RISK FACTORS ASSOCIATED WITH NEURAL TUBE DEFECTS AMONG INFANTS ATTENDING KIJABE HOSPITAL AND THE AFFILIATE SIX SATELLITE CLINICS

BY:

CHRISTINE JEPCHUMBA KEITANY

THESIS SUBMITTED TO THE SCHOOL OF PUBLIC HEALTH, COLLEGE OF HEALTH SCIENCES, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN FIELD EPIDEMIOLOGY.

MOI UNIVERSITY

2020

DECLARATION

Declaration by Candidate

This thesis is my original work and has not been presented for a degree in any other university. No part of this project may be reproduced without prior permission of the author and/or Moi University.

Signature: _____ Date: _____

Christine J. Keitany

SPH/PGH/FE/13/15

Declaration by the Supervisors

This thesis has been submitted with our approval as university supervisors.

Signature: _____ Date: _____

Prof. Grace A.K. Ettyang (PhD)

Department of Health Policy Management & Human Nutrition

School of Public Health

Moi University

Eldoret-Kenya

Signature: _____ Date: _____

Dr. Maurice Ope MPH Msc (Epidemiology)

Senior Public Health Specialist/Medical Epidemiologist

Division of Global Migration and Quarantine

Centres of Disease Prevention and Control (CDC)

Nairobi-Kenya

DEDICATION

This work is dedicated to my parents Mr Silvester K. Keitany & Mrs Hellen Bernice J. Keitany, My Daughter Faith Jebet Kite Keitany, my Sister Professor Gladys J. Kiptiony and my friend and partner Dr Martin K. Mwangi for their continued support and inspiration.

ABSTRACT

Introduction: Neural tube defects (NTDs) are preventable birth anomalies of the spine and brain that occur in the first trimester of pregnancy, leading to increased morbidity and mortality in children <5 years. Globally an estimated 300,000 cases of NTD occur, where 276,000 die during infancy, majority in low income countries. In Kenya World Health Organization (WHO) estimates prevalence of NTDs at 6/10,000 live births as at 2013. NTDs account for 0.5-2.0/1000 pregnancies annually and is as high at 10.0/1000 in certain geographical locations.

Objective: Determine factors associated with NTDs among infants attending Kijabe Hospital and affiliated satellite clinics.

Methods: Matched case-control study conducted between July and November 2017. Cases were infants diagnosed with NTD at Kijabe Hospital and its affiliated satellite clinics, controls were infants with no history of hospitalization, not known /or diagnosed with any congenital anomaly. Controls were selected randomly from Maternal Child Health Clinics in County and Sub-County Hospitals proximal to the satellite clinics. Controls were matched to cases for -age, -sex and -geographical location. Maternal risk factor information collected included, maternal socio-demographic information, maternal Health, Obstetric, and nutrition information. Neonates information collected were Age, sex, weight, length, type of NTD, gestation age at birth, birth order. Risk factor information were FA supplementation, consumption of FA fortified foods, folate rich foods, drug use, alcohol use, medication in pregnancy, smoking, previous family history of NTDs/ or birth defects. Principal Components Analysis (PCA) was used to generate wealth index for the respondents based on a set of household characteristics, and asset ownership. Conditional logistic regression was used to evaluate risk factors for NTDs.

Results: A total of 60 cases and 120 controls were enrolled. Of 60 cases, 59 (98.3%) had spinabifida and 1 (1.7%) encephalocele. Among spinabifida cases, 29 (48%) were meningoceles, 29 (48.0%) myelomeningocele and 25 (41.7%) spina bifida occulta 5(8.4 %). Median age of infant- participants was 5.5 (range 3 -52) weeks, median gestational age at birth was 39 (range 34-42) weeks. Infant gender 111(61.7%) were boys of whom 37 were cases, and 69 (38.3%) girls with 23 being cases. Mothers with unplanned pregancy were 101 (55.2%) and 166 (92.2%) attended antenatal clinic (ANC). Mothers tested for HIV were 158 (87.8%) of whom 12 (6.6%) were on antiretrovirals, with only one being a case. Factors associated with increased risk of NTD were history of pregnancy loss (aOR=6.4, 95% CI 2.4-17.4), multiparity (aOR=6.59, 95% CI 2.16-20.1), periconception consumption of folic acid fortified flour (aOR=6.9, 95% CI 1.61-29.6), use/contact with pesticides (aOR=6.4, 95% CI 1.3-13.9). Preconception folic acid supplementation was protective (aOR 0.13, 95% CI 0.045-0.5).

Conclusions: Risk factors associated with NTDs identified were multiparity, previous pregnancy loss, use/ contact to pesticides. Folic acid supplementation before conception was protective. Periconceptional consumption of folic acid fortified flour 3 times/week was not protective against NTDs.

Recommendations: Use of personal protective gear, contact to pesticides was associated with increased NTDs risk. Encourage periconceptional FA supplements to women of reproductive age and post market surveillance of levels of FA in fortified flour.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS/ACRONYMS

ACK	Anglican Church of Kenya
AFP	Alpha fetoprotein
AIC	Africa Inland Church
APDK	Association of the Physically Disabled of Kenya
BMI	Basal Metabolic Rate
ICD-10	International Classification of Disease
MH-OR	Mantel-Haenszel Odds ratio
NTD	Neural Tube Defects
QOL	Quality of life
SB	Spina Bifida
WHO	World Health Organization

DEFINITIONS

Anencephaly- Absence of bony covering over the back of the head or missing bones around the front and sides of the head

Encephalocele; - Presence of a sac-like protrusion of the brain and the membrane that cover it through openings in the skull

Hydrocephalus- Rapid increase in head circumference due to an increase in cerebrospinal fluid within the cranial cavity

Meningocele- type of spinal bifida cystica characterised by herniation of meninges through a defect bony in the spine or skull.

Myelomeningocele- type of spina bifida cystica characterised by herniation of spinal cord contents through an abnormal defect in the spinal cord.

Neural tube defects (NTD) - Any form of spina bifida, anencephaly or encephalocele

Peri-conceptional – Relating to or done during the period before conception to early pregnancy

Spinal Bifida Occulta - The lesion is covered by skin and meninges do not herniate through the bony defect.

ACKNOWLEDGEMENTS

I would like to appreciate my supervisors; Prof. Grace Ettyang and Dr. Maurice Ope for the valuable input and support throughout this process.

I would also like to acknowledge the support received from the faculty of the Field Epidemiology and Laboratory Training Program (Kenya) who served to impart the necessary knowledge as well as provide technical and financial support required to conduct this study.

I wish to express my gratitude to the members of staff of Kijabe Hospital particularly the Bethany Kids at Kijabe Hospital team.

Finally, I wish to also thank the mothers of children with neural tube defects who participated in the study. Their bravery and fortitude in the face of misfortune is greatly admired and for control mothers for willingly participating in this study.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Neural tube defects (NTDs) are preventable, serious birth defects affecting the skull and the spine, it results from failure of closure of the neural tube during development. It is a major cause of death and disability worldwide (Mitchell,et al., 2005). Neural Tube Defect occurs in the first trimester of pregnancy (below 22 weeks) most often before the woman knows she's pregnant (Christianson,et al., 2006), and can be classified as open when the nervous tissue is exposed, or closed when the nervous tissue is covered by skin. Classification of NTDs can also be based on the site affected. Those affecting the skull (cranium) include anencephaly and encephalocele while those affecting the spinal cord include rachischisis, menigomyelocele, meningocele, and spinal bifida occulta. Neural tube defects affecting the spinal cord, collectively termed as spina bifida account for about 90% of all spinal defects (De Marco et al., 2011).

Globally an estimated 300,000 to 400,000 neonates are born each year affected with neural tube defects (Liu et al., 2012). Approximately 88,000 deaths and 8.6 million disabilities can be attributed to NTDs. In the western world, 2500 and 4500 live births with NTDs occur annually in the USA and Europe respectively. A study in China estimates the number of live births with NTDs to be about 100,000 annually (Yi, Lindemann, et al., 2011). In Africa the prevalence of NTDs ranges from 5.2 per 10,000 live births in Nigeria to 75.4 per 10000 live births in Algeria (Zaganjor et al., 2016a). In East Africa the prevalence is estimated to be 13% (Christianson et al., 2006a). The World Health Organization (WHO) estimates prevalence of birth defects in Kenya is at 6 per 10,000 live births as at 2013. This estimate is almost double the prevalence (3.3 /

10,000 live births) reported in a cross-sectional study done at Kijabe Mission hospital (Muga, et al., 2009).

Neural tube defects are a major cause of morbidity and mortality in children below two years(Christianson et al., 2006a). They are associated with various forms of complications including developmental delays, paralysis and orthopaedic problems in the case of spinal bifida, seizures, motor impairment, and visual deficits in those with encephaloceles. Other long term problems include hydrocephalus, neurogenic bladder, kidney involvement and psychosocial consequences (Yi et al., 2011). Anencephaly is incompatible with life with most pregnancies ending in still births or neonatal death shortly after delivery (BOTTO, et al., 1999). Children with spinal bifida have lifelong physical and mental disabilities; those who survive to adulthood are unable to function independently resulting in great economic burden to families and care givers (Bowman, McLone., 2010).

Several factors have been associated with an increased risk of NTDs. These include environmental factors, genetic factors and maternal nutrition (De Marco et al., 2011a). Studies have shown that up to 70% of NTDs can be prevented with folic acid consumption during early pregnancy (BOTTO et al., 1999). In 2003, a study done in South Africa after the introduction of fortification of staple food with folic acid, showed a significant decline in NTDs of about 30.5% from 1.41 to 0.98 per 1000 births (Sayed, Bourne, et al., 2008). Economic benefits following prevention of NTDs through food fortification greatly exceeded the costs of folic acid implementation (Bannink, et al., 2015). The decline in NTDs following mandatory introduction of food fortification with folic acid was consistent in several countries like Chile, Canada, Costa-Rica, and United States of America (Viswanathan et al., 2017).

1.2 Problem Statement

Neural tube defects are a major cause of morbidity and mortality in children below five years contributing significantly to global burden of disease and disability, it is estimated to affect about 300,000 births each year worldwide leading to serious birth defects, paralysis and death (Wu, Poenaru et al., 2013). Up to 70% of NTDs are preventable with folic acid supplementation administered before conception and end of the first trimester (De Marco et al., 2011).

Global prevalence of NTDs is high and it is estimated to be at 6.0 per 10,000 live births (Bowman, et al.,2010). Birth defects are the leading cause of death in the first year of life and infants who survive have increased risk of long term disabilities. Globally birth defects affects 1 in 33 infants and 3.2 million birth defects related disabilities each year (Flores, et al., 2014). South-East Asian regions had a prevalence of 15.8 per 10,000 births while Latin America had 11.5 per 10,000 births.

World health organization estimates that 94% of severe birth defects occur in low and middle income countries either due to maternal malnutrition, exposure to teratogenic agents, micronutrient deficiency and or maternal illnesses (World Health Organization, 2014). In East and sub-Saharan Africa neural tube defects are common due to or in part from high birth rates, lack of maternal pre/post conception use of folic acid among women of reproductive age. Modification of dietary habits and increase level of poverty leading to high challenges brought about from poor or low knowledge of the African woman. Other factors include poor healthcare infrastructure, and resource limited settings, lack of epidemiological studies and scarcity of data (Njamnshi et al., 2008).

In Kenya, data on prevalence and risk factors for NTDs is scanty. A cross sectional study done in 2009 examined births at Kenyatta National Hospital from 1980-1984 and stated a hospital prevalence of 20 neural tube defects per 10,000 live births (Muga et al., 2009). Another population based study conducted at Kijabe Hospital in 2013 found that the prevalence of congenital malformation was at 6.3 per 1000 and children with spina bifida had the highest disease burden (Wu et al., 2013).

Inadequate pre and post conception use of folic acid is one of the risk factors for NTDs. Many studies have proven the benefits and role of folic acid in prevention of neural tube defects. Other associated risk factors include exposure to toxins leading to teratogenic effects, maternal hyperthermia in early pregnancy, diabetes mellitus, hyperinsulinemia, anti-epileptic drugs, psychosocial or emotional problems, genetic susceptibilities (De Marco et al., 2011). In 2012 Kenya introduced mandatory fortification of cereals and oil, and women attending antenatal clinics are regularly given folic acid supplements yet little is known about the factors that influence the occurrence of neural tube defects in Kenya because data is still very limited (Government of Kenya, 2012).

1.3 Justification

Studies conducted in Africa and Kenya have concentrated more on the NTD child, on clinical outcomes and burden of NTD to the primary health care systems and to the affected families (Wu et al., 2013), some have described the role of folic acid in the prevention of the disease and decreasing disease burden (Viswanathan et al., 2017). The risk factors associated with NTDs have been studied extensively (Mitchell, 2005), however few studies have been conducted in Kenya.

In 2012 the Kenyan government passed a bill requiring fortification of maize, wheat flour and vegetable oil (Government of Kenya, 2012). From May 1, 2014, the Ministry of Health in Kenya made it mandatory for folic acid supplements to be available to all pregnant women attending ANC. Despite this, women still seek care late when the window period to confer protection to the unborn child has closed.

This study aimed to provide baseline information about factors associated with neural tube defects in Kenya, including adequate use of folic acid supplementation and severe consequences of folic acid deficiency. Accessibility to folic acid fortified foods by women of reproductive age.

Despite passing the mandatory cereal and oil fortification bill by the Kenya government in 2012, it is not known if this initiative has had any major impact in reduction of NTDs in Kenya since its implementation because there is still limited data, and studies. Information on factors causing NTD in Kenya is still lacking therefore it is important to assess the outcome of the above implementations by assessing risk factors of NTD in order to provide evidence critical for policy formulation.

The findings from this study will provide better understanding on the risk factors that lead to of occurrence of NTDs in Kenya and contribute in development of evidence based information critical for formulation of interventions and policies for prevention of NTDs in future.

1.4 Research Question

What factors are associated with NTDs among infants attending kijabe hospital and the affiliate satellite clinics?

1.5 Hypothesis

Null hypothesis: There was no significant in risk factors factors between infants with neural tube defects and those without.

Alternative hypothesis: There was significant risk factors between infants with neural tube defects and those without.

1.6 Objectives of the study

1.6.1 General objectives

Assessment of risk factors associated with neural tube defects among children attending Kijabe Hospital and its affiliated satellite clinics.

1.6.2 Specific objectives

1. To determine maternal social demographic characteristics
2. To determine effect of maternal health on risk of neural tube defects.
3. To determine maternal pre-conceptional factors associated with neural tube defects
4. To determine effect of consumption of folic acid fortified foods and folic acid supplements among cases and controls on risk of neural tube defects.
5. To compare the risk factors for neural tube defects among cases and controls.

CHAPTER TWO

LITERATURE REVIEW

2.1 Embryology of Neural Tube Defects

Despite extensive studies causes of NTDs are not well understood (Botto et al., 2006). Neural tube forms three weeks post conception and develops from three germ layers involved in gastrulation giving rise to the primordial tissues. These layers are the ectoderm, mesoderm, and endoderm. Development of the neural plate from the ectoderm occurs in the 19th day giving rise to the central nervous system which consists of the brain and spinal cord forming the neuroaxis (Mckeever, 2004). The nervous system develops from the neural crest cells of the embryonic ectoderm leading to development of peripheral nervous system. The notochord and paraxial differentiate into the neural crest which form peripheral nervous system and central nervous system while neural plate forms brain and spinal cord. Neurulation is the formation of the nervous system and neural plate with the thickening of the ectoderm located along the mid-dorsal region of the embryo. Neural plate develops into neural tube where two stages of neurulation are involved. While the notochord forms the future exoskeleton including the spinal vertebra. This is primary neurulation and secondary neurulation (Moore, Persaud, et al, 2013).

Primary Neurulation begins when lateral edges of the neural plate elevate as cells differentiate and migrate towards the edges of the neural plate forming neural folds during the early part of the fourth week (22 weeks) which leads to neural tube formation. It involves folding of the neuroepithelium (Moore et al., 2013). Secondary neurulation occurs distal to the primary neural tube where mesenchymal cells forms a space connecting the lumen of the primary neural tube (Z. Z. Li et al., 2011).

Neural tube defects are caused by abnormal closure of the neural tube during embryogenesis at the 28th day after conception (1st trimester) where the neural tube usually develops into spinal cord and brain. When neural tube fails to close properly it leads to development of NTDs which can either be open example myelomeningocele and anencephally or closed- spinal bifida (Mitchell, 2005).

