume wako (kama umeolewa), kuhusu familia yako, mimba iliyopo na uzazi uliyopita na pia kuhusu maisha yako kisha

nitachukua damu ili niweze kupeleka katika mahabara kupima kiwango cha madini ya “folate” kwenye damu.

Madhara/ hatari ya kushiriki

Hatutarajii madhara au hatari yoyote kwako ukishiriki katika utafiti huu lakini utahisi uchungu kidogo tutakapotoa damu; ili kuhakikisha hujapata uchungu zaidi, tutachukua damu wakati daktari atakapokuwa akichukua damu ambayo huchukuliwa kuchunguza hali ya afya ya mama wakati anapoanza kuja cliniki ya wanawake wajawazito. Tukifanya hivyo, tutazuia kukudunga mara mbili. Kwa hivyo unaombwa kushiriki bila wasiwasi wowote.

Manufaa

Hakuna gharama yoyote utakayopitia kwa kushiriki katika utafiti huu. Tutakujulisha wewe na muuguzi matokeo ya mahabara pindi tu yatakapokuwa tayari na ikiwa utapatikana na upungufu wa madini ya “folate”, utapokea matibabu. Unaposhiriki katika utafiti huu, utapewa nasaha kuhusu madini ya “folate”, umuhimi wake na umuhimu wa kutumia unga ulioongezwa madini. Matokeo ya utafiti huu yatasaidia kubuni sera na mikakati ya kutatua shida hii katika jamii.

Hifadhi ya utafiti

Mahojiano yatafanyika faraghani. Habari na mahojiano ya utafiti huu itawekwa kwa umakini na siri. Ingawa tutakuwa na jina lako, Jina lako halitatajwa kwenye ripoti ya utafiti huu. Habari yako ya kibinafsi haitatolewa kwa mtu yeyote bila ya idhini yako.

SEHEMU YA PILI: Fomu ya idhini

Arifa ya mhojiwa wa hiari

Mimi Bi…….. natoa ruhusa kwa Bi Elizabeth Adhiambo Mgamb kunihusisha kwa utafiti, 'Nini inayosababisha upungufu wa madini ya folate mwilini kwa wanawake wajawazito wanaoukuja katika cliniki ya wajawato ya hospitali ya Pumwani Maternity, Kenya'. Nimesoma nakala ya habari kuhusu utafiti huu, nimeelewa madhumuni ya utafiti huu na pia yatakayotarajiwa kwangu nikikubali kushiriki. Maswala yote kuhusu kuhusika kwangu kwenye utafiti huu yamejibiwa kikamilifu. Nimeelewa kwamba ninaweza kukoma kushiriki bila ya mimi kuchukuliwa hatua yoyote. Naelewa nitahojiwa, nitapimwa tumbo na damu yangu itachukuliwa ili ipelekwe kwenye mahabara kupima madini ya "folate". Nakubali kwa hiari yangu kushiriki kwenye utafiti huu.

Sahihi ya mhojiwa (alama yakidole gumba kushoto) ……

Tarehe……

Jina la anayepewa ruhusa……..

Sahihi……..Tarehe

APPENDIX 2: RESEARCH QUESTIONNAIRE

Questionnaire No: _____

Date: _____

ANC No: _____

Phone No: _____

Serum Folate: _____

SECTION A: SOCIODEMOGRAPHIC INFORMATION

1. What is your date of birth? (If can't remember the date then record the year of birth) _____
2. What is your religion?

   ☐ Christian

   ☐ Muslim

   Others (Specify) _____

3. What is your level of education?

   ☐ None

   ☐ Primary

   ☐ Secondary

☐ College

☐ University

4. What is your employment status?

☐ Employed

☐ Self-Employment

☐ Unemployed

5. What is your marital status?

☐ Married

☐ Single

☐ Divorced/Separated

☐ Widowed

☐ Cohabiting

If not married, skip to question 9

6. What is your spouse's level of education?

☐ None

☐ Primary

☐ Secondary

☐ College

☐ University

7. What is your spouse's employment status

☐ Employed

☐ Self Employed

☐ Unemployed

SECTION B: OBSTETRIC AND GYNAECOLOGIC INFORMATION

8. When was your last menstrual period? (Date) _____
9. Gestation in weeks (estimate from the date of the last menstrual period) _____
10. Fundal Height (from review of ANC register) _____
11. How many biological children do you have? _____

If this is the first pregnancy, skip to question 18.