Open NTDs occur when the neural folds fail to meet and fuse during primary neurulation whereas incomplete secondary neurulation leads to closed NTDs (Moore et al., 2013).

Anencephally implies that the bones of the skull do not develop therefore the brain is partially or completely absent, this condition is incompatible with life. Encephalocele implies that the brain and meninges have herniated through a defect in the skull. Defects of the spinal cord reffered as spinl bifida are most common and represent about 90% of all neural tube defects are a major cause of childhood disability (Mckeever, 2004). Spinal defects include spinal bifida occulta, meningocele and myelomeningocele and present with paraplegia, bladder problems and impaired bowel function (Pitkin, 2007).

In summary therefore NTDs are malformation of the Neuroectoderm and abnormalities in the surrounding mesodermal structures.

Table 1.1: Summary of central nervous system malformations

Gestation in days	Development	Defects
18-20	Formation of neural plate and groove	Anterior-midline defects/spina bifida
21-23	Forming of Optic vessels	Hydrocephalus
24-26	Closure of the anterior neuropore	Anencephally Spina bifida cystica Spina bifida occulta

Summary of central nervous system malformations. (Moore et al., 2013).

The developing human: clinically oriented embryology (10th Ed.). Philadelphia: Saunders. p. 70-74, 463

2.2 Overview of Nural Tube Defects

Neural tube defects (NTDs), are a major cause of morbidity, mortality and disabilty in children under five years globally. Neural tube defects are preventable and mainly affects the spine and brain, they contribute significantly to pubic health burden. Three hundred thousand babies born each year globally are affected, result in 88,000 deaths and 8.6 million disability adjusted life years (DALYs). In low income countries 29% of neonatal deaths are from observable birth defects (Zaganjor et al., 2016). Numerous studies have proven that folic acid and food fortification reduces the occurrence and prevalence of neural tube defects.The World Health Organization estimates birth defects affect an estimated 1 in 33 infants leading to 3.2 million disabilities from birth defects with 270,000 neonatal deaths globally (Flores et al., 2014).

2.3 Global Burden of Neural Tube Defects

Occurrence Neural tube defects varies according to social economic status, geographical area, ethnic background. The march dimes global report on birth defects

ranks countries with birth defects prevelance from the highest to the lowest. According to this report the western world highest rates were seen in ireland and scotland with frequency of 10 per 1000 births, lowest seen in United States of America (Pitkin, 2007). In the middle east the highest number of cases was seen in South East Asia between 55-65 per 1000 live births. In Africa Sudan had the highest cases at 82 per 1000 live births (Christianson, et al., 2006b). Great social and emotional costs are associated with NTDs to both families patients and the health care systems.

Data abstraction and risk bias assessment study done between 1990 to July 2014 using systematic reviews and meta analysis guideline results represented data from 75 countries among whom were World Health Organization (WHO) member states. This review demonstrated great variability of neural tube defects prevalence with a global range of 0.3-199.4 per 10,000 live births (Zaganjor et al., 2016b). It estimated that global neural tube prevalence was high at 6.0 per 10,000 live births.

This surveillance noted that the type of neural tube defect data collected globally was from national surveillance registries. It was noted that there was limitation of data from African and South East Asian countries ,and presence of NTD surveillance was increased with the countries economic status, low income countries were at 0%, while high income countries at 91%. Data would therefore be useful to establish burden of birth defects and NTDs in particular. This will determine level of need to inform prevention against neural tube defects, monitor trends through time and evaluate impact of prevention through time, and develop services for those affected (Zaganjor et al., 2016b)

2.4 Economic Burden of Neural Tube Defects

Neural tube defects contributes significantly to the public health impact especially in low and middle income countries due to the impact of disease burden, economic burden and emotional burden to affected children and their care givers predisposing them with lifelong difficulties (Christianson, et al., 2006).

A study in Chile estimated that the expense of raising a child with spinal bifida from birth to adulthood estimated to cost about $120,000 this contributes significantly to poverty to the affected families especially in developing countries where care is insufficient and no policies in place to support this children. (Lo, Polšek, et al., 2014).

2.5 Risk Factors Associated with Neural Tube Defects

Aetiology of NTDs is not known though several factor have been linked to increase the risks of NTD. Some of these factors include the female sex, a family history of NTDs, maternal hispanic discent, obesity, folate deficiency, pregestational diabetes mellitus, use of anticonvalsant drugs and hyperthermia. Many of the reported associations have been weak and not consistently replicated.

2.5.1 Maternal nutritional status

2.5.1.1 Folic acid deficiency

Folic acid deficiency has been associated with an increase in the risk of NTDs especially spinal bifida and anaencephally (Christianson et al., 2006a). Folic acid is the oxidised and active form of the vitamin (Pitkin, 2007). Studies have indicated that women who took 400mcg of folic acid during pre pregnancy period and first trimester had a low prevalence of NTDs with rates reduced from six to one per 1000 live births in Northern China (M. Wang et al., 2013). Fortification of wheat and maize flour with folic acid has been recomended and adopted by many countries both in Africa, Asia,

and America as a way of increasing serum folate among women of reproductive age (Christianson,et al., 2006b). Fortification is reccommended because folic acid supplementation is initiated late in pregnancy after development of the central nervous system is complete. The rates of unplanned pregnancies is also high so folic acid supplimentation may not have been initiated, fortification would therefore reach all women of reproductive age regardless. Consumption of foods rich in folate such as spinach liver also help increase level of circulating folate in blood(Flores,et al., 2014).

2.5.1.2 Maternal pregestational diabetes

Pregestational diabetes predisposing to hyperglyceamia is teratogenic it predisposes to chromosomal defects. Type 1 and type 2 diabetes mellitus predisposes to congenital abnormalities with a high proportion of defects being NTDs. Good glyceamic control has been shown to reduce the outcome of NTDs (Garne et al., 2012).

2.5.1.3 Obesity

Maternal obesity during pregnancy is a well known teratogen that increases risks of NTDs by affecting gene expression in the developing embryo (Cawley et al., 2016) . Developing embryo lacks beta-cells which develop after seven weeks of gestation, at this stage embryos could receive excess glucose from the mother and are unable to regulate the excess glucose, leading to oxidative stress and depletion of inositol which has been implicated in abnormal closure of developing neural tube (Mitchell, 2005).

2.5.2 Social Economic Status

Low level maternal education has been implicated to be associated with of increased risk of NTD affected child. Studies show that women with less than high school education and living in neighbourhoods where most residents did not graduate from high school or college and with a high rate of low education, unemployment, poverty,

overcrowding, are at increased risk of having children with NTDs (Grewal,et al., 2009), this is because low maternal education may impact dietary habits, access to medical care, and may not have sufficient knowledge and resources about importance of folic acid supplementation and prevention of NTDs. Fertility rate tend to be increased in this group (Blanco Muñoz et al., 2005). Women with a higher level of education tend to have more independence, knowledgeable, and are able to make decisions that affect their health and outcome of their pregnancies and children (Blanco Muñoz et al., 2005).

2.5.3 Alcohol

Alcohol is a known teratogen and it is thought to induce NTDs in animal studies. Human studies have failed to replicate the same results. Alcohol induces NTDs through excessive cell death of premigratory neural crest cells leading to few cells to close the neural tube. Alcohol is also thought to also reduce plasma folate levels (De Marco et al., 2011b).

2.5.4 Enviromental factors

2.5.4.1 Use of drugs

Use and exposure to social drugs such as cigarette smoking, are known to be teratogenic and predisposes to NTDs, this can be modified through altering behaviour(Flores et al., 2014). Alcohol exposure during early pregnancy showed a small association with NTD, while caffaine intake was associated with an increased risk (De Marco et al., 2011b).

2.5.4.2 Maternal exposure to chemicals

Exposure to medications, certain pesticides, radiation during first trimester of pregnancy may increase the risks of NTDs. Exposure can occur through occupation,

area of residence, ingestion of food contaminated by mycotoxins, chlorination disinfection by-products in drinking water (Blatter, et al., 1994). Anti-convulsant drugs have been associated with major teratogenic effects predisposing to NTDs (Kondo et al., 2013). Maternal exposures to certain medications during pregnancy increase the incidence of NTD. Anticonvulsants like carbamazepine, valproic acid used in the management of epilepsy have been associated with increased risk of spinal bifida (De Marco et al., 2011a).

2.5.4.3 Maternal hyperthermia

Hyperthermia in pregnancy has been shown to increase the risks of NTDS upto two fold in a developing embryo during the first trimester. A mexican-american study conducted from 1995-2000 generated information from maternal febrile illnesses (Suarez,et al., 2004). Exposure to heat generated from external sources, hyperthermia inducing exercises, showed a risk effect (OR) of 2.9 concluding that maternal hyperthermia increased the risk of having NTD affected child, and as such pregnant women or those intending to become gravid should avoid intense exposures to heat and careful treatment of maternal febrile illnesses(Li, Ren, et al .,2006).

2.5.5 Genetic risk

NTDs have occasionaly been caused by chromosomal abnormalities, single gene defects and mutations involving methylenetetrahydrofolate reductase gene (C677T) susceptible (Christianson et al., 2006b).

2.6 Diagnosis of NTDs

2.6.1 Biochemistry

Alphafeto protein (AFP) is elevated in maternal serum AFP and in amniotic fluid. AFP is elevated in encephalocele, meningomyelocele, and in anencephally (Driscoll, et

al.,2009). Maternal screening for AFP levels is usually done by collecting maternal blood serum, and amniotic fluid to measure levels of AFP and acetylcholinestrase. It detect anencephally and /or myelomeningocele (Bowman, et al.,2010). Analysis is prefferably done between between 15 to 20 week gestation. If AFP is elevated in amniotic fluid it is 90-95% accurate in identifying an affected pregnancy with an open NTD, while acetylcholenistarase assay is 99% specfic for neural tissue. AFP is also increased in other medical states such as multiple pregnancy, anterior abdominal wall defects and as such other tests should be carried out such as ulrasound imaging to confirm diagnosis (Driscoll, et al., 2009).

2.6.2 Imaging

Ultrasonography is an imaging technique that is used to detect any malformations in the developing spine, it is done before the 12^{th} week and detects myelomeningocele (Bowman, et al,.2010). More studies need to be done in East and Sub-saharan Africa to evaluate need for early screening and diagnosis for NTDs as studies and data are still very limited.

2.7 Clinical features of NTDs

Globally NTDs have an incidence of 0.79-6.39 per 1000 live births (Bowman,et al., 2010). Neurological damage caused by NTDs is irriversible and affects quality of life of the affected children and their families (Van't Veer et al., 2008). Spinal bifida presents either as spinal bifida occulta which is closed type and defect is not visible. Spinal bifida aperta is open, this can presents as a myelomeningocele, meningocele, or lipomeningocele.spinal aperta is the most common type and contain a sac with meninges, cerebrospinal fluid,nerves and a dysplastic spinal cord, this can lead to hydrocephalus and chiari malformation (Corneg- Blokland, et al,. 2011).

This malformation present with various neurological symptoms like paralysis, spasticity of the lower limbs, urinary incontinence, fecal incontinence, neurocogcognitive retardation (B. Warf et al., 2009).

2.8 Management of NTDS

It is multifaceted, complex and involves pediatricians, surgeons, occupational therapist, speech therapist and the main aim is to prevent and or reduce complications and improving the quality of life of the patient (Bowman, et al., 2010).

2.8.1 Infant Examination

Examination of an NTD affected neonate after delivery should be done under sterile conditions with examiner wearing non-latex gloves to minimise latex sensitivity. Small defects are covered with sterile saline soaked dressing while large defects with a plastic wrap to minimise heat loss (Mckeever, 2004). Prophylaxis with broad spectrum antibiotic to reduce risks of infections and apply appropriate wound care (Botto,et al. , 1999).

2.8.2 Surgical management

Surgical closure of spinal defects are done within 72 hours after delivery. This is the standard care of management, which later prevents or minimises bladder complications (Bowman, et al., 2010). In neonates with hydrocephalus myelomeningocele repair and Ventriculoperitoneal (VP) shunt placement, and those with chiari malformation appropriate decompression is done to reduce chiari symptoms (Warf,et al., 2011).

2.8.3 Long Term Management

Children with NTDs require a life long commitment by the patient, family and the medical personnel in order to maintain a stable neurological function throughout a

patient lifetime to decrease overall morbidity and mortality and improve the quality of life (Van't Veer et al., 2008). Occupational and physical therapy are included in long term management inorder to improve neurological function and muscular-skeletal function (Bowman, et al., 2010). Most of this patients have acute flaccid paralysis of ankles and knees and may require brace and crutches.

2.9 Complications of NTDs / Long term sequelae

Complications from spinal bifida include Renal failure, kyphosis, allergy to latex, pressure sore and recurrent infections (Corneg-Blokland et al., 2011). Management is complex and mutifaceted involving different specialities in order to minimise complications. Clean intermittent catheterization is important and should be done under aseptic procedures inorder to prevent renal failure and urinary incontinence (Corneg-Blokland et al., 2011). Bladder and bowel problems are present in nintey percent of children with meningomyelocele, to prevent complications regular toileting, use of stool softeners and addition of fibre in the diet to prevent constipation and soiling (Botto et al., 2006).

2.10 Prevention of NTDs

2.10.1 Folic acid Fortification

Flour and oil fortification and folic acid suppliments in early pregnancy , has markedly lead to decline of NTDs (Sayed, et al., 2008). Many studies have suggested that increased intake of synthetic folic acid which is the bioavailable form reduces the risks of NTDs when consumed during the preconception period and in the first trimester of pregnancy (upto 12 weeks), and prevents prevalence of NTDs by upto 50-70% of cases if taken at 0.4mg (400micrograms) (Youngblood et al., 2013). Women with a previous history of NTD affected pregnancy should receive information on the risks of recurrence and should be adviced on the protective effect of preconceptional folate

suppliment taken at a higher dose of 4mg(4000 micrograms) before conception (Youngblood et al., 2013).

Countries that have introduced Fortification of staple foods including grain, wheat and oil have reported a significant decline in NTDs and the cost and benefit ratio decline (Sayed et al., 2008).

2.10.2 Preconception care

In most developing countries less than thirty percent of women receive folic acid supplementation (Taiwo, et al.,2014). Many studies have proven that at least daily intake of 400mcg folic acid is significant in prevention of NTDs, food fortification with folic acid therefore remains most important strategy to increase mass consumption of fortified foods and so preventing NTDS (Christianson et al., 2006a).

2.10.3 Antenatal care

Focused Antenatal care mainly aims to achieve a positive outcome to the mother and baby and prevent possible complications during pregnancy, labour, delivery and postpartum period, aims for four maternal antenatal care visits (Ministry of Health, 2010). However, the World Health Organization recommends that all pregnant women should be able to have at least eight or more contact ANC visits for effective monitoring and utilization of maternal Health services and receive positive maternal experience (World Health Organization (WHO), 2018), this recommendation has barely been achieved in most African countries including Kenya where most women hardly achieve the four visits previously recommended by WHO (Lincetto,et al., 2013). Majority of mothers are therefore unable to receive medical care including screening, supplementation, treatment, and care throughout pregnancy(Salih, et al., 2014).

2.11 Fortification of food with folic acid

Supplementation with folic acid has been shown to be effective in prevention of NTDs when used in periconceptional period or in early pregnancy. Despite this it is still not adequately effective since most women seek care late when the window period for effective prevention of NTDs has passed (H. Wang et al., 2016). Fortification of foods regularly consumed such as cereals, flour, and oils is therefore a more effective way of preventing folate insufficiency and NTDs. In 2006 it was estimated that current fortification initiatives have prevented about twenty-two thousand (9%) of NTDs and an annual global decrease of about six thousand, six hundred (6,600) folic acid preventable neural tube defects (Youngblood et al., 2013). In the NTD high risk Shanxi province in china, a cross sectional study conducted between August 2006 to November 2007 showed an increase in serum folate in women consuming fortified foods , this marked a decline in prevalence of NTD affected births from 229.1 per 10,000 live births to 72.9 per 10,000 live births compared to controls (H. Wang et al., 2016). This examples clearly show that fortification of foods regularly consumed is effective in the decline of NTD affected pregnancies. In countries such as America, Canada, Australia, South Africa where fortification of foods is mandatory, data clearly shows marked decrease in neural tube defects (Flores et al., 2014).

In Kenya the Ministry of Health implemented the national food fortification program with the aim of fortifying maize, wheat flour and vegetable oil with the aim of reaching about 95% of the population (Government of Kenya, 2012). Data on the outcome of this government directive and burden of NTD since its implementation of mandatory fortification is scanty.

CHAPTER THREE

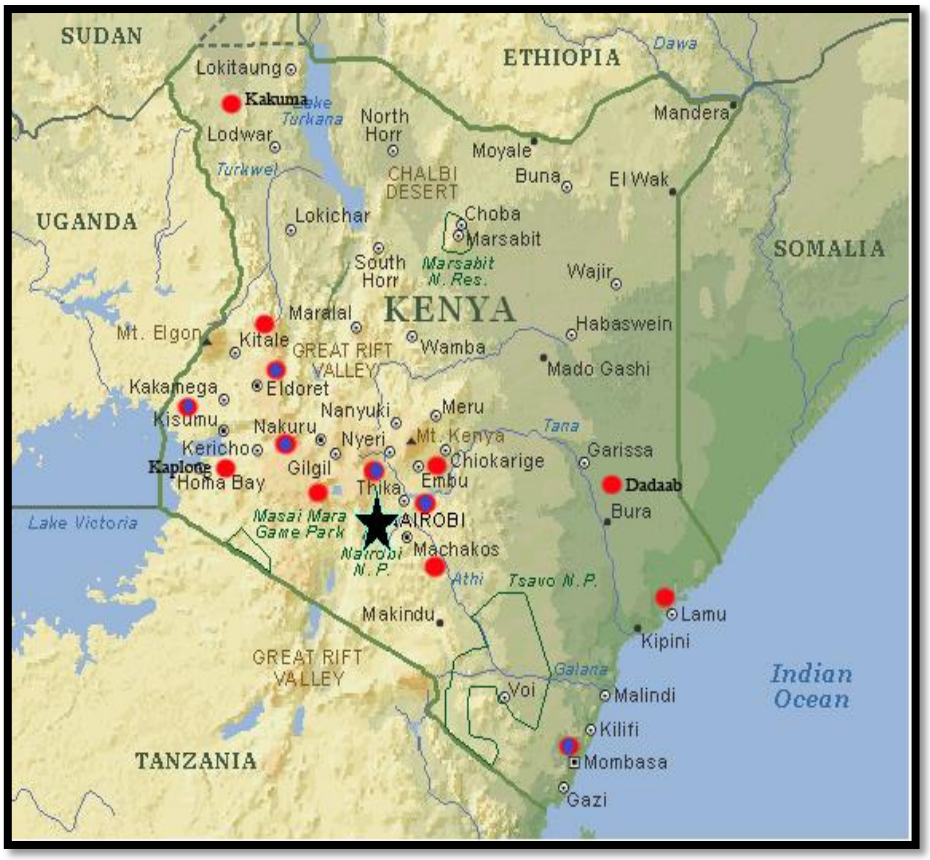
MATERIALS AND METHODS

3.1 Study Site

The study was conducted at six Satellite Clinics associated with Kijabe Hospital and county/sub-county hospitals proximal to these clinics. The satellite clinics were located in Kisumu, Eldoret, Nakuru, Kiambu, Nairobi,and Mombasa.

Kijabe Hospital is located in Lari subcounty Kiambu County Kenya. It has about sixteen affiliated satellite clinics spread across the country (figure 1). It was first establlished in 1903 as a Mission station for Africa Inland Missionaries. There are 16 affilliated satellite clinics and includes the following; Westlands Clinic Nairobi, Thika Joy Town Primary, Meru Level Five, Machakos Cathedral, Kibwezi AIC Church, Mombasa Association of the physically disabled of Kenya (APEDK) Portreiz, Narok Catholic Church, Kaplong St.Claires Hospital, Kisii Level Five, Kisumu Level Five Hospital-Russia, Kitale ACK, Eldoret ACK, Nakuru level five, Nyahururu St. Matthew, Naivasha AIC church.

Kijabe hospital has a paediatric neurosurgical centre and sixteen satellite clinics spread across the country. Satellites clinics began providing followup neurologic services in November 2004 and specialised care in dealing with NTDs and Orthpaedic complications occuring as a sequele of NTDS. Kijabe Hospital serves as a referral hosptial for paediatric patients with neurological conditions countrywide and for new cases of NTDs, while the clinics act as followup centres. Once a child is discharged from Kijabe Hospital they are followed up at a satellite clinic close to their geographical residence. Kijabe is also a training and teaching centre for health professionals and treatment centre for paediatric patients with other disease conditions.



Satellite-clinics

6 Selected study sites

Kijabe Hospital

Figure 3.1: Location of Study Sites

Out of the sixteen clinics, six satellites were selected for our study for cases. This was done by selectively selecting the centres that had high volume of case patients. This satellite clinics were Kisumu, Eldoret, Nakuru, Kiambu, Nairobi, and Mombasa clinics respectively while controls were recruited from County and Sub-County public hospitals close to the satellite clinics.

3.2 Facilities Used In Enrolment Of Research Subjects

Cases were enrolled from Centres affiliated to Kijabe Hospital while controls were selected from County and Sub-County public Health facilities as listed in table 3.1.

Table 3.1: Facilities used for Enrolment of Cases and Controls.

Regions	Cases	Controls
Eldoret	ACK Church	Huruma Sub County hospitals
Kiambu	Thika Joy Town	AIC Kijabe Hospitals
Kisumu	Kisumu level 5 Hospital	St. Elizabeth Hospital Chiga
Mombasa	APEDK Mombasa	Likoni Sub-County Hospital
Nairobi	Westlands clinic	St. Francis Community Hospital Kasarani
Nakuru	APEDK clinic Provincial General Hospital (PGH)	MCH-Clinic-(PGH)

3.3 Distribution Of Cases And Their Matched Controls From Selected Health Facilities

Cases were interviewed at selected satellite clinics from the selected regions and controls from Public County and Sub-County Health facilities.

Table 3.1: Distribution of cases and controls by County

County where participants were enrolled	Case(n=60)	Control(n=120)	Total(n=180)
Eldoret	8 (13.3)	16 (13.3)	24 (13.3)
Kiambu	13 (21.7)	26 (21.7)	39 (21.7)
Kisumu	10 (16.7)	20 (16.7)	30 (16.7)
Mombasa	4 (6.7)	8 (6.7)	15 (8.3)
Nairobi	14(23.3)	28 (23.3)	42(23.3)
Nakuru – PGH Level Five Hospital ╬	11 (18.3)	22 (18.3)	33 (18.3)

3.4 Study Design

This was a Case Control study on infants with neural tube defects matched with controls by age,sex an geographical location attending the selected six Kijabe Hospital satelite clinics across the country from July to December 2017.

3.5 Case Definition

A case was defined as an infant with a neural tube defect, which was either a spinal bifida, anencephally, myelomenigocele, meningocele, encephalocele based on the ICD-10 criteria, who were attending Kijabe Hospital or its affiliated satellite clinic.

A control was defined as an infant who had never been hospitalized since birth and never been diagosed with any known congental anomaly, seeking immunization services at public Health facilities proximal to the Kijabe Hospital satellite clinics where cases were enrolled.

3.6 Inclusion and Exclusion Criteria

3.6.1 Inclusion criteria

All infants whose mothers or legally authorized guardians were available and had the mother child cards were eligible for enrolment. The presence of the mother/guardian with the mother and child card was to enable assessment of factors that could predispose to NTDs before and during pregancy.

3.6.2 Exclusion criteria

Infants who had multiple congenital anomalies and infants whose mother/guardian refused to give consent to participate in the study were excluded.

3.7 Study Population

The study was conducted among infants with NTDs who were attending neurosurgery outpatient clinic at the six satellite Clinics affiliated with Kijabe Hospital.

Controls were selected from the county and subcounty hospital that was neighboring the satellite clinics. Two controls were selected for every case of NTD.

3.8 Sample Size Calculation

We calculated the sample size using the formula by Fleiss for analytic studies as follows:

$$n_1 = \frac{\left[z_{\alpha/2}\sqrt{(r+1)\bar{p}\bar{q}} + z_{1-\beta}\sqrt{rp_1q_1 + p_2q_2} \right]^2}{r(p_1 - p_2)^2} \qquad n_2 = r \times n_1$$

And:

Where:

Variable	Case-control study	value
n1	number of cases	60
n2	number of controls	120
zα/2	z-score for two-tailed test based on α level	1.96
z1-β	z-score for one-tailed test based on β level	0.84
r	controls : cases	2
p1	proportion of cases with exposure	
q1	1 – p1	
p2	proportion of controls with exposure	0.26
q2	1 – p2	0.74
OR	Odds ratio	3.35

Where,

n_1 = the number of cases

q_1 = Proportion of exposure among controls (58%)

Zα/z= Desired alfa level at 95% (1.96)

Z$1-\beta$= desired power level set at 80% (0.84)

p_1 = Proportion of exposure among cases(26) Exposure use of folic acid four weeks after conception (De Marco et al., 2011)

r = ratio of cases to controls (2)

OR = desired odds ratio (2) Exposure use of folic acid after conception (De Marco et al., 2011)

3.8.1 Parameters used in calculation of the sample size

The main exposure variable used for calculaiton of the sample size was exposure to folic acid supplements within 4 weeks after conception. A prospective study done in Gaslini hospital Italy between March 2000 and January 2008 indicated that those who used folic acid after conception was 26% and those who never used at all was at 70% among mothers of cases (De Marco et al., 2011). In this study they defined a case as white italian caucasian with open or closed spinal Dysraphism obtained within 24 months and had clinical records. A lot of studies have been done in africa and kenya on NTDs but we didn't find a similar study. The probability of erroneuosly detecting an association between folic acid supplementation and NTDs (type I error) was taken as 0.05. The probability of erroneously not finding an association between folic acid supplementation and NTDs when one truly exists (type II error) was taken as 0.2 (power of study is 80%). The odds ratio considered important to detect was 3.35. Since NTDs are a rare condition, there was need to increase the power of the study. A case to control ratio was taken as 1:2

The minimum sample size calculated for cases was 60 and number of controls was 120. The total minimum sample size required was therefore 180 participants.

3.9 Sampling Method

All Infants with neural tube defects seen at Kijabe Hospital and the 6 affiliated satellite clinics during the study period were recruited into the study after seeking consent from their parents/ guardians. Cases were recruited both prospectively and restropectively from Kijabe hospital and the 6 satellite clinics. Retrospective recruitment involved identifying all infants with neural tube defects while prospective recruitment involved identifying any infant born with an NTD after commencemnt of the study and was eligible for enrolment as a case. Both retrospective and prospective selection was done to achieve the study sample size because NTD is a rare disease. All infants diagnosed with NTDs from the selected satellite clinics were enrolled into the study. Clinics that had more case patients were included in the study.

Controls were randomly selected from the maternal child health clinics (MCH) from the county and subcounty. Matching of cases to controls was done by age, sex and geographical location. Mothers of children with NTD were interviewed as cases and mothers of infants without NTDs as controls.

3.10 Enrollment Procedure

Project staff were identified in each neurosurgical affliated satellite clinics of Kijabe hospital and the potential county/sub-county hospital where participants were enrolled from. The project staff underwent training which covered, identification of cases and controls, consent seeking and administration of questionnaires. The project staff enrolled cases and controls by random sampling.

Cases were recruited both prospectively and restropectively. Our aim was to enrol all case infants who met the criteria at each satellite clinic. Trained project staff scrutinized the records at neurosurgical/affliated clinics of Kijabe hospital to identify infants newly

diagnosed with neural tube defects and attending the clinics for follow up. The parents and / or guardians of infants were approached, screening questions were asked, and were requested to show the MCH card if available. The study was explained to the parents/guardians and informed consent obtained before enrollment into the study. Through the screening questions it was possible to keep track of the demographic characteristics and number of participants who declined to participate.

Controls were recruited from maternal child health (MCH) clinics in the nearest county / subcounty Hospitals nearest to the satellite clinics where cases come from. For example, if a case is enroled from Kisumu Nyanza Refferal Hospital, then two controls were selected from the nearest county/sub-county hospital as the case, which in this case will be Kisumu sub-county Hospital. Matching of controls to cases was done by geographical region, age and sex . Age groups were: 0-<4 months, 4-<6 months, 6-<9 months and 9-<12 months.

Project staff who enrolled a case, notified the principal investigator of the age and nearest county/sub-county hospital where the case was recruited from using the screening form. The principal investigator then determined the required age group of the control and the MCH clinic where the control was enrolled from. The principal investigator notified the relevant project staff to identify and enroll the controls. Upon receiving guidance from the principal investigator, the project staff went to the MCH clinic and begun enrolling from a random number and identified every 3rd eligible child, and proceeded to ask screening questions, after explaining the study and obtaining an informed consent before enrolling them into the study. In case the participant declined to participate the next eligible participant was approached until all controls were enrolled. The procedure was repeated until 2 eligible controls were enrolled.

The principal investigator kept track of enrolment of cases and controls in all sites and notified all project staff to terminate enrolment of additional participants once the required sample size was achieved.

3.11 Wealth Quantiles

We used the Principal Components Analysis (PCA) to generate a wealth index for the respondents based on a set of household characteristics and asset ownership (Firestone, 2014). The conceptual characteristics were:

1. Possession of household items: television, video or dvd player, radio, refrigerator, telephone (mobile or other
2. House have: house-help, sanitation (toilet), rented or family house, electricity
3. own an agricultural land

Based on this wealth index, we determined wealth quintiles in which each respondent fell into by dividing the indices into equal quintiles, a fifth (20%) of the respondents. Table below summarizes the cut-off points for the wealth index into the quintiles.

Table 3.3: Cut-off points used for grouping the wealth index into wealth quintiles

Wealth	**Wealth Score**	
Quintiles	**Min**	**Maximum**
Poor	-3.695198	-2.10337
Second poor	-2.026607	-0.96371
Middle	-0.9601527	-0.23797
Second rich	-0.1721852	2.452282
Rich	2.512264	6.211455

3.12 Data Collection Instruments

3.12.1 Questionnaire

A structured questionnaire was used for data collection. The questionnaire was administered to the mother through a face to face interview.

3.12.2 Variables

Variables selected included:

Mother's demographic information: Age, weight, height, occupation, residence, marital status, education level, religion, wealth index.

Mother's obstetric history: parity, history of birth defects in previous pregnancies, medical conditions during pregnancy. This information was abstracted from the mother child card.

Risk factor information: folic acid supplementation, consumption of folic acid fortified foods, folate rich foods, drug and /or alcohol use, medication in pregnancy, smoking, family history of NTDs/birth defects.

Neonate's information: Age, sex, weight, length, type of NTD, gestation age at birth, birth order.

3.13 Data Management and Analysis

3.13.1 Data collection, entry and storage

Data collection was done using a questionnaire administered through face to face interviews. The data was uploaded daily into excel and checked for errors and duplication before storage and back up. Data was stored under a password protected database that was accessible to the principal investigator only.

3.13.2 Data analysis

Data analysis was done using Epi info7 (CDC Atlanta GA USA) and Microsoft excel 2007 (Microsoft, Seattle, WA, USA). Univariate analysis was done and reported as frequencies and proportions with a confidence level of 95% for categorical data and summary statistics for continuous variables. The outcome variable was a case or

controls (binary variable). The measure of association was an odds ratio and 95% confidence intervals calculated around the odds ratio. Bivariate analysis was done using chi square test as a measure of association to determine the factors associated with neural tube defects. Factors with a p- value < 0.1 from bivariate analysis and those that are known from the literature to be associated with NTDs were entered into a multivariate condition logistic regression model to yield factors that are independently associated with neural tube defects. Conditional logistic regression model was used to calculate Odds ratio, and multivariate analysis was done using stepwise backward elimination method criteria.

3.14 Ethical Considerations

Ethical approval of the study was sought from Moi University's Institutional Review Ethics Board (IREC). Prior to commencement of the study, permission was also sought from Kijabe Hospital Ethics Review Board and all County and Sub County public health facilities.

Informed consent was sought from all mothers after establishing eligibility for the enrollment into the study. A standard explanation was provided to all mothers of participants in a written informed consent before administering the questionnaires. The consent form described the purpose of the study, procedures followed the risks and benefits of participation in the study. Parents of participants signed the informed consent document and a thumb print was taken in case the mother was illiterate.

3.14.1 Confidentiality

All collected information from the participants was treated with confidentiality. We maintained confidentiality through use of codes in place of participants' names. All data was stored in password protected computer accessible to the Principal investigator.