12. How many of your children are below 5 years old? _____
13. How many pregnancies have you carried to nine months? _____
14. Have you ever delivered any of the babies prematurely? (explain the definition of prematurity)

    ☐ Yes

    ☐ No

15. If yes, how many of the babies have you delivered prematurely? _____
16. When was your last pregnancy? (not counting this one)_____
17. Have you used any family planning methods before?

    ☐ Yes

    ☐ No

18. If yes, which family planning method have you used before this pregnancy? (check all that apply)

    ☐ None

    ☐ Oral contraceptives

    ☐ Injection

☐ Implant

☐ Intra Uterine Contraceptive Devices

Others, specify _____

19. How long have you used the family planning methods marked above? _____
20. Antenatal profile (from the file)

Hemoglobin _____

VDRL _____

SECTION C: FACTORS ASSOCIATED WITH FOLATE DEFICIENCY

21. In a week, how many times do you eat ugali? _____
22. Which type of maize flour do you use?

☐ From local mills

☐ From large scale millers

☐ Sometimes from local mills and sometimes from large scale mills

23. If from large scale millers, which brand? _____

24. If sometimes local and sometimes from large scale millers, which one did you use in the last one month?

☐ From local mills

☐ From large scale millers

25. If from large scale millers, which brand did you use? _____

26. Which type of wheat flour do you use?

☐ From local mills

☐ From large scale millers

27. If from large scale manufacturers, which brand do you use? _____

28. How many times in a week do you eat chapattis? _____

29. In a week, how many times do you eat mandazi? _____

30. Have you ever heard about folic acid or folate?

☐ Yes

☐ No

If no to question 31, skip to question 34

31. If yes, where did you hear about folic acid?

☐ Television

☐ Radio

☐ Health Care Worker

☐ Neighbors

☐ Friends

Others, specify _____

32. If you have heard about folic acid, do you know why it is important for women of childbearing age to consume it? (check that apply)

☐ Prevents birth defects

☐ Prevents anemia

☐ To prevent preterm births

☐ To prevent delivery of babies with low birth weight

Others, specify_____

33. Did you use any folic acid supplements before this pregnancy?

☐ Yes

☐ No

34. If yes, when did you begin? _____

35. What dose/how many tablets did you take? _____

36. How often did you take the supplements? _____

37. Did you use any folic acid supplements during this pregnancy?

☐ Yes

☐ No

If No to question 38, skip to question 42

38. If yes, when did you begin? _____

39. What dose/how many tablets did you take? _____

40. How often did you take the supplements?

41. Have you ever heard about fortified flour?

☐ Yes

☐ No

If no to question 42, skip to question 49

42. If yes, how did you know about fortified flour?

☐ Radio

☐ Television

☐ Health Care Worker

☐ Friend

☐ Neighbor

Others, specify _____

43. Why is flour fortified?

☐ To prevents birth defects

☐ To prevents anemia

☐ To prevent preterm births

☐ To prevent low birth weight

Others, specify_____

44. When you go to buy maize or wheat flour, will you be able to know whether it is fortified or not when you look at the packet?

☐ Yes

☐ No

45. If yes to question 45, how do you know whether it is fortified? _____
46. Do you think the maize or wheat flour you consume is fortified flour?

☐ Yes

☐ No

47. If no, why do you consume the unfortified flour? _____

☐ It is expensive

☐ Unaware

☐ No access to fortified flour

☐ Don’t know

48. Do you suffer from any chronic illnesses?

☐ Yes

☐ No

If no, go to question 56

49. If yes, which illness? (check all that apply)

☐ Diabetes Mellitus

☐ Hypertension

☐ Epilepsy

☐ Asthma

☐ Heart Disease

☐ Liver Disease

Others, specify _____

50. When were you diagnosed with the disease? (Record the year)_____
51. Are you currently on medications to treat that disease (s)?