3.14.2 Risks and benefits

There were no risks to the participants during administration of the questionnaire. Participants did not benefit directly from this study but result findings will be contribute in prevention of future occurrence of NTDs.

3.14.3 Criteria for termination of study

Mothers who consented to participate in the study were allowed to terminate the interview at any point during the study. Once the required sample size had been obtained then the study stopped at that point.

3.15 Dissemination of Findings

Research findings will be shared with Counties, Ministry of Health and Kijabe Hospital. Findings will also be published to this forums and publish in a peer review journal.

CHAPTER FOUR

RESULTS

4.1 Description of Study Participants

A total of 60 case infants and their mothers and 120 controls were recruited and interviewed during the study period.

4.1.1 Characteristics of enrolled mothers

4.1.1.1 Distribution of Participants by age and education

The median age of mothers was comparable in the two groups, cases had a median age of 27 (range of 19 to 42) years and controls 27 (range 15-42) years. Case mothers who were <24 years were 29 (31.7%) and control mothers were 36 (30%), while cases >35 years were 8 (13.3%) and 13 (10.8%) control mothers. Fig.4.1 shows the age distribution of mothers.

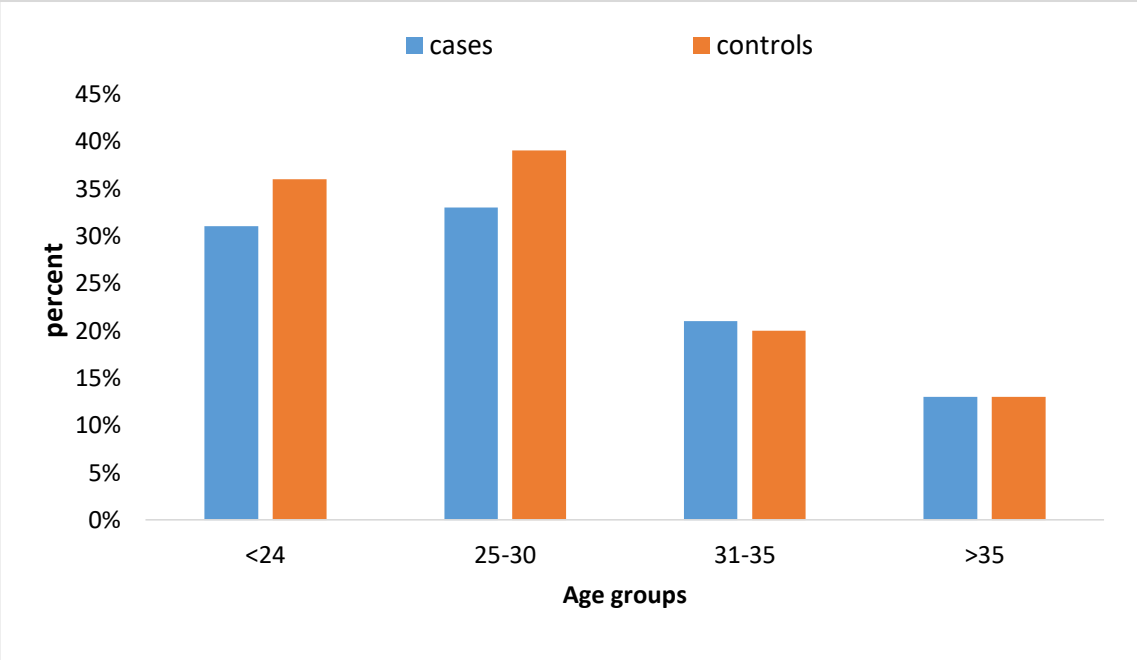


Figure 4.1: Age distribution of cases and controls enrolled in the study

Most of the mothers of enrolled infants (65.6%) had at least secondary level of education. Only 41.7% of case mothers and 30.1% of control mothers had primary level

of education or below as shown in table 4.1. Most of the case mothers had achieved primary level of education (n=25, 41.7%) whereas most of the control mothers had achieved post-secondary education (n=44, 36.7%) this indicates that 99% of the enrolled participants had attained at least primary level of education.

Table 4.1: Distribution of Cases and Controls by level of Education

Variable	Cases	Controls	Total
Median (range) number of years of education completed	10.5 (4, 16)	12 (0, 23)	12 (0, 23)
Education level		[n (%)]	
No education	0 (0.0)	2 (1.7)	2 (1.1)
Primary	25 (41.7)	35 (29.2)	60 (33.3)
Secondary	18 (30.0)	39 (32.5)	57 (31.7)
Post-secondary	17 (28.3)	44 (36.7)	61 33.9)

4.1.1.2 Distribution of Cases and Controls by marital status, occupation, parity and religious affiliation.

Most of the enrolled participants reported being Christians (90.0%) while a few were Muslims (8.3%). Majority of women in both groups were married (85.6%) with 51 (85%) case-mothers being married. The majority of case respondents were either farmers/casual workers (n=14, 23.3%) or in business (n=18, 30.0%) while in the control group majority were housewives (n=39, 32.5%) and in formal employment (n=35, 29.2%) as shown on table 4.2. Most enrolled mothers were multiparous (parity of ≥2) with cases being 51(85.0%) and controls 62(51.7%). In the wealth quantile index, majority of case mothers were grouped as poor 21 (35) and majority of the controls grouped as second rich 30 (24.6).

Table 4.2: Distribution of Cases and Controls by occupation, marital status, parity, religious affiliation and wealth

Variable	Cases (n=60) n (%)	Controls (n=120) n (%)	Totals (n=180) n (%)
Maternal characteristics			
Religion			
Muslim	3 (5.0)	12 (10.0)	15 (8.3)
Christian	55 (91.7)	107 (89.2)	162 (90.0)
Other	2 (3.3)	1 (0.8)	3 (1.7)
Marital status			
Never married	5 (8.3)	17 (14.2)	22 (12.2)
Married	52 (86.7)	102 (85.0)	154 (85.6)
Parity			
Para 1	9 (15.0)	58 (48.3)	67 (37.2)
Para >=2	51 (85.0)	62 (51.7)	113 (62.8)
Occupation			
House wife	13 (21.7)	39 (32.5)	52 (28.9)
Farmer/casual	14 (23.3)	9 (7.5)	23 (12.8)
Business	18 (30.0)	24 (20.0)	42 (23.3)
Formal job	10 (16.7)	35 (29.2)	45 (25.0)
Others	5 (8.3)	13 (10.8)	18 (10.0)
Wealth Quantiles			
Poor	21 (58.3, 34.4)	15 (41.7, 12.3)	36 (19.7)
Second poor	11 (31.4, 18.0)	24 (68.6, 19.7)	35 (19.1)
Middle	11 (31.4, 18.0)	24 (68.6, 19.7)	35 (19.1)
Second rich	7 (18.9, 11.5)	30 (81.1, 24.6)	37 (20.2)
Rich	10 (16.4)	27 (22.1)	37(20.2)

4.1.2 Characteristics of infants

There was no significant difference in age and gender of the case and control-infants as shown on table 4.3. The median age of infants with NTD was 3.5 months (range of 3 weeks to 12 months), while that of control infants was 5.4 months (range of 2 weeks to 12 months). Median gestational age at birth was similar among case-infants and control infants being 39 (range 36-42) weeks and 39 (range 34-42) weeks respectively. Majority of the enrolled infants were boys (61.7%). Case infants who were early

preterm (28-32 weeks gestation at birth) were 10 (16.7%) while majority were born at between 39-40 weeks gestational age (full term) were 47 (78.3%).

Table 4.3: Characteristics of Case and Control infants

Infant characteristics	**Cases (n=60)**	**Controls (n=120)**	**Totals (n=180)**	**P-value**
Age of infant in months				
Median (range)	3.5 (0.2, 12)	5.4 (0.04, 12)	5.4 (0.04, 12)	0.18
Sex [n (%)]				
Boys	37 (61.7)	74 (61.7)	111 (61.7)	1.00
Girls	23 (38.3)	46 (38.3)	69 (38.3)	
Gestational age at birth				
Median (range)	39 (36, 42)	39 (34, 42)	39 (34, 42)	0.03
Gestational age at birth [n (col %)]				
Late preterm (34-36weeks at birth)	1 (1.7)	10 (8.3)	11 (6.1)	0.24
Early term (28-32 weeks at birth)	10 (16.7)	26 (21.7)	36 (20.0)	
Full term (37-42 weeks at birth)	47 (78.3)	80 (66.7)	127 (70.6)	
Post term (>42 weeks at birth)	2 (3.3)	4 (3.3)	6 (3.3)	

4.1.3. Neural tube defects

As shown in Fig.4.2, majority of enrolled case infants (59, 98.3%) had spina bifida while only 1(1.7%) infant had encephalocele. Among the spina bifida cases, those who had meningocele were the majority with 29(48%), followed by myelomeningocele 25(41.7%) and spina bifida occulta 5(8.4 %).

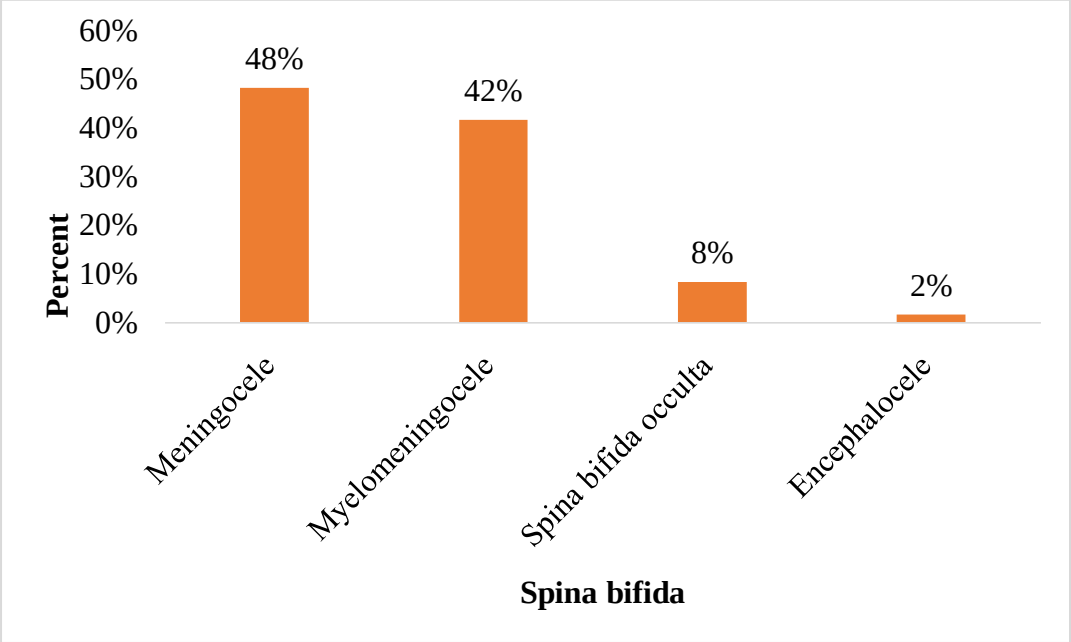


Figure 4.2: Type of Neural tube defects in Case infants enrolled in the study, July-November 2017, Kenya

4.2 Socio-Demographic Characteristics of Mothers Who Delivered Infants With NTDs

Case mothers who delivered NTD affected infants had an average median age of 27(range 19, 42) majority had primary level education, were married, Multiparous and majority were farmers/casual workers and some were involved in a small-scale business.

4.3 Effect of Maternal Health Risk Factors for NTDs

On bivariate analysis, history of illness before and during pregnancy was associated with a fourfold increased odds of an NTD affected infant (MH-OR 4.21 CI= 1.94, 9.15). This association was statistically significant. The specific maternal illnesses that were significantly associated with NTDs on bivariate analysis included maternal fever and hypertension. Maternal fever before and during first twelve weeks of pregnancy was associated with more than 8 times increased odds of having an NTD affected infant (MH-OR 8.86 CI= 3.58, 21.92). Maternal diabetes and malaria were not significantly associated with NTDs on bivariate analysis as shown in table 4.4.

Table 4.1: Maternal Factors associated with NTD risk among Cases and Controls

Variables	Cases n(%)	Controls n (%)	MH Odd Ratio	OR 95% CI		P-value
History of a disease before and during pregnancy						
Yes	27 (44.3)	21 (17.2)	4.21	1.94 - 9.2		<0.01
No	33 (54.1)	99 (81.1)	Ref			
Maternal Fever						
Yes	48 (78.7)	41 (33.6)	8.86	3.58	21.9	<0.01
No	12 (19.7)	79 (64.8)	Ref			
Malaria Diagnosis						
Yes	5 (8.2)	6 (4.9)	1.76	0.5	6.2	0.38
No	55 (90.2)	114 (93.4)	Ref			
Increased maternal blood pressure						
Yes	4 (6.6)	29 (23.8)	0.18	0.06	0.6	<0.01
No	56 (91.8)	91 (74.6)	Ref			
Maternal diabetes						
Yes	1 (1.6)	5 (4.1)	0.35	0.04	3.4	0.36
No	59 (96.7)	115 (94.3)	Ref			

Other proxies of maternal health investigated included medication taken during the peri-conceptional period either prescribed by a health care worker or purchased over

the counter. On bivariate analysis, use of antibiotics, antiretroviral, non-steroidal anti-inflammatory drugs and herbal medicines were significantly associated with NTDs. Those who took antibiotics had up to threefold increased odds of NTD (MH-OR 3.08 CI= 1.5, 6.32) compared to those who did not take antibiotics and this association was statistically significant. Among those who took antibiotics, Doxycycline was associated with 6 times increased risk, but despite the high OR it was not statistically significant (MH-OR 6.00 CI= 0.62 57.68). Taking non-steroidal anti-inflammatory drugs (NSAIDs) either for fever or pain had a statistically significant 7 fold increased odds (MH-OR-7.87 CI= 3.36, 18.45). Similarly, consuming herbal medicines during periconception period was significantly associated with NTDs (MH-OR 3.4 CI= 1.12, 10.34). In this study, few respondents (n=13, 7.2%) reported being on antiretroviral treatment for HIV. This notwithstanding this study found that mothers on ARVs treatment during the periconceptional period were significantly protected from delivering babies with NTDs.

Table 4.2: Bivariate analysis of use of drugs and effect on NTDs

Variable/risk factor	Cases n (%)	Control n (%)	MH Odds	95% CI	p-values
Took antibiotic					
Yes	23 (37.7)	19 (15.6)	3.08	1.5	<0.01
No	37 (60.7)	101 (82.8)	Ref	6.3	
Took doxycycline					
Yes	3 (5.0)	1 (0.8)	6	0.6	0.08
No	57 (95.0)	119 (99.2)	Ref	6.7	
Took antimalarial					
Yes	8 (13.1)	11 (9.0)	1.5	0.6	0.39
No	52 (85.2)	109 (89.3)	Ref	3.9	
Took ARVs					
Yes	1 (1.6)	12 (9.8)	0.09	0.01	0.03
No	59 (96.7)	108 (88.5)	Ref	1.1	
Took herbal medicine					
Yes	9 (14.8)	6 (4.9)	3.4	1.1	
No	51 (83.6)	114 (93.4)	Ref	10.3	0.03
Took NSAIDs					
Yes	49 (80.3)	43 (35.2)	7.87	3.4	<0.01
No	11 (18.0)	77 (63.1)	Ref	18.5	

4.4 Maternal Preconception Risk Factors for NTDs

We were unable to determine the effect of smoking cigarettes on NTDs since among all study participants, only one control-mother reported to have smoked cigarettes in the periconceptional period. Only 5 (2.8%) of participants reported use of alcohol during pregnancy. Although the proportions of case-mothers (4.9%) who used alcohol during pregnancy was higher than control-mothers (1.6%), this difference was not statistically significant (Table 4.6). Use or contact with pesticides during periconception period had more than fivefold increased odds of NTD (MH-OR 4.79 CI= 1.7, 13.5) compared to those who did not have contact with pesticides.

Table 4.1: Effect of periconception factors on NTDs

Variable/risk factor	Cases n (%)	Controls n (%)	MH Odds	95% CI	p-values
Smoke cigarettes when pregnant					
Yes	0 (0.0)	1 (0.8)	Ref	-	-
No	60 (100)	119 (99.2)			
Used alcohol during pregnancy					
Yes	3 (4.9)	2 (1.6)	3	0.5	0.23
No	57 (93.4)	118 (96.7)	Ref	17.9	
Use/contact pesticides					
Yes	14 (23.0)	8 (6.6)	4.79	1.7,	
No	46 (75.4)	112 (91.8)	Ref	13.5	<0.01

4.5 Effect of Consumption of Folic Acid Fortified Flour and Folic Acid Supplementation

The proportion of women who took periconceptional folic acid were significantly fewer (11.5 %) among cases than controls (40.2 %). Periconceptional folic acid supplementation was significantly protective of NTDs (Table 4.7). However only 1 (1.7 %) of the case participants initiated folic acid before conceiving while majority 43, (71.7 %) initiated folic acid supplementation during pregnancy. Most of the participants (80.1%) initiated folic acid supplementation in the 2nd or 3rd trimester of pregnancy.

Although the proportion of case-mothers who initiated folic acid supplementation before conceiving were lower (1.7%) than control-mothers (5.8%), this difference did not achieve statistical significance.

Majority of participants reported to have consumed unfortified flour (n=156, 86.7%). Those who reported to have consumed folic acid fortified flour at least three times per week were 42(70%) among cases and 58(48.3%) in the control group. Those who consumed folic acid fortified flour for only 3 times per week, had statistically significant threefold increased odds (MH-OR 3.17 CI= 1.45-, 6.9) of NTDs compared to those who did not.

Table 4.1: Folic acid supplementation and effect of consumption of folic acid fortified flour on NTDs among Cases and Controls

Variable	Case (n=60)	Controls (n=120)	OR	95% CI	p-values
Took folic acid 3 months before becoming pregnant and in the first 3 months of pregnancy					
Yes	7(11.5)	49(40.2)	0.17	0.05- 3.6	< 0.01
No	53(86.9)	71(58.2)	Ref		
Folic acid supplementation initiated before pregnancy					
Yes	1 (1.7)	7 (5.8)	0.29	0.04, 2.2	0.18
No	59 (98.3)	113 (94.1)	Ref		
Folic acid supplementation initiated during pregnancy					
Yes	43 (71.7)	103 (85.8)	0.41	0.9- 0.2	0.02
No	17 (28.3)	17 (14.2)	Ref		
Trimester of initiation of folic acid supplementation*					
First trimester	11 (25.6)	18 (17.5)	2.07	0.98- 4.4	0.21
Second trimester	24 (55.8)	52 (50.5)			
Third trimester	8 (18.6)	33 (16.5)			
Consumed unfortified flour					
Yes	57 (95.0)	99 (82.5)	3.5	1.07 - 11.4	0.02
No	3 (5.0)	21 (17.5)	Ref		
Consumed folic acid fortified flours (maize or wheat) at least 3 times per week for the first 3 months of pregnancy					
Yes	42 (70.0)	58 (48.3)	3.17	1.45- 6.9	<0.01
No	18 (30.0)	62 (51.7)	Ref		

**17 case mothers and 17 control mothers did not report the gestation at which they initiated folic acid supplementation*

4.6 Effect of Other Factors Investigated for Association with NTDs

All case-mothers (n=60) and 106(88.3%) control-mothers attended antenatal clinic (ANC), however more than half of the participants found out they were pregnant one month into the pregnancy. Multiparity was associated with a fivefold increase in risk while, a previous pregnancy loss had fourfold increase risk of having NTD affected infant. Those who reported to have a known family history of birth defect was 4 (6.7%) among case-mothers and 4 (3.3 %) among the controls. Although there was a twofold increase in odds of NTDs among those with family history of birth defect, this association was not statistically significant. In the wealth quantile index majority of cases were below the 50% quantile 40 (66.7) while majority of controls were above 50% index 53 (44.2).

Table 4.1: Effect of other factors investigated for association with NTDs in Cases and Controls

Antenatal practices of mothers					
Variables	**Cases (%)**	**Controls (%)**	**OR**	**(95% CI)**	**P-value**
Parity					
Para 1	9 (15.0)	58 (48.3)	4.7	2.12, 10.3	<0.01
Para >=2	51 (85.0)	62 (51.7)			
Previous Pregnancy Loss					
Yes	21(34.4)	11(9.0)	4.1	2.12, 10.3	<0.01
No	39(63.9)	109(89.3)	Ref		
How far along were you when you found that you were pregnant					
0-4 weeks	30 (50.0)	60 (50.0)			
5-8 weeks	19 (31.7)	36 (30.0)	4.56	1.82, 11.4	0.01
Above 8 weeks	11 (18.3)	24 (20.0)			
Family history of birth defect					
Yes	4(6.7)	6 (4.9)	2	0.5, 0.8	0.32
No	56 (93.3)	108 (96.4)			
Obesity					
Obese	3(5.0)	21(17.5)	3		
Non- obese	57(95.0)	99(82.5)		0.5. 17.9	0.23
Wealth class					
<50%	40 (66.7)	50 (41.7)	Ref		
50%-90%	18 (30.0)	53 (44.2)	0.32	0.10, 1.0	0.01
Top 10%	2 (3.3)	17 (14.2)	0.12	0.00, 2.9	

4.6.1 Factors entered in the multivariate model

Table 4.11 shows all factors that were included in the multivariate model to find the independent risk factors.

Table 4.9: Factors entered in the multivariate model to assess the risk of NTDs

Characteristic/risk factor	Cases (%)	Controls (%)	MH odds ratio	95% CI)	p-value
Attended ANC					
Yes	60 (98.4)	106 (86.9)	**7.62**	**0.01, 10.1**	p=0.006
No	0 (0.0)	14 (11.5)			
History of Birth Defect					
Yes	4 (6.6)	4 (3.3)	5.9	1.28, 26.9	0.1220
No	5 (91.8)	8 (6.6)			
Had fever				3.59, 21.9)	
Yes	48 (80.0)	41 (34.2)	8.86		0.01
No	12 (20.0)	79 (65.8)	Ref		
Parity				(1.45, 23.2)	
Para 1	9 (15.0)	58 (48.3)	Ref		0.15
Para >=2	51 (85.0)	62 (51.7)	5.79		
House wife was a farmer or casual	13 (21.3)	39 (32.0)	Ref		
	14 (23.0)	9 (7.4)	4.25	(1.53, 11.9)	0.01
Took folic acid before pregnancy					
Yes	43 (71.7)	103 (85.8)	1.00	0.02, 0.5	0.13
No	17 (28.3)	17 (14.2)			
Consumed folic acid fortified flour before pregnancy or in the first trimester					
Yes	42 (70.0)	58 (48.3)	7.19	2.25, 22.9	0.01
No	18 (30.0)	62 (51.7)	Ref		
Wealth class					
<50%	40 (66.7)	50 (41.7)	Ref		
50%-90%	18 (30.0)	53 (44.2)	0.32	0.10, 1.00	0.01
Use or often in contact with pesticides					
Yes	14 (23.3)	8 (6.7)	Ref	0.73, 32.1	0.18
No	46 (76.7)	112 (93.3)	4.83		

4.7 Multivariate Analysis of Independent Factors Associated with NTD Risk Among Cases and Controls and Factors Found to be Protective

Those who consumed folic acid fortified flour 3 times per week in the preconception period (aOR 6.9; 95% CI 1.61-29.6) , those who had a history of previous pregnancy

loss (aOR 6.4; 95% CI 2.4-17.4), use/contact with pesticides (aOR 44.12; 95% CI 1.270 -13.88),and multiparity (aOR 3.4; 95% CI 2.16 - 20.1) were significantly associated with risk of having an NTD affected infant, while use of folic acid supplements was found to be protective (aOR 0.13; 95% CI 0.045- 0.348).

Table 4.10: Multivariate Analysis of independent risk factors associated with NTD risk among cases and controls and factors found to be protective.

Characteristic/risk factor	Cases (%)	Controls (%)	**Adjusted odds ratio**	**OR 95% CI**
Consumed folic acid fortified flours (maize or wheat) at least 3 times per week for the first 3 months of pregnancy				
Yes	42 (70.0)	58 (48.3)	6.9	1.61-
No	18 (30.0)	62 (51.7)	Ref	29.6
Previous history of pregnancy loss				
Yes	21(34.4)	11(9.0)	6.4	2.4-
No	39(63.9)	109(89.3)	Ref	17.4
Use or often in contact with pesticides				
Yes	14 (23.3)	8 (6.7)	4.2	1.3-
No	46 (76.7)	112 (93.3)	Ref	13.9
Parity				
Para 1	9 (15.0)	9 (15.0)	Ref	
Para >=2	51 (85.0)	62 (51.7)	6.59	2.16 - 20.1
Took folic acid before pregnancy				
Yes	43(71.7)	103(85.8)	0.125	0.045-
No	17(28.3)	17(14.2)	Ref	0.4

CHAPTER FIVE

DISCUSSION

5.1 Maternal Social Demographic Characteristics

This was a case control study that looked at risk factors for NTDs in 6 Counties in Kenya. Mothers of infants with NTD were of varied ages with the youngest being 15 and the oldest was 42 years this was indicative of a mixed age group, majority being young mothers possibly due to the fact that women in low to middle income countries tend to have their index babies when they are still very young compared to developed nations (Lo, Polšek, et al., 2014). This finding is comparable to a demographic health survey conducted between 2005- 2015 in 45 countries(Neal, et al., 2018), which demonstrated that women who were young when they had their index babies were at an increased risk of delivering babies with NTDs (spina bifida). This could be due to lack of knowledge about folic acid and low folic acid uptake in this group (Bourouba, et al., 2018). In our study we found that mothers who delivered NTD affected infants were between the ages of 15-42 years.

Majority of case mothers had primary level education compared to controls. Most were housewives, farmers/ casual workers and small-scale business. Low level of education among women of reproductive age, has been associated with a higher risk of NTD affected infant. This could be attributed to either lack of knowledge and information on importance of early uptake of folic acid, unable to find employment predisposing to low social economic status (Taiwo Akeem Lawal1, 2014).

5.2 Antenatal Care Practices

Majority of mothers in both case and control groups in this study attended ANC. This was comparable with the Kenya national KDHS findings (KDHS, 2014) where ninety-six percent of women received ANC from a skilled health care provider.

Despite this impressive figure, majority of mothers in this study attended ANC at varied gestational stages. Those who attended clinics in the first trimester were less compared to those who had their first visit in the second trimester. The world health organization recommends at least eight or more contact ANC visits for effective monitoring and utilization of maternal Health services and receive positive maternal experience (World Health Organization (WHO), 2018), this recommendation has barely been achieved in most African countries including Kenya where most women hardly achieve the four visits previously recommended by WHO (Chorongo et al., 2016). This could be attributed from either lack of information, distance and travelling time, cost of service, or experience with previous births (Yadufashije, et al., 2017), leading to late folic acid supplementation when window period to provide protection against NTDs has elapsed (Bannink, Larok, et al, 2015). The First ANC visit is recommended in the first trimester (8-12 weeks) where pregnancy is confirmed, and screening tests carried out either prevent and manage disease are done and expected date of delivery postulated. Second visit at second trimester (24-26 weeks), third visit at 32 weeks and fourth visit at third trimester (36-38 weeks), (Lincetto et al., 2013). This ANC findings of low rate of utilization of folic acid in the periconceptional period are similar to those found in three teaching hospitals in Addis Ababa Ethiopia (Gedefaw, et al., 2018).

5.3 Characteristics of Infants with NTDs

Our study findings indicated that male infants with NTD were more than the females. This finding is contrary to a study done in South-America that analysed changes in sex ratios during pre-fortification phase. Females diagnosed with NTDs were more and during post- fortification indicated that males with NTD were more than the females (Poletta, et al., 2018). Although fortification policy passed in 2012 in Kenya, there was a delay in implementation of fortification because mothers in our study areas still consume both fortified and locally milled flour so these areas were still in the pre-fortification phase.

Majority of the NTDs in our study were spina bifida 98.3%, with meningocele leading with 48%, myelomeningocele 42%, spina bifida occulta 8.2% and encephalocele 1.7%. This findings are similar to a study conducted in Nigeria (Leonard, et al., 2017), but contrary to a study conducted in three hospitals in Addis Ababa where the most common NTD diagnosed prenatally was anencephaly 54%, followed by spina bifida 40.5%, and encephalocele 5.4% (Gedefaw et al., 2018). This study looked at live births excluding still births and abortions, there is a high chance we missed important data on the true number of NTDs like anencephaly which are incompatible with life.

5.4 Effect of Maternal Health on Risk of NTDs

Independent risk factors found to be associated with increased risk of NTD affected infant in this study were multiparity and pregnancy loss.

5.4.1 Multiparity

Majority of case mothers with NTD affected infants were multiparous (85%), grand multiparity is a common feature in many African societies either due to sociocultural or gender reasons (Maduabuchukwu et al., 2017). Multiparity predisposes these

mothers to micronutrient deficiencies, either by change in attitude leading to decline in use of folic acid supplements. Genetic aberrations leading to chromosomal abnormalities which increase with maternal age (Kitova, Karaslavova, et al., 2013). This finding is comparable to study done in Tigray Ethiopia that demonstrated an increased risk of NTD among multiparous mothers (Alem, et al., 2018).

5.4.2 Pregnancy loss

Previous history of pregnancy loss was found to be associated with increased risk of NTD in our study. This finding is similar to studies that have shown that previous abortions could predispose to future cases of NTD either because the aborted foetus had an NTD, or the trophoblastic cell rests remaining from a previous aborted foetus interfere with normal embryogenesis of subsequent pregnancies (Chen, 2007).

Several factors that have been identified by many studies to be associated strongly with NTDs in the periconceptional period, are Illness in the periconceptional and fever. We found these variables to be significant on bivariate analysis and included in the multivariate model but we did not find them to be independent risk factors in the final multivariate model.

5.4.3 Illness in the periconceptional

Illness in the periconceptional was associated with increased NTD risk in our findings. This finding is comparable to studies that have demonstrated that an illness in the periconceptional period whether acute or chronic is associated with genetic mutations, decline in immunity and so increased risk of co-morbidities leading increased risk for NTD (Luche tadesse, et al., 2014).

5.4.4 Febrile illness

Fever a known teratogen was found to be a risk factor in our bivariate analysis but not in the multivariate, contradicting findings from previous studies that found fever to be an independent risk. Febrile illness in the first 12 weeks of pregnancy could result from an infectious process during the periconceptional period. Studies have associated a febrile process with a threefold increase in NTD affected pregnancy (Suarez et al., 2004). Fever was strongly correlated with use of analgesics and antipyretics used to relieve discomfort of fever and pain, analgesics/antipyretics has also been linked to an increased risk of NTDs (Li, et al., 2006). A study among American women of Mexican heritage demonstrated that maternal fever in the periconceptional period carried a two to four fold increase in NTD affected pregnancy (Suarez et al., 2004). Fever causing disease common in our setup include malaria, a parasitic illness that presents with fever and is caused by plasmodium falciparum which uses the host plasma folate as its main source of folate for its growth causing impairment of folate mediated carbon metabolism pathway. Malaria infection in pregnancy causes increased plasma homocysteine levels leading to placenta impairment and consequently causing NTD in affected embryos (Verhoef, et al., 2017). A study in North-West Nigeria demonstrated that women who had malaria infection in the first trimester and were treated with antimalarial drugs led to an NTD affected infant. Antimalarial drugs are known to be anti-folate modulators and was associated with increase in spina bifida cystica cases (Emejulu et al., 2012).

5.4.5 Maternal diabetes

Although maternal diabetes is a known risk factor for NTDs, it did not achieve statistical significance on bivariate analysis because only a few participants had reported diabetes in this study. This study relied on reported diabetes and never tested

the mothers for the levels of blood sugar nor glycosylated haemoglobin to determine how well controlled their diabetes was. Undiagnosed diabetes was therefore not included in the analysis even though it could lead to NTDs. This misclassification was likely non-differential and could only mask the strength of association.

Maternal Diabetes is a known teratogen and pre-pregnancy obesity, and states of hyperinsulinemia, intake of foods with a higher glycaemic index was demonstrated to have two-fold to tenfold increase in NTDs.

Diabetes affects embryogenesis by disrupting expression of genes that control developmental processes in the embryo leading to oxidative stress induced deficient gene causing development of NTDs. The effect of hyperglycaemia to the developing embryo inhibits Myo-Inositol which is critical to the developing embryo (Anyanwu, 2015). Women of reproductive age with known insulin dependent diabetes mellitus should have a strict glycaemic control in order to maintain an optimal glucose homeostasis to prevent incidences of NTDs. However, on bivariate analysis, this study found that maternal diabetes was a protective factor for NTDs but was not statistically; this finding can be attributed to low numbers of mothers found to have diabetes in our study.