☐ Yes

☐ No

52. If yes, which medication? _____
53. Since when are you taking the medication
54. How often do you take the medication
55. Since you became pregnant, have you suffered from any of the following?

☐ Fever

☐ Malaria

☐ Pneumonia

☐ Urinary tract infection

Others, specify_____

If no, skip to question 61

56. If yes, when were you ill? (record the date or month) _____
57. Which medication did you use? _____
58. When did you started taking the medication?
59. How often did you take the medication?
60. Have you ever smoked cigarettes?

☐ Yes

☐ No

If no, go to question 65

61. If yes, when did you start smoking? (enter the year) _____
62. Currently do you smoke?

☐ Yes

☐ No

63. If no, when did you stop smoking? (enter the year) _____
64. Have you ever taken any type of alcohol?

☐ Yes

☐ No

If no, the interview is over.

65. If yes, when did you start taking alcohol? (enter the year) _____

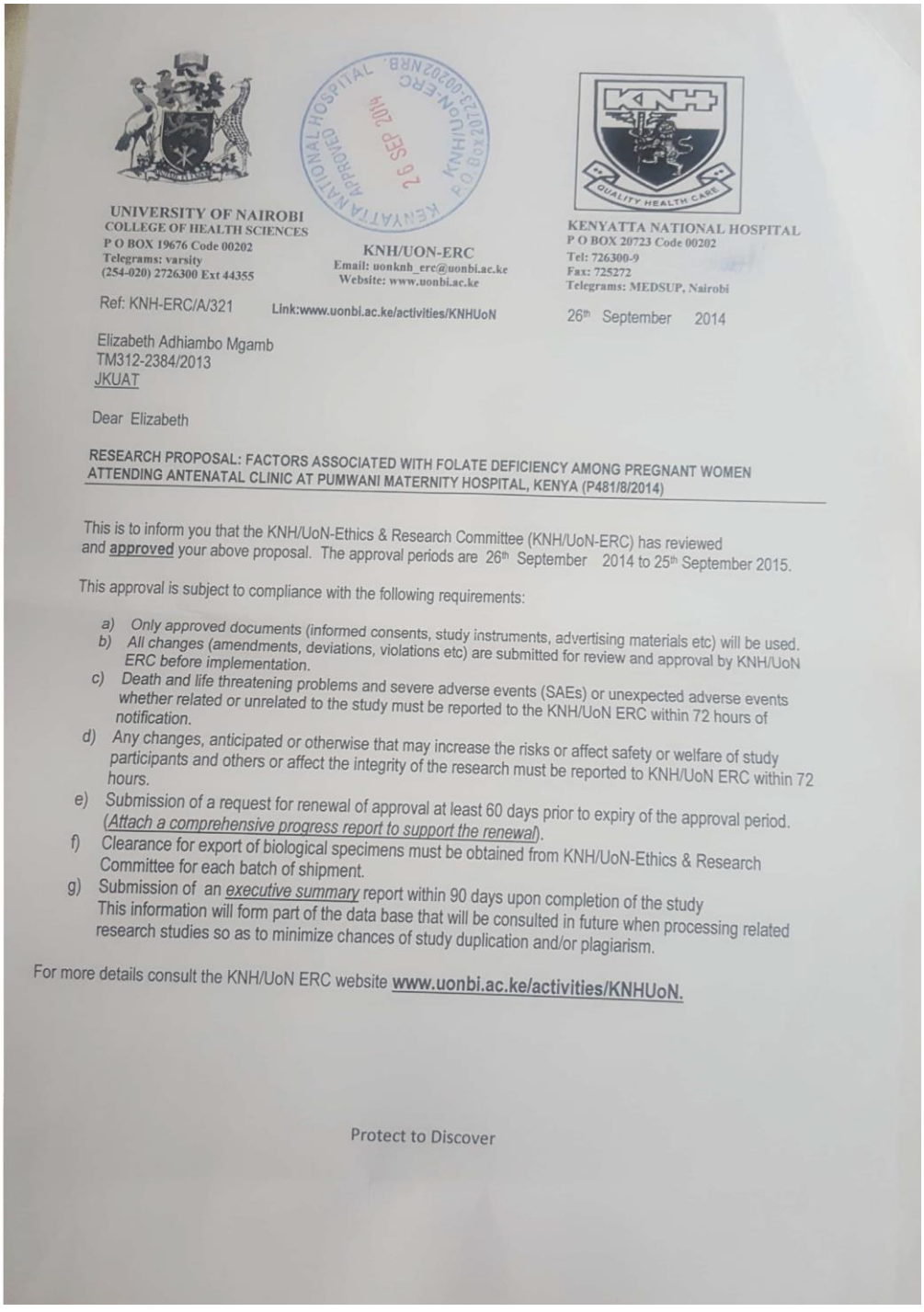
66. Currently do take alcohol?