5.4.6 Antiretroviral therapy

Anti-retro viral drugs (ARVs) and more so Dolutegravir (DTG) has recently been implicated with a seven fold increase in cases of NTD in Botswana which was one of the first countries to recommend its use in pregnancy (Schomaker, Davies,et al., 2018). Despite this finding few mothers reported to be on ARVs. It is possible that some participants were reluctant to report their HIV status resulting in a misclassification bias. Anti-retroviral (ARVs), were protective against NTDs in this study. This was

surprising it could be explained by the small sample size of those who were on ARVs thus was unlikely to find it statistically significant. Whereas some ARVs have been associated with NTDs we did not look at the specific ARV that the participants took during pregnancy probably masking the strength of association. Further studies should be carried out to assess the impact of Dolutegravir (DTG) and other ARVs as risk factors for NTD in our setting.

5.5 Other risk factors found to be associated with increased NTDs risk

5.5.1 Maternal age

Maternal age < 19 and above 35 years was found to be a risk factor. This finding is similar to a study conducted in a North Indian Province that demonstrated that a higher maternal age was linked to a higher incidence of NTD in women above forty years (Kadian, 2017). In this study, the highest maternal age was 42 years while the youngest was 15. Studies have also shown that some NTDs are more increased in certain age groups with women below nineteen years having a strong predilection to have infants with spina bifida compared to above 35 years who have anencephaly affected infants (De Marco et al., 2011b).

5.5.2 Previous birth complicated by a birth defect

Studies have demonstrated this risk to have three to five fold increase risk of NTD affected outcome in subsequent pregnancies. This could be due to genetic aberrations or consanguinity among parents (Glinianaia, et al., 2017). History of maternal illness before pregnancy and into the first trimester was has been associated with higher odds of increased NTD affected pregnancy (Taye, et al., 2018).

5.5.3 Wealth Quantile

Our study findings demonstrated that a wealth quantile above 50% translating to middle level class or rich had low odds of NTD affected infants. This group have better health seeking behaviour, employment translated to better income, access to health information and health care and can afford balanced diet, while low income would have the opposite outcome (Lo et al., 2014).

5.6 Periconceptional factors Associated with increased risk for NTDs

Some maternal lifestyle and occupational factors in the periconceptional period has been associated with increased NTD risk (De Marco et al., 2011b).

5.6.1 Pesticides

This study demonstrated that periconceptional exposure to pesticides used either in a residential setting or occupational (farms, animals) used either in high or low doses demonstrated significantly increased risk of NTDs**.** From our study the most common source of pesticides was seen in those working in the farm and either came in contact with pesticides by spraying crops or animals, or working in flower farms where, 23% of cases were farmers/casual labourers working in farms of whom14 farmers among cases all of whom were exposed to pesticides. Among 9 farmers who were controls, 8 were exposed to pesticides.

Further investigation is required to determine the type of pesticides that predisposed to NTDs in our setup. Chemicals and pesticides have been shown to cross the placental barrier and alter neuroepithelial cells proliferation and negatively impacting embryogenesis causing NTD (Ren et al., 2011)**.**

5.6.2 Herbal medicines

On Bivariate analysis we found that exposure to herbal medicines was statistically significant but was not significant risk factor on final multivariate model though many studies have associated it with NTD risk. Herbal medicine is taken frequently in many African countries as part of traditional culture and to maintain general wellbeing and good health of the mother and the growing foetus. It has also been linked to contain lead which is a Bivalent ion that competes with zinc in the gut leading to a decrease in zinc as it's replaced by lead. Zinc is vital in the absorption and synthesis of folate and its deficiency leads to impaired folate affecting DNA repair and synthesis of nucleic acids in the developing embryo leading to development of NTDs (Leonard et al., 2017).

5.6.3 Alcohol

Although a higher proportion of cases drunk alcohol compared to controls on bivariate analysis, this difference was not statistically significant. This can be explained by the fact that only few women reported drinking alcohol during pregnancy. Drinking of alcohol has been shown by previous studied to be associated with NTDs. A study conducted in Amhara region in Ethiopia where alcohol both beer and traditional brew is consumed by the community including women, noted that there was an increase in congenital abnormalities in women who took alcohol with frequency of NTDs being about 30% (Taye et al., 2018).

It is likely that there was underreporting of alcohol consumption in this study because it is not socially acceptable in this setting.

5.6.4 Smoking

Cigarette Smoking has been shown to have a positive correlation whether be active and or passive cigarette smoking with increased risk of NTD as demonstrated by

(Kondo et al., 2013), and (Kallen, et al., 1998). Despite this study findings we were unable to determine the effect of smoking on risk for NTDs. While Smoking among women in the area of our study is not socially acceptable and is frowned upon it is likely that smoking was under reported in this study thus leading to a misclassification bias. None of the case mothers reported to have smoked while one control reported to have smoked cigarettes. In this regard, we were unable to assess whether smoking cigarettes during pregnancy was a risk factor for NTD in this population.

5.7 Effect of Folic Acid Supplementation and Consumption of Folic Acid Fortified Flour on Neural Tube Defect

5.7.1 Folic acid

This study found folic acid supplementation in the peri-conception period to be an independent protective of NTDs. This finding is consistent with many other studies done in Africa (Sayed, et al, 2008), Europe (De Marco et al., 2011b) and Americas (Viswanathan et al., 2017). It is however notable in our study that only a small proportion of case mothers (1.7%) and control mothers (5.8%) initiated folic acid supplementation before pregnancy. Majority of mothers (81%) reported to have initiated folic acid supplements during pregnancy most (81%) of which had been initiated in a health facility. Even when initiated during pregnancy, 74% of the mothers initiated folic acid supplementation in the second and third trimester of pregnancy, which is too late to prevent NTDs since the defect results from abnormal closure of the neural tube during embryogenesis in the 28^{th} day of conception within the 1^{st} trimester (Mckeever, 2004).

The Proportion of Women who took folic acid during the periconceptional period was very low in our study compared to findings from developed countries where

periconceptional folic acid supplement is initiated early (44%) (Cawley et al., 2016) Majority of mothers were taking folic acid late into the last weeks of the first trimester and early into the second trimester. Previous studies have shown that low uptake of folic acid in low and middle income countries could be due to lack of information on importance of early intake of folic acid, low social economic status, low education (Tsawe & Susuman, 2014).

At the time we conducted our study, not much sensitization had been done to health workers and women of reproductive age on the association between folic acid and NTDs, our finding was similar to that by (Githuku et al., 2014). Consequently, much of the folic acid supplementation initiated during pregnancy is aimed at preventing anaemia in pregnancy which is a major cause of morbidity and mortality during pregnancy (Kimiywe, et al, 2017) . Besides, even if folic acid supplementation was to be initiated to prevent NTDs, another challenge that exists in Kenya and other parts of Africa is that many women do not plan their pregnancies and supplementation would therefore not be initiated early enough during the preconception period to prevent NTDs. This situation is not unique to Kenya but has been found in other countries in Africa (Taiwo et al, 2014), Europe (Cawley et al., 2016) and America (Blanco Muñoz et al., 2005). Lack of folic acid supplementation during preconceptional period is the strongest risk factor of having an NTD affected infant (De Marco et al., 2011a).

5.7.2 Consumption of folic acid fortified foods

Consumption of folic acid fortified flour 3 times per week was associated with increased risk of NTDs in this study. This was a new finding and contradicted previous studies which found that consumption of folic acid fortified foods would protect women of reproductive age even if they had unintended pregnancies from delivering children

with NTDs (R. Sayed et al., 2008). Majority of mothers in this study consumed locally milled flour. Consumption of store bought FA fortified flour 3 times per week was insufficient to confer protection to the foetus from NTDs. A possible explanation for this unusual finding could be due to the fact that, the amount of folic acid consumed daily in this group did not meet the minimum recommended daily dose of 400ug (Youngblood et al., 2013), required to prevent NTDs in the long run in the population (Singer M, *et al*, 2016). An article in the local Kenyan daily could also support this, published on 12th July 2018 reported that only about 28% of maize and wheat flour sold locally met the stipulated micronutrient quantities recommended by the government, which is about 200 mg/ 100 gm of flour (Ombogo, G. standard newspaper 2018 Ed).

In June of 2012 the Kenya government passed a policy for mandatory fortification of maize and wheat flour (Government of Kenya, 2012). Despite this policy change, implementation of the policy was delayed and later revised on 24th July 2015 and food fortification strategic plan final press was signed in August 2018 when folic acid fortified flour should be available in the market (Ministry of Health, 2018). Since this study did not measure the level of folic acid in the fortified flour, it is unclear whether the fortified flour in the market at the time the women conceived had the required level of folic acid. Many studies have demonstrated that folic acid supplementation in the periconceptional period provides protection against NTDs. A daily dosage of 400 mg daily supplemented with fortified flours and foods rich in folate sources decreasing the incidence of NTD affected pregnancies (Viswanathan et al., 2017).

In summary studies have found that women with fever and or have a diagnosis of malaria or those with a history of previous pregnancy affected by NTD, or those on anti-folate medicines, diabetic women, active smokers or women exposed to passive

cigarette smoke, should be supplemented with higher doses of folic acid up to 5 mg per day during the periconceptional period since low folate leads to elevated homocysteine which has been linked to increase in NTD (Verhoef et al., 2017).

5.8 Limitations

1. We enrolled infants born alive and excluded abortions/still births. Severe NTDs like anencephaly, could have contributed to cases of abortions and still births. Therefore, we were unlikely to detect exposures that are associated with severe NTDs incompatible with life.
2. Inherent in the design of case control studies is recall bias. Cases are more likely to remember events than controls. To minimise recall bias and missing details of folic acid use in the preconceptional period, infection and major events that could have occurred during periconceptional period of mothers of infants were enrolled in the study and we excluded children above one year.
3. We did not test for laboratory levels of folate in maternal blood and amount of folic acid in the fortified foods so were not able to estimate the serum folate levels or folate deficiency among the respondents.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusions

1) Majority of both case and control mothers attended ANC, with most starting late into the 1st trimester and majority in the 2nd trimester.
2) Mothers who supplemented folic acid in periconceptional period was too low. Majority took folic acid late into the first trimester and majority in the early period of second trimester when the window period to confer protection from NTDs had closed.
3) Intake of folic acid fortified flour was very low in case and control groups of women.
4) Exposure to pesticides was associated with increased risk of NTDs
5) Multiparity and previous history of pregnancy loss were associated with increased risk of NTDs.
6) Early initiation of FA supplementation before pregnancy was protective of NTDs.
7) Periconception consumption of FA fortified flour was not protective from NTDs.

6.2 Recommendations

1. Create awareness through public forums, health institutions by leaders and stakeholders in Health, Agriculture, Education sectors on risk of birth defects associated with exposure to pesticides and encourage use of personal protective gear

2. Sensitize and capacity build Health care workers to encourage and support women of reproductive age visiting Health facilities through health education on benefits of periconceptional FA supplements
3. Post market surveillance by Government and private regulatory bodies to ensure that millers are complying with the recommended levels of FA in fortified flour.

REFERENCES

Alem, B., Leul, A., Berhe, Y., & Magana, T. (2018). Maternal risk factors associated with neural tube defects in Tigray regional state of Ethiopia Maternal risk factors associated with neural tube defects in Tigray regional state of Ethiopia. *Brain and Development*, (July), 0–8. https://doi.org/10.1016/j.braindev.2018.07.013

Anyanwu, et al. (2015). T Nigehe Prevalence of Neural Tube Defects in Live Born Neonates in Kano , North-westernria, 105–109. https://doi.org/10.4103/2384-5147.164417

Bannink, F., Larok, R., Kirabira, P., Bauwens, L., & Hove, G. van. (2015). Prevention of spina bifida: folic acid intake during pregnancy in Gulu district, northern Uganda. *Pan African Medical Journal*, *20*, 1–9. https://doi.org/10.11604/pamj.2015.20.90.5338

Blanco Muñoz, J., Lacasaña, M., Borja Aburto, V. H., Torres Sánchez, L. E., García García, A. M., & López Carrillo, L. (2005). Socioeconomic factors and the risk of anencephaly in a Mexican population: a case-control study. *Public Health Reports (Washington, D.C. : 1974)*, *120*(1), 39–45. Retrieved from http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1497685&tool=pmcentrez&rendertype=abstract

Blatter, B. M., van der Star, M., & Roeleveld, N. (1994). Review of neural tube defects: risk factors in parental occupation and the environment. *Environmental Health Perspectives*, *102*(2), 140–145. https://doi.org/10.1289/ehp.94102140

Botto, L. D., Lisi, A., Bower, C., Canfield, M. A., Dattani, N., De Vigan, C., … Mastroiacovo, P. (2006). Trends of selected malformations in relation to folic acid recommendations and fortification: An international assessment. *Birth Defects Research Part A - Clinical and Molecular Teratology*, *76*(10), 693–705. https://doi.org/10.1002/bdra.20307

BOTTO, L. O. D. B., C YNTHIA A. M OORE , .D., M. U. J. K. H., & RICKSON, A. J. D. A. E. (1999). FEATURES. *Medical Progress*, *341*(20), 1509–1519.

Bourouba, et al. (2018). The Egyptian Journal of Medical Human Genetics Risk factors of neural tube defects : A reality of Batna region in Algeria. *Egyptian Journal of Medical Human Genetics*, *19*(3), 225–229. https://doi.org/10.1016/j.ejmhg.2017.10.003

Bowman, R. M., & McLone, D. G. (2010). Neurological Management of Spina Bifida: Research Issues. *Developmental Disabilities Research Reviews*, *16*(1), 82–87. https://doi.org/10.1002/ddrr.100

Cawley, S., Mullaney, L., McKeating, A., Farren, M., McCartney, D., & Turner, M. J. (2016). An analysis of folic acid supplementation in women presenting for antenatal care. *Journal of Public Health (Oxford, England)*, *38*(1), 122–129. https://doi.org/10.1093/pubmed/fdv019

Chen, C. (2007). C HROMOSOMAL A BNORMALITIES A SSOCIATED WITH N EURAL T UBE D EFECTS (I): F ULL A NEUPLOIDY, *46*(4), 325–335.

Chorongo, D., Okinda, F. M., Kariuki, E. J., Mulewa, E., Ibinda, F., Muhula, S., … Muga, R. (2016). Factors influencing the utilization of focused antenatal care services in Malindi and Magarini sub-counties of Kilifi county, Kenya. *The Pan African Medical Journal*, *25*(Supp 2), 14. https://doi.org/10.11604/pamj.supp.2016.25.2.10520

Christianson, A., Howson, C., & Modell, B. (2006a). March of Dimes. Global report on birth defect. The hidden toll of dying and disabled children. *New York*, 10–16. Retrieved from http://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:MARCH+OF+DIMES+GLOBAL+REPORT+ON+BIRTH+DEFECTS#6

Christianson, A., Howson, C., & Modell, B. (2006b). March of Dimes. Global report on birth defect. The hidden toll of dying and disabled children. *New York*, 10–16. Retrieved from http://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:MARCH+OF+DIMES+GLOBAL+REPORT+ON+BIRTH+DEFECTS#6

Corneg??-Blokland, E., Jansen, H. E., De Jong-De Vos Van Steenwijk, C. C. E., & Poenaru, D. (2011). Quality of life of children with spina bifida in Kenya is not related to the degree of the spinal defects. *Tropical Medicine and International Health*, *16*(1), 30–36. https://doi.org/10.1111/j.1365-3156.2010.02680.x

De Marco, P., Merello, E., Calevo, M. G., Mascelli, S., Pastorino, D., Crocetti, L., … Capra, V. (2011a). Maternal periconceptional factors affect the risk of spina bifida-affected pregnancies: An Italian case-control study. *Child's Nervous System*, *27*(7), 1073–1081. https://doi.org/10.1007/s00381-010-1372-y

De Marco, P., Merello, E., Calevo, M. G., Mascelli, S., Pastorino, D., Crocetti, L., … Capra, V. (2011b). Maternal periconceptional factors affect the risk of spina bifida-affected pregnancies: An Italian case-control study. *Child's Nervous System*, *27*(7), 1073–1081. https://doi.org/10.1007/s00381-010-1372-y

Driscoll, D. A., & Gross, S. J. (2009). Screening for fetal aneuploidy and neural tube defects. *Genetics in Medicine*, *11*(11), 818–821. https://doi.org/10.1097/GIM.0b013e3181bb267b

Emejulu, J. K. C., & Okwaraoha, B. O. (2012). Peculiarities in cases of spina bifida cystica managed recently in South-East Nigeria: Could antimalarial drugs be a major but unrecognized etiologic factor? *Pediatric Neurosurgery*, *47*(3), 194–197. https://doi.org/10.1159/000334255

Firestone, R. (2014). Nationally Representative, (October). https://doi.org/10.13140/2.1.3118.0806

Flores, A., Vellozzi, C., Valencia, D., & Sniezek, J. (2014). Global Burden of Neural Tube Defects, Risk Factors, and Prevention. *Indian Journal of Community Health*, *26*(Suppl 1), 3–5. Retrieved from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4480200/

Garne, E., Loane, M., Dolk, H., Barisic, I., Addor, M. C., Arriola, L., … Wiesel, A. (2012). Spectrum of congenital anomalies in pregnancies with pregestational diabetes. *Birth Defects Research Part A - Clinical and Molecular Teratology*, *94*(3), 134–140. https://doi.org/10.1002/bdra.22886

Gedefaw, A., Teklu, S., & Tadesse, B. T. (2018). Magnitude of Neural Tube Defects and Associated Risk Factors at Three Teaching Hospitals in Addis Ababa , Ethiopia, *2018*.

Githuku, J. N., Azofeifa, A., Valencia, D., Ao, T., Hamner, H., Amwayi, S., … Arvelo, W. (2014). Assessing the prevalence of spina bifida and encephalocele in a Kenyan hospital from 2005–2010: implications for a neural tube defects surveillance system. *The Pan African Medical Journal*, *18*. https://doi.org/10.11604/pamj.2014.18.60.4070

Glinianaia et al. (2017). Risk estimates of recurrent congenital anomalies in the UK: A population-based register study. *BMC Medicine*, *15*(1), 1–14. https://doi.org/10.1186/s12916-017-0789-5

Government of Kenya. (2012). Kenya: mandatory fortification amendment, 497–499.

Grewal, J., Carmichael, S. L., Song, J., & Shaw, G. M. (2009). Neural tube defects: An analysis of neighbourhood- and individual-level socio-economic characteristics. *Paediatric and Perinatal Epidemiology*, *23*(2), 116–124. https://doi.org/10.1111/j.1365-3016.2008.00992.x

Health, M. O. F. (2018). Kenya National Food Fortification Strategic Plan, 2018–2022.

Kadian, et al. (2017). Neural tube defects and maternal characteristics in a North Indian Province. *African Journal of Medical and Health Sciences*, *16*(1), 25. https://doi.org/10.4103/2384-5589.209484

Kallen, K. (1998). Maternal Smoking , Body Mass Index , and Neural Tube Defects Etiologic factors in neural tube defects (NTDs) have been studied extensively during the past decades , and a great number of studies evaluating exposures and Decreasing trends over time , sea, *147*(12), 1103–1111.

kenya demographic and health survey, 2014. (2014). Kenya. *Kenya Demogrphic Health Survey*, 149–155.

Kimiywe, J., Ahoya, B., & Kavle, J. (2017). Barriers to Maternal Iron-Folic Acid Supplementation and Compliance in Kisumu and Migori , Kenya, (January).

Kitova, T. T., Karaslavova, E. G., Masmoudi, A., & Gaigi, S. S. (2013). Maternal factors and associated anomalies in NTD fetuses from Tunisia, *8*(6), 707–712. https://doi.org/10.2478/s11536-013-0238-6

Kondo, A., Morota, N., Ihara, S., Saisu, T., Inoue, K., Shimokawa, S., … Watanabe, T. (2013). Risk factors for the occurrence of spina bifida (a case-control study) and the prevalence rate of spina bifida in Japan. *Birth Defects Research Part A - Clinical and Molecular Teratology*, *97*(9), 610–615. https://doi.org/10.1002/bdra.23179

Leonard, A., Ezeome, I. V, & Ozumba, B. (2017). A 10 Year Review of Neural Tube Defects in South-East Nigeria, (March 2018).

Li, Z., Ren, A., Zhang, L., Guo, Z., & Li, Z. (2006). A population-based case-control study of risk factors for neural tube defects in four high-prevalence areas of Shanxi province, China. *Paediatric and Perinatal Epidemiology*, *20*(1), 43–53. https://doi.org/10.1111/j.1365-3016.2006.00694.x

Li, Z. Z., Ren, A., Zhang, L., Guo, Z., Li, Z. Z., De Marco, P., … Capra, V. (2011). A population-based case-control study of risk factors for neural tube defects in four high-prevalence areas of Shanxi province, China. *Child's Nervous System*, *20*(7), 43–53. https://doi.org/10.1111/j.1365-3016.2006.00694.x

Lincetto, O., Mothebesoane-anoh, S., Gomez, P., & Munjanja, S. (2013). Antenatal Care: Opprotunities for Africa's Newborns. *International Journal of Scientific & Technology Research*, *2*(2), 51–62. https://doi.org/10.1111/phn.12242

Liu, L., Johnson, H. L., Cousens, S., Perin, J., Scott, S., Lawn, J. E., … Black, R. E. (2012). Global, regional, and national causes of child mortality: An updated systematic analysis for 2010 with time trends since 2000. *The Lancet*, *379*(9832), 2151–2161. https://doi.org/10.1016/S0140-6736(12)60560-1

Lo, A., Polšek, D., & Sidhu, S. (2014). Estimating the burden of neural tube defects in low– and middle–income countries. *Journal of Global Health*, *4*(1). https://doi.org/10.7189/jogh.04.010402

luche tadesse. , fikru tafesse, hanan hammy. (2014). Communities and Community Genetics in Ethiopia, *8688*, 1–7. https://doi.org/10.11604/pamj.2014.18.115.3172

Maduabuchukwu, I. E., & Ayuba, I. I. (2017). Grandmultiparity : The Reasons Women Give for High Parity in South-South Nigeria, *6*(3), 92–98. https://doi.org/10.11648/j.cmr.20170603.16

Mckeever, P. E. (2004). Central Nervous System. *Sternberg´s Diagnostic Surgical Pathology*. https://doi.org/10.1161/ATVBAHA.116.307023

Ministry of Health. (2010). National Guidelines for Quality Obstetrics and Perinatal Care. *Moh 2010*, 1–410.

Mitchell, L. E. (2005). Epidemiology of neural tube defects. *American Journal of Medical Genetics Part C: Seminars in Medical Genetics*, *135C*(1), 88–94. https://doi.org/10.1002/ajmg.c.30057

Moore, K., Persaud, V., & Torchia, M. (2013). *The Developing Human. Clinically Oriented Embryology*.

Muga, R., Mumah, S., & Juma, P. (2009). Congenital Malformation among newborns in Kenya. *African Journal of Food Agriculture Nutrition and Development*, *9*(3). https://doi.org/10.4314/ajfand.v9i3.43008

Neal, S., Channon, A. A., & Chintsanya, J. (2018). The impact of young maternal age at birth on neonatal mortality: Evidence from 45 low and middle income countries. *PLoS ONE*, *13*(5), 1–16. https://doi.org/10.1371/journal.pone.0195731

Njamnshi, A. K., Djientcheu, V. de P., Lekoubou, A., Guemse, M., Obama, M. T., Mbu, R., … Kago, I. (2008). Neural tube defects are rare among black Americans but not in sub-Saharan black Africans: The case of Yaounde - Cameroon. *Journal of the Neurological Sciences*, *270*(1–2), 13–17. https://doi.org/10.1016/j.jns.2008.01.010

Pitkin, R. M. (2007). Folate and neural tube defects. *The American Journal of Clinical Nutrition*, *85*(1), 285S-288S. Retrieved from http://ajcn.nutrition.org/content/85/1/285S

Poletta, et al. (2018). Neural tube defects: Sex ratio changes after fortification with folic acid. *PLoS ONE*, *13*(3), 1–12. https://doi.org/10.1371/journal.pone.0193127

Ren, A., Qiu, X., Jin, L., Ma, J., Li, Z., Zhang, L., … Zhu, T. (2011). Association of selected persistent organic pollutants in the placenta with the risk of neural tube defects. *Proceedings of the National Academy of Sciences*, *108*(31), 12770–12775. https://doi.org/10.1073/pnas.1105209108

Salih, M. A., Murshid, W. R., & Seidahmed, M. Z. (2014). Epidemiology, prenatal management, and prevention of neural tube defects. *Saudi Medical Journal*, *35*(05), S15–S28.

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. https://doi.org/10.1002/bdra.20442

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. https://doi.org/10.1002/bdra.20442

Schomaker, M., Davies, M. A., Cornell, M., & Ford, N. (2018). Assessing the risk of dolutegravir for women of childbearing potential. *The Lancet Global Health*, *6*(9), e958–e959. https://doi.org/10.1016/S2214-109X(18)30326-7

Singer M, Deutschman CS, Seymour C, & et al. (2016). Prevention of neural tube defects. *JAMA*, *315*(8), 801–810. https://doi.org/10.1001/jama.2016.0287

Suarez, L., Felkner, M., & Hendricks, K. (2004). The effect of fever, febrile illnesses, and heat exposures on the risk of neural tube defects in a Texas-Mexico border population. *Birth Defects Research Part A - Clinical and Molecular Teratology*, *70*(10), 815–819. https://doi.org/10.1002/bdra.20077

Taiwo Akeem Lawal1, A. O. A. (2014). Determinants of folic acid intake during preconception and in early pregnancy by mothers in Ibadan, Nigeria, *8688*, 1–6. https://doi.org/10.11604/pamj.2014.19.113.4448

Taye, et al. (2018). Factors associated with congenital anomalies in Addis Ababa and the Amhara Region, Ethiopia: A case-control study. *BMC Pediatrics*, *18*(1), 142. https://doi.org/http://dx.doi.org/10.1186/s12887-018-1096-9

Tsawe, M., & Susuman, A. S. (2014). Determinants of access to and use of maternal health care services in the Eastern Cape, South Africa: a quantitative and qualitative investigation. *BMC Research Notes*, *7*(1), 723. https://doi.org/10.1186/1756-0500-7-723

van't Veer, T., Meester, H., Poenaru, D., Kogei, A., Augenstein, K., & Bransford, R. (2008). Quality of life for families with spina bifida in Kenya. *Tropical Doctor*, *38*(3), 160–162. https://doi.org/10.1258/td.2007.070053

Verhoef, H., Veenemans, J., Mwangi, M. N., & Prentice, A. M. (2017). Safety and benefits of interventions to increase folate status in malaria-endemic areas. *British Journal of Haematology*, *177*(6), 905–918. https://doi.org/10.1111/bjh.14618

Viswanathan, M., Treiman, K. A., Kish-Doto, J., Middleton, J. C., Coker-Schwimmer, E. J. L., & Nicholson, W. K. (2017). Folic Acid Supplementation for the Prevention of Neural Tube Defects. *Jama*, *317*(2), 190. https://doi.org/10.1001/jama.2016.19193

Wang, H., De Steur, H., Chen, G., Zhang, X., Pei, L., Gellynck, X., & Zheng, X. (2016). Effectiveness of folic acid fortified flour for prevention of neural tube defects in a high risk region. *Nutrients*, *8*(3). https://doi.org/10.3390/nu8030152

Wang, M., Wang, Z. P., Gao, L. J., Gong, R., Zhang, M., Lu, Q. Bin, & Zhao, Z. T. (2013). Periconceptional factors affect the risk of neural tube defects in offspring: A hospital-based case-control study in China. *Journal of Maternal-Fetal and Neonatal Medicine*, *26*(11), 1132–1138. https://doi.org/10.3109/14767058.2013.770461

Warf, B. C., Wright, E. J., & Kulkarni, A. V. (2011). Factors affecting survival of infants with myelomeningocele in southeastern Uganda. *Journal of Neurosurgery: Pediatrics*, *7*(2), 127–133. https://doi.org/10.3171/2010.11.PEDS10428

Warf, B., Ondoma, S., Kulkarni, A., Donnelly, R., Ampeire, M., Akona, J., … Nsubuga, B. K. (2009). Neurocognitive outcome and ventricular volume in children with myelomeningocele treated for hydrocephalus in Uganda. *Journal of Neurosurgery. Pediatrics*, *4*(6), 564–570. https://doi.org/10.3171/2009.7.PEDS09136

World Health Organization. (2014). Birth defects surveillance: a manual for programme managers, 126. Retrieved from http://www.who.int/iris/handle/10665/110223

World Health Organization (WHO). (2018). WHO Recommendations on Antenatal Care for a Positive Pregnancy Experience: Summary. *World Health Organization*, *10*(January), 1–10. https://doi.org/10.1186/1742-4755-10-19.5

Wu, V. K., Poenaru, D., & Poley, M. J. (2013). Burden of surgical congenital anomalies in Kenya: A population-based study. *Journal of Tropical Pediatrics*, *59*(3), 195–202. https://doi.org/10.1093/tropej/fmt001

Yadufashije, D. C., Sangano, G. B., & Samuel, R. (2017). Barriers to Antenatal Care Services Seeking in Africa. *Ssrn*, (January), 0–14. https://doi.org/10.2139/ssrn.3034150

Yi, Y., Lindemann, M., Colligs, A., & Snowball, C. (2011). Economic burden of neural tube defects and impact of prevention with folic acid: a literature review. *European Journal of Pediatrics*, *170*(11), 1391–1400. https://doi.org/10.1007/s00431-011-1492-8

Youngblood, M. E., Williamson, R., Bell, K. N., Johnson, Q., Kancherla, V., & Oakley, G. P. (2013). 2012 Update on global prevention of folic acid-preventable spina bifida and anencephaly. *Birth Defects Research Part A - Clinical and Molecular Teratology*, *97*(10), 658–663. https://doi.org/10.1002/bdra.23166

Zaganjor, I., Sekkarie, A., Tsang, B. L., Williams, J., Razzaghi, H., Mulinare, J., … Rosenthal, J. (2016a). Describing the Prevalence of Neural Tube Defects Worldwide: A Systematic Literature Review. *PLoS ONE*, *11*(4). https://doi.org/10.1371/journal.pone.0151586

Zaganjor, I., Sekkarie, A., Tsang, B. L., Williams, J., Razzaghi, H., Mulinare, J., … Rosenthal, J. (2016b). Describing the Prevalence of Neural Tube Defects Worldwide: A Systematic Literature Review. *PLOS ONE*, *11*(4), e0151586. https://doi.org/10.1371/journal.pone.0151586

APPENDICES

Appendix 1: Questionnaire

NEURAL TUBE DEFECT CASE CONTROL STUDY: QUESTIONNAIRE

Information contained on this Questionnaire which could permit identification of any individual or any establishment has been collected with an assurance that it will be held in strict confidence by the investigator, will be used only for purposes stated in this study, and will not be disclosed or released to anyone other than those authorized.

PART I: IDENTIFYING INFORMATION

Date of interview (dd/mm/yyyy) _____ / _____ /_____

Participant identification number_____

Case status: O Case O Control

Date of birth of infant……..

Sex: O Male O Female O Indeterminate sex

Name of county of birth_____

Name of sub-county of birth_____

Name of hospital where participant is enrolled from…….

For cases only:

Diagnosis…….Date of diagnosis:…….

Description of birth defect:……

Photographs taken:……

Interviewer name _____Telephone contact_____

Maternal/guardian name……Telephone contact……..

Reporting Hospital	County	Outcome Of Birth

FORM COMPLETED BY……..

PART II: MATERNAL DEMOGRAPHIC AND OBSTETRIC HISTORY

Demographic information

5. Mothers age in years? _____

6. Where do you currently live? Village _____ Ward_____ Sub-county_____ County _____

7 .Was this your residence three months before your pregnancy and throughout the entire pregnancy?

☐ Yes ☐ No

8. If no, where did you live? ----------

9. What is your religion? ☐ Muslim ☐ Christian ☐ Traditional ☐ Other (specify)_____

10. What is the highest level of education that you have attained?
☐ Lower primary ☐ Upper primary ☐ Secondary school ☐ College/University

11. Are you tenants in the house you live or is it owned by the family?
☐ Rented ☐ Family House

12. How many bedrooms are there in the house you live in? _____

13. Is there electricity in the house you live in?
☐ Yes ☐ No

14. Do you have any of the following items in your house?
☐ Radio ☐ TV ☐ Telephone ☐ Fridge
☐ Mobile Phone ☐ House-help ☐ Motorcycle ☐ Car

15. Do you or your spouse own any agricultural land?
☐ Yes ☐ No

16. What is your marital status?
☐ Single ☐ Married ☐ Widowed
☐ Divorced ☐ Cohabiting

17. How many times have you been pregnant? ____

18.