☐ Yes

☐ No

67. If no, when did you stop drinking? (enter the year) _____

APPENDIX 3: ETHICAL APPROVAL



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Ref: KNH-ERC/A/321 Link:www.uonbi.ac.ke/activities/KNHUoN

26th September 2014

Elizabeth Adhiambo Mgamb
TM312-2384/2013
JKUAT

Dear Elizabeth

RESEARCH PROPOSAL: FACTORS ASSOCIATED WITH FOLATE DEFICIENCY AMONG PREGNANT WOMEN ATTENDING ANTENATAL CLINIC AT PUMWANI MATERNITY HOSPITAL, KENYA (P481/8/2014)

This is to inform you that the KNH/UoN-Ethics & Research Committee (KNH/UoN-ERC) has reviewed and **approved** your above proposal. The approval periods are 26th September 2014 to 25th September 2015.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc) are submitted for review and approval by KNH/UoN ERC before implementation.
c) Death and life threatening problems and severe adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH/UoN ERC within 72 hours of notification.
d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH/UoN ERC within 72 hours.
e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
f) Clearance for export of biological specimens must be obtained from KNH/UoN-Ethics & Research Committee for each batch of shipment.
g) Submission of an *executive summary* report within 90 days upon completion of the study This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/or plagiarism.

For more details consult the KNH/UoN ERC website **www.uonbi.ac.ke/activities/KNHUoN.**

Protect to Discover

APPENDIX 4: SERUM FOLATE MEASUREMENT PROTOCOL

Elecsys® Folate RBC

Electrochemiluminescence immunoassay (ECLIA) for the in vitro quantitative determination of folate in erythrocytes (red blood cells, RBC)

Indication

Folate is essential for normal metabolism, DNA synthesis and red blood cell regeneration. Untreated deficiencies may lead to megaloblastic anemia. For diagnosis of folate deficiency, it is recommended to perform determinations not only in serum but also in erythrocytes.5 More than 95 % of folate occurs in the red blood cells. The folate concentration in erythrocytes more truly reflects the overall folate concentration in the tissue while the serum or plasma level reflects the preceding uptake of folates from the food and fluctuates significantly with the diet. Following dietary deprivation of folate, serum levels decline within 3 weeks, but RBC folate levels remain the same for 3 – 4 months.2, 3, 4 Therefore, the Elecsys Folate RBC assay is used as an aid in the diagnosis of folate deficiency in erythrocytes.

Preparation of the hemolysate sample

Whole blood treated with anticoagulants (heparin or EDTA) is mixed with ascorbic acid solution and incubated for approximately 90 minutes at 20 – 25°C. Lysis of the erythrocytes takes place, with liberation and stabilization of the intracellular folate. The resulting hemolysate sample is then used for subsequent measurement. 3.0 mL of Folate RBC Hemolyzing Reagent 100 µL of well-mixed whole blood Incubate with closed caps for 90±15 minutes at 20–25°C Hemolysate sample ready for measurement

Test principle: Competition principle Folate bound to its binding proteins Measurement Free Folate 9 min 9 min 9 min Ruthenium labeled folate binding protein Ru Ru R u Ru Biotinylated folate Streptavidin microparticle Ru + +

1st incubation (9 minutes)

By incubating 25 µL of sample with the folate pretreatment reagents 1 and 2, bound folate is released from endogenous folate binding proteins.