Year of delivery	Alive /Dead	Diagnosis

Have you ever delivered a child with any other congenital malformation? (Cleft lip, missing limbs, additional limbs)(Show illustrations of other birth defects)

☐ Yes ☐ No

Year of delivery	Alive /Dead	Diagnosis

2.3. Residence_____

2.4. Date of Admission to Hospital (dd/mm/yyyy) _____/_____ /_____

2.5. Expected date of delivery (dd/mm/yyyy) _____/_____ /_____

2.6 What is the total number of years of Education you have had? _____

2.8. Occupation _____

2.9. Parity _____

2.10. Gestation age at birth_____

2.11. Date of delivery _____ / _____ /_____

2.12. Diagnosis_____

2.13. Pregnancy outcome _____

2.14 Have any of your family members ever had a child with a birth defect?

O Yes O No O Don't know

If yes,

What is your relationship with the mother of the child with birth defect?.....

Which birth defect did the child have (describe if not known)?.....

OPENING STATEMENT

In this interview we will be asking you questions about your family, health, lifestyle habits, and work history. The questions cover many topics because we don't know what causes most neural tube defects. We will study the answers from several mothers hoping to learn something new about the causes of neural tube defects. Your individual responses are being collected with an assurance of confidentiality.

Maternal Weight……..Kg Maternal Height: …….cm

I am going to ask many questions about the year before birth when you had a pregnancy affected by a neural tube defect. In order to do this, I need to start by asking you some dates.

A1. What was the date of birth of the affected pregnancy (dd/mm/yyyy_____/_____/_____?

A2. What date did the doctor give you as a due date of birth of the affected pregnancy____/_____/_____?

A3. In this pregnancy, how many babies were you carrying? PROBE: Did you have a single baby, twins, or more babies?_____

PREGNANCY HISTORY

A4. How many times have you been pregnant before the pregnancy that ended? Including pregnancies that may have ended in miscarriages, stillbirths, abortion, or a tubal or molar pregnancy? _____

(a) Live births…….. (b) Still births……

(c) Ectopic pregnancies……. (d) miscarriages/abortions……..

A5. In your 1st/ 2nd/ 3rd pregnancy, was there a health problem with your pregnancy?

Yes/ No _____

If yes specify the health problem _____

A6. When did the pregnancy last before the affected pregnancy ended?_____

Did the mother smoke cigarettes when pregnant with the index child?

O Yes O No O Don't know

If yes, how many sticks per day?

O 1-5 O 6-10 O > 10

Did the mother drink alcohol when pregnant with the index child?

O Yes O No O Don't know

If yes, how many glasses per day

O 1-4 O 5-8 O>9

When you got pregnant with the index child, were you trying to get pregnant at that time?

O Yes O No O Don't know

During this interview we will be asking you questions about different aspects of your life from 3 month before you became pregnant to the beginning of your pregnancy to the end.

RESIDENCE DURING PREGNANCY

A7. We would like to know where you lived from the time you became pregnant to the time you delivered in order for us to study possible environmental exposures.

A8. Where did you live during your 1st/2nd/3rd pregnancy? List all

i……..

ii ……

iii……

A9. When did you start living there? Dd/mm/yy

A10. Are you still living there? YES/ NO

A11. What month and year did you stop/ move from there? Dd/mm/yy

PREGNANCY HISTORY FOR INDEX BABY

A12. In the 3 months before you became pregnant were you on any form of contraception? _____Yes/ No

A13. If yes which type of contraception. List

i…….

ii……

A14. Did you stop using contraception in order to conceive? YES/NO ————————

ANTENATAL CARE

A15. How far along were you before you found out that you were pregnant? (Tick and indicate number)

Weeks.....

Months……..

A16. Did you have any antenatal care supplements (medicines) with this pregnancy (probe for the known supplements)? YES/NO ————————

A17. When was your first visit to the antenatal clinic? (Do not include date when pregnancy was confirmed verify from MCH card) dd/mm/yy

A17. Were there tests done during your pregnancy? YES/NO / DN_____

A18. If yes list them (amniocentesis/ blood profiles/ H.I.V/ urinalysis)_____

A19. In the 2 months before becoming pregnant did you take any medications? YES/NO/ DN _____

If yes list them if she remembers or use hospital MCH cards if available

A20. After becoming pregnant, did you take any medications to prevent pregnancy complications such as vomiting, headaches etc.? YES/ NO _____

If No, move to question number A24

	A21. Name of medicine (list all medication taken)	A22. How frequent did you take the medication?	A23. When did you stop taking the medicines?
1		O Once a day O Twice a day O Other specify…….	
2		O Once a day O Twice a day O Other specify…….	
3		O Once a day O Twice a day O Other specify…….	
4		O Once a day O Twice a day O Other specify…….	

SECTION B: MATERNAL HEALTH

At this time, and at other times during this interview, I will be asking you about illnesses you may have had and various kinds of medications or remedies you may have used. Please include medications prescribed by a health care practitioner and medications you might have obtained without a prescription from stores, pharmacies, friends or relatives, as well as herbal or home remedies. Now I have some questions about your health.

A24. Did you have any disease before and during pregnancy? YES/ NO/ DN_____

A25. If yes which one _____

Maternal Fever……

In the first 3 months before pregnancy and the first 3 months did you have any fever? YES/ NO……

How high was temperature…..? (^{O}C) highest temperature recorded

How long did the fever last...?

Did you have a rash at the time? YES /NO….

Medication taken (list)…….

How long did you use the medicines...?

What diagnosis was made after visiting the doctor?.....malaria/urinary tract infection?

Others……..

Maternal-Diabetes

When it was first diagnosed……

Which type…gestational (during pregnancy), type I insulin dependent/non-insulin dependent.

Medications……insulin/ oral tablets…….

Any associated complications...

Was there diet modification during pregnancy? YES/NO …..

How often did you measure your blood glucose level...? (Confirm blood sugar control)

Maternal Seizures

Have you ever had a seizure YES/NO/DN...?

Were you ever told by a doctor that you have epilepsy? YES/NO/DN……

Did you ever take any medications or remedies in the first 3 months of your pregnancy? YES/NO …

What did you take? (List) use ANC booklet……

Maternal Blood pressure

Did your health care provider ever tell you have a high blood pressure/ pre-eclampsia /eclampsia? YES/ NO

When you were first diagnosed? Dd/mm/yy _____

Were you pregnant at the time YES/NO? _____

Did you take pressure medications in the first 3 months of pregnancy? YES/NO……

If yes what did you take? Use maternal antenatal clinic booklet- list the drugs_____

How long did you use the medicines? _____

Other maternal illnesses

From 3 months before pregnancy to the end of your pregnancy are there illnesses we haven't already talked about? YES/ NO/ DN……

Did you take any medication that we haven't talked about? YES/ NO/ DN

If yes list……

Have you ever been diagnosed with any chronic illness we haven't talked about? Example cancer/ autoimmune disease/ sickle cell? YES/ NO……..

If yes specify _____

MATERNAL SCANS/ XRAYS

In the first 3 months before pregnancy and in the first 3 months of pregnancy, did you have any x-rays or scans done not related to pregnancy? YES/NO _____

If yes, when was the exposure to x-rays? …….. (dd/mm/yyyy)

MEDICATION

We are interested in some medicines that you may have taken from 3 months before you became pregnant, to the end of your pregnancy. These would include prescription and non-prescription medicines. Some of these medicines we may have already discussed. I will read you a list of medications. As I read the list, please tell me Yes or No whether you took the medicine. During this time period, did you take any of the following medications?

Paracetamol…….

Aspirin…….

Valproic acid……..…….

Ipubrufen…….

Augmentin……

Septrin……..

Levofloxacin……

Doxycycline…….

Cigarettes…….....

Alcohol……

Anti-retro viral (which ones example) list: ……

Herbal medicines…….…..

Multivitamins……

Others specify…….…..

Maternal diet

During the 3 months before pregnancy and first 3 months of pregnancy did you eat flour bought from the shop? Yes_____ No_____ _____

Flour ground from a posh mill? Yes_____/No_____

Did you consume folic acid fortified flours (maize or wheat) at least 3 times per week during the following periods?

Three months before pregnancy/conception O Yes O No O Don't know

First 3 months of pregnancy O Yes O No O Don't know

FOLIC ACID SUPPLIMENTATION

Did you take folic acid in the 3 months before becoming pregnant and in the first 3 months of pregnancy? YES/~~NO~~

When was folic acid supplementation initiated?

Before conception O Yes O No O Don't Know

Before pregnancy O Yes O No O Don't Know

During pregnancy O Yes O No O Don't Know

If during pregnancy, please indicate the trimester…….

Name of folic acid supplements (Confirm in the ANC booklet)	Strength of folic acid supplement (mg/pill)	Frequency per week (7 days)

NTD identified

BIRTH DEFECT DESCRIPTION

Appendix 2: Consent form

MOI UNIVERSITY COLLEGE OF HEALTH SCIENCES INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE (IREC) INFORMED CONSENT FORM (ICF)

Study Title: A CASE-CONTROL STUDY OF RISK FACTORS FOR NEURAL TUBE DEFECTS AMONG INFANTS ATTENDING SIX SATELLITE CLINICS AFFILIATED TO KIJABE HOSPITAL -2017.

Name of Principal Investigator: Christine Jepchumba Keitany

SPH/PGH/FE/13/15

Co Investigators:

Name of Organization: Field Epidemiology and Laboratory Training Program

Informed consent for: Mothers with children with NTD attending six satellite clinics affiliated with Kijabe Hospital.

Informed Consent Form for Mothers: with Infants with neural tube defects

This Informed Consent Form has two parts

Information sheet (to share information about my with you)

Certificate of consent (for signatures if you choose to participate)

You will be given a copy of the signed Informed Consent Form

Part I: Information Sheet

Introduction:

You are being asked to take part in a research study. This information is provided to tell you about the study. Please read the form carefully. You will be given a chance to ask questions. If you decide to be in the study, you will be given a copy of this consent form to sign.

Taking part in this research study is voluntary, you may choose not to take part in the study. Saying No will not affect your rights to health care services. You are also free to withdraw from the study at any point in time. If after data collection you decide to quit, you can request that the information provided by you be destroyed under supervision and will not be used in the research. You will be notified if new information becomes available about the risks or benefits of this research, then you can decide if you want to stay in the study.

Purpose of the Study

To find out risk factors that can lead to development of Neural tube defect.

Type of Research Project

This research will involve asking you questions about your pregnancy, before you pregnancy, any illness during that period, medication, and family history.

Why have I been identified to Participate in this Study?

You have been picked because your child has a Neural tube defect and so suitable for this research.

How long will the study last?

You will be in this study for less than six hours because we will be asking you afew questions.

What will happen to you during the study?

We are requesting you to help us learn more about Neural Tube Defects.if you accept we will ask you few questions. There will be no embarrassing or sensitive questions. (Question only applies to cases with an NTD)

Volunteer information

Your agreement to participate in this study is voluntary,

You may withdraw from the study at any time,

Refusal to participate in the research will not in any way affect the treatment given in the hospital.

Purpose of the study

The purpose of this study is to understand factors associated with development of NTDs and how to prevent their occurrence.

Procedure

During the interview you will be asked how your pregnancy progressed when did you first started taking folic acid, any history of chronic disease like diabetes or febrile illnesses in the first trimester of pregnancy, or use of medication.

You must have agreed to take part

You must be able to communicate with the researcher

Risks and discomforts

No risks or discomforts are associated with this study. The researcher will have access to your confidential information but maximum confidentiality will be maintained.

Benefits

The findings of this study will be used to educate women of reproductive age on important risk factors associated with neural tube defects, prevention and importance

of early use of folic acid, will also enable national government and ministry of health and county governments to formulate policies in prevention and care of NTDs.

Confidentiality:

All the information you have given will be kept private as allowed by law, to protect your privacy we will keep the records using documents numbers and not names. We will also keep the records in secure files and only the principal investigator will have access to your records. Your name or other things that may point to you will not appear in all reports that will be given to the institution and other stakeholders.

Voluntary participation

Decision to take part in this study is your choice. You may choose to participate without any consequence as to the quality of care you will receive.

Cost and compensation:

You will not be charged for any information. There will be no laboratory testing in this study. And we are also not paying those who have agreed to be part of this study.

Persons to Contact:

In case you have any questions regarding this study contact Christine Keitany 0722389861: Email address ckeitany88@gmail.com

Questions about your rights as a research subject: you may contact Institutional Ethics Committee (IREC) 053 33471 Ext.3008

Part II

Consent for subjects

I have read or have had read to me the description of the research study. The research assistant has explained to me the study and has answered the questions I had at this time .I have been told that there are no potential risks or discomforts in this study and that I will not benefit directly from it but it is for the good of the whole community. I

understand that any information obtained for the purposes of this study will be held in strict confidentiality.

I freely volunteer to take part in this study.

_____ _____

Name of Participant Signature of subject/thumb print

Date and Time_____

(Witness to print if the _____

Subject is unable to write

Name of Representative/Witness Relationship to Subject

_____ _____ _____

Name of person Obtaining Consent Signature of person Date

Obtaining Consent

_____ _____ _____

Printed name of Investigator Signature of Investigator Date

Appendix 3: Letters of Approval

FC

INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE (IREC)

MOI TEACHING AND REFERRAL HOSPITAL
P.O. BOX 3
ELDORET
Tel: 33471//2/3

MOI UNIVERSITY
SCHOOL OF MEDICINE
P.O. BOX 4606
ELDORET

Reference: IREC/2016/FELP/P
Approval Number: 0001828

2nd March, 2017

INSTITUTIONAL RESEARCH & ETHICS COMMITTEE
02 MAR 2017
APPROVED
P. O. Box 4606-30100 ELDORET

Christine Jepchumba Keitany,
Moi University,
School of Public Health,
P.O. Box 4606-30100,
ELDORET-KENYA.

Dear Ms. Keitany,

RE: FORMAL APPROVAL

The Institutional Research and Ethics Committee has reviewed your research proposal titled: -

"A Case-Control Study Risk Factors for Neural Tube Defects among Infants Attending Kijabe Hospital and Six Satellite Clinics - 2016".

Your proposal has been granted a Formal Approval Number: ***FAN: IREC 1828*** on 2nd March, 2017. You are therefore permitted to begin your investigations.

Note that this approval is for 1 year; it will thus expire on 1st March, 2018. If it is necessary to continue with this research beyond the expiry date, a request for continuation should be made in writing to IREC Secretariat two months prior to the expiry date.

You are required to submit progress report(s) regularly as dictated by your proposal. Furthermore, you must notify the Committee of any proposal change (s) or amendment (s), serious or unexpected outcomes related to the conduct of the study, or study termination for any reason. The Committee expects to receive a final report at the end of the study.

Sincerely,

PROF. E. WERE
CHAIRMAN
INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE

cc CEO - MTRH Dean - SOP Dean - SOM
Principal - CHS Dean - SON Dean - SOD

MEDICAL EDUCATION AND RESEARCH DIVISION

PO Box 20 Kijabe 00220 Kenya

Tel: 020-324-6637 fax: 020-3246335

E-mail:researcher.kh@gmail.com

July 06, 2017

Dear Christine Jepchumba Keitany

RE : A CASE-CONTROL STUDY OF RISK FACTORS FOR NEURAL TUBE DEFECTS AMONG INFANTS ATTENDING KIJABE HOSPITALAFFILIATED SIX SATELLITE CLINICS

The institutional review board having carefully reviewed your above title proposal grants you approval to conduct this study at Kijabe hospital.

This approval is for a period of one year from 06/07/17. Kindly note that if you intend to continue this study beyond 07/07/2018 then you will need to apply for approval from the institutional review board.

Kijabe IRB requires you to provide regular updates reports, from the study, for monitoring purposes.

We look forward to receiving the results of the interim analysis

We wish you all the best in the study. Kindly furnish this office with a copy of your results.

Thank you,

Sincerely,

Peter Halestap, MD
Chair, Kijabe Hospital IRB

"Health Care to God's Glory"