2nd Incubation (9 Minutes)

By incubating the pretreated sample with the ruthenium labeled folate binding protein, a folate complex is formed, the amount of which is dependent upon the analyte concentration in the sample.

3rd incubation (9 minutes)

After addition of streptavidin-coated microparticles and folate labeled with biotin, the unbound sites of the ruthenium labeled folate binding protein become occupied, with formation of a ruthenium labeled folate binding protein-folate biotin complex. The entire complex becomes bound to the solid phase via interaction of biotin and streptavidin.

Measurement

The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

Elecsys® technology

ECL (ElectroChemiLuminescence) is Roche's technology for immunoassay detection. Based on this technology and combined with well-designed, specific and sensitive immunoassays, Elecsys delivers reliable results. The development of ECL immunoassays is based on the use of a ruthenium-complex and tripropylamine (TPA). The chemiluminescence reaction for the detection of the reaction complex is initiated by applying a voltage to the sample solution resulting in a precisely controlled reaction. ECL technology can accommodate many immunoassay principles while providing superior performance.

Elecsys® Folate RBC test characteristics

Testing time	27 minutes
Test principle	Competitive assay
Calibration	2 point
Traceability	Standardized against the Folate III assay / RBC application
Sample material	Hemolysate prepared from whole blood treated with anticoagulants (Na-heparin or K3-EDTA).
Sample volume	100µL whole blood
LoB, LoD, LoQ	20.0 ng/mL, 46.5 ng/mL, 120 ng/mL
Measuring range	120 – 620 ng/mL or 272 – 1'407 nmol/L
Intermediate	cobas e 411 analyzer: 4.7 –14.2 %

imprecision	cobas e 601/e 602 modules: 3.4 – 10.4 % Lowest conc. measured: 61.0 ng/mL
Expected values	Whole blood folate (from hemolysate samples) Europe: 212 – 534 ng/mL, Australia: 241-584 ng/mL RBC folate (folate in erythrocyte fraction) Europe: 523 – 1’257 ng/mL, Australia: 629 – 1’453 ng/mL, (2.5th – 97.5th percentile

APPENDIX 5: PUBLICATION

Folate deficiency and utilization of folic acid among pregnant women attending antenatal clinic at Pumwani Maternity Hospital, Kenya, 2015

Elizabeth Mgamb, Zeinab Gura, Peter Wanzala, Jane Githuku, Anselimo Makokha

The Pan African Medical Journal. *2017;28 (Supp1):8.* ***doi:****10.116 04/pamj.supp.2017.28.1.9296*

Abstract

Introduction: in 2012, the Government of Kenya amended the Food, Drug and Chemical Substances Act to make the fortification of maize and wheat flour with folic acid mandatory. We assessed folate deficiency, awareness and use of folic acid fortified flour among pregnant women receiving antenatal care (ANC) at a clinic at Pumwani Maternity Hospital, Kenya, 2015.

Methods: we conducted a cross-sectional survey at Pumwani Maternity Hospital between October and November 2014. We enrolled pregnant women who received ANC and interviewed them using a semi-structured questionnaire after obtaining informed consent. Blood samples were collected from all study participants and serum folate level was analyzed by electrochemiluminescence immunoassay. Folate deficiency was defined as serum folate of < 10nmols/L and borderline folate deficiency was defined as serum folate of between 10nmols/L and 15nmols/L.

Results: among the 247 study participants, two (1%) had folate deficiency. One hundred and seventy-nine (73.4%) had heard about folic acid, but only 56 (23%) had heard about folic acid fortified flour. Overall, 198 (80%) study participants consumed fortified brands of maize flour and 205 (84%) consumed fortified brands of wheat flour; only four (2%) and two (1%) of study participants consumed specific brands of maize and wheat flour respectively because they were fortified.

Conclusion: the prevalence of folate deficiency was low and this may have been because of the availability of fortification programs. Although there was limited knowledge of fortified flour, utilization was high. The Kenyan Ministry of Health should enforce implementation of the legislation on maize flour and wheat flour fortification by all milling industries